Microbial Risk Assessment for Recreational Use of the Kranji Reservoir, Singapore

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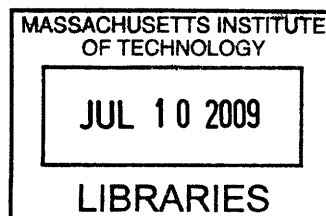All rights reserved

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Abstract

The Public Utilities Board of Singapore is responsible for management of the Kranji drinking water reservoir and wishes to open the reservoir for recreational water use as part of their "Active, Beautiful, and Clean Waters Programme". A field campaign was conducted at the Kranji Reservoir to determine the microbial water and sediment quality of the reservoir for use in a model that predicts the risk of gastrointestinal illness due to recreational use of the reservoir.

Water samples were collected at seven locations throughout the reservoir and sediment samples were collected at two locations located near the shore. The samples were then analyzed for *Enterococci* concentrations using a most probable number method. The measured geometric mean concentrations found during the field campaign were 13.3 *Enterococci* colony forming units (CFU) per 100 ml water and 1400 *Enterococci* CFU per gram sediment.

Based on the strengths and weaknesses of available statistics-based risk models, a model by Wiedenmann was chosen based on the flexibility of the model and the quality of the underlying epidemiological study. Using the model, no-observed-adverse-effect-level guideline concentrations of 25 *Enterococci* CFU per 100 ml for swimming, 51 *Enterococci* CFU per 100 ml for kayaking and 860 *Enterococci* CFU per gram sediment for wading were calculated.

Based on all available bacterial measurements of the Kranji Reservoir, an interim geometric mean guideline of 25 *Enterococci* CFU per 100 ml water and 860 *Enterococci* CFU per gram sediment is suggested. Single-sample maximums for a monitoring program should be set to 96 *Enterococci* CFU per 100 ml water and 2,500 *Enterococci* CFU per gram sediment. These guidelines should be applied to the area of the reservoir open to recreation, which should be restricted to the northern main section of the reservoir. Entry and exit from the reservoir and wading should be restricted to a smaller area of shoreline until more sediment samples are taken to determine safe entry and exit areas. Final geometric mean and single-sample maximum guidelines should be based on a study of the pathogen-to-indicator-bacteria ratios in the Kranji Reservoir.

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1 Introduction

The Singapore Public Utilities Board (PUB) is responsible for management of the water systems of Singapore. This includes drinking water treatment and supply, wastewater treatment, and storm water management. As part of their storm water collection program, PUB manages a series of coastal estuaries that have been dammed to form freshwater drinking water reservoirs. One of the largest of these reservoirs is the Kranji Reservoir on the north-west side of the country. In recent years, PUB has implemented a masterplan to provide more recreational opportunities to Singaporeans, as part of an effort to increase the appreciation of Singapore's water resources (PUB 2007a).

This masterplan calls for the Kranji Reservoir to be opened up for recreational water activities. Before doing so, PUB wants to evaluate potential illness risks to recreators participating in the three proposed levels of recreation for the Kranji; swimming, kayaking, and/or wading. In January 2009, a team from the Massachusetts Institute of Technology visited Singapore, and completed a microbial water quality study of the Kranji Reservoir. The data from this study are combined with the data from a previous water quality study of the reservoir to determine appropriate guidelines and recommendations.

Section 1 of this thesis was written as part of a collaborative effort with Carolyn Hayek, Jessica Yeager, Kathleen Kerrigan, and Jean Pierre Nshimyimana.

1.1 Singapore and Water Supply

1.1.1 Physical Location

Singapore is an island nation in Southeast Asia, just South of Malaysia (Figure 1) with a total land area of only 682.7 square kilometers (CIA 2009). For the purposes of fresh water capture and management, PUB has divided Singapore into three main catchment areas: the Western Catchment, the Central Catchment, and the Eastern Catchment. This study focuses on the Kranji Reservoir in the Kranji Catchment (Figure 2), which is located within the Western Catchment. The Kranji Reservoir is located in the northwestern corner of the island (1°25'N, 103°43'E) (NTU 2008).

Figure 1:Map of Southeast Asia with Singapore Highlighted (CIA 2009)
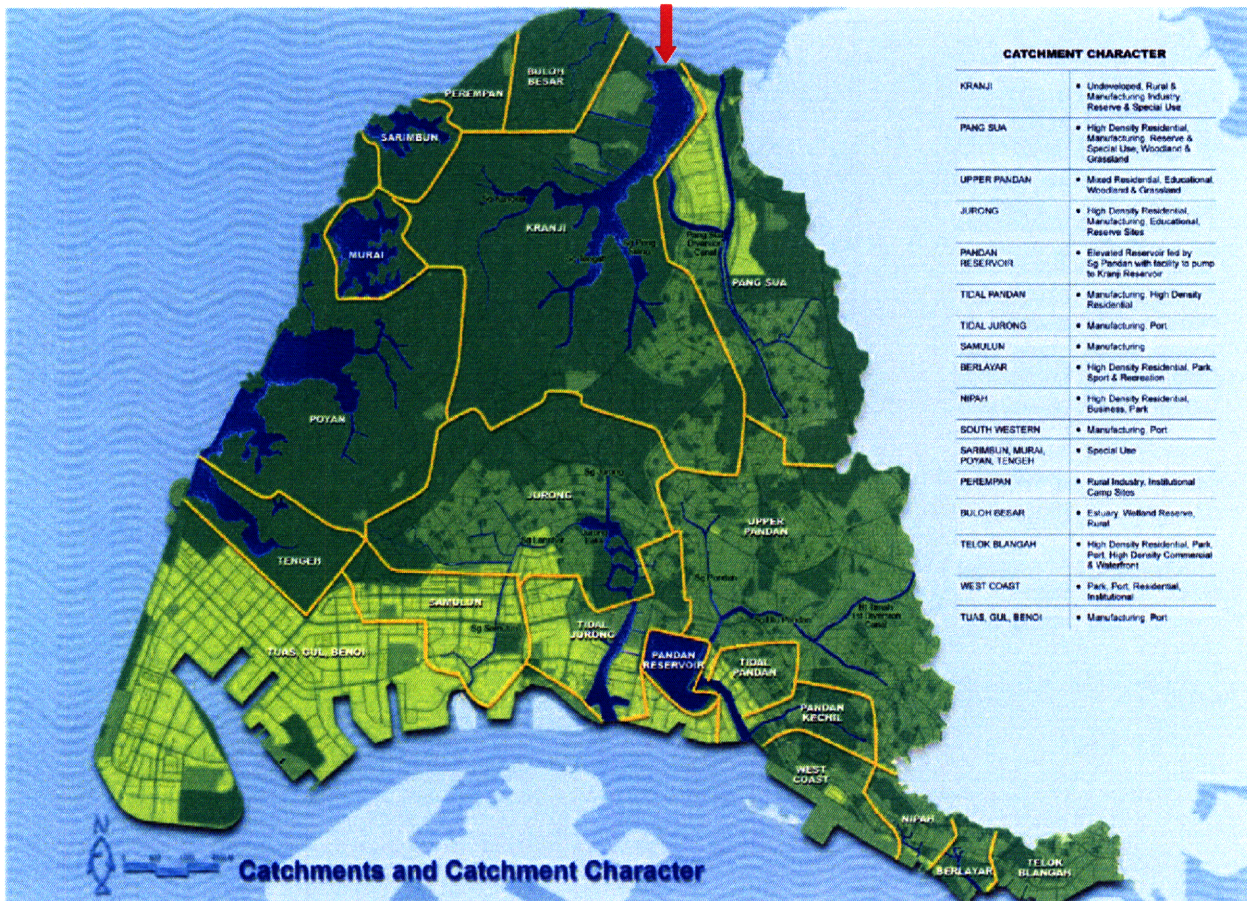
Figure 2 Map of Singapore Western Catchment with Kranji Reservoir Marked (PUB 2007b)

The Kranji Reservoir was created in 1975 by the damming of an estuary which drained into the Johor Straits that separate the Malaysian mainland from Singapore. The reservoir is approximately 647 hectares in area and the catchment has four tributaries, Kangkar River, Tengah River, Pengsiang River in the south, and Pangsua River in the north (NTU 2008). The Kranji Catchment is approximately 6076 hectares in area (NTU 2008). The Catchment has a variety of land uses, including forests, reserved areas, agriculture, and residential areas.

1.1.2 A Brief History

Singapore was established as a British port in 1819 due to its location and function as a hub for trade with India and China. After World War II, Britain felt that the country was too small to be a sovereign nation and instead granted them increasing liberties with time. Singapore joined the federation of Malaya in 1963, but the union was short-lived due to internal conflicts. In contrast to the other federation members, Singapore's majority population was Chinese. This racial diversity spurred the call for a "Malaysian Malaysia," leading to several race riots in Singapore. Singapore exited the federation and became an independent nation in 1965.

1.1.3 Issues of National Water Security

Water security can be defined from a national perspective as the ability to supply a sufficient amount of water to all of a nation's inhabitants. Singapore has 4.4 million people and a water demand of 1.36 billion liters per day (Madslien 2008). While Singapore receives a significant amount of rainfall—approximately 2400 millimeters per year (Tortajada 2006)—it is considered water scarce. Singapore has no natural aquifers or lakes and due to its small size there is little space to store water for use.

Prior to becoming a sovereign nation, Singapore negotiated treaties for water purchases from Malaysia to meet their water demand. The first treaty was signed in 1960 and expires in 2011, while a second treaty was signed in 1961 and expires in 2061. The two countries have already met to discuss the terms of new treaties that will take the place of existing water treaties once they have expired. However, Malaysia is demanding a price that is fifteen to twenty times higher than that negotiated under the previous

contract, which was S$0.026 per ten cubic meters (Tortajada 2006).

In response to Malaysia's demands, the Prime Minister of Singapore has called for water self-sufficiency by 2061, such that when the treaties on water exchange with Malaysia expire, there will no longer be a need to import water. Recognizing that meeting the country's water needs can be viewed as a problem of insufficient supply as well as one of high demand, PUB has taken actions to both increase Singapore's internal water supply and to reduce the national water demand through a strategy known as "Water for All: Conserve, Value and Enjoy." The campaign to increase supply consists of steps to re-use more wastewater, increase the supply of desalinized water, and capture as much of the considerable rainwater Singapore receives each year as is possible. By taking this two-pronged approach, Singapore is well on its way to becoming self-reliant in terms of its water needs.

1.1.4 Rainwater Catchments

Rainwater catchments are an important part of the water supply for Singapore. Stormwater is collected through a network of drains, canals and river channels and directed towards one of the nation's fourteen reservoirs. These reservoirs currently collect water from about half of Singapore's land surface. It is expected that additional catchments will be built by 2011 to bring the total rainwater capture area from one half to approximately two thirds of the country's land surface. The Kranji Reservoir is one of these catchments.

1.2 Catchment Masterplans

PUB wants to use its water management system not just for providing water, but to provide enjoyment to the people of Singapore as well. The Kranji Reservoir is an important part of this plan, since it is located near some of the last remaining undeveloped land in Singapore.

1.2.1 Active Beautiful Clean Waters Programme

An important part of the "Conserve, Value and Enjoy" campaign is the ABC Waters Programme. PUB launched the ABC Waters Programme in an effort to achieve national waters that are:

- <u>Active</u> – open for different recreational activities such as boating or fishing.
- <u>Beautiful</u> – aesthetically pleasing in a way that the nation's inhabitants can enjoy.
- <u>Clean</u> – of sufficient quality for domestic, industrial, and recreational uses.

The program involves a variety of methods, one of which is using drinking water reservoirs for recreation. By improving the quality, aesthetics, and access to Singapore's waterways, PUB hopes to foster a greater sense of ownership and respect for water in Singaporean communities.

1.2.2 The Western Catchment Master Plan and the Kranji Reservoir Project

The Western Catchment encompasses the western third of the country and is home to about 1 million people or 27% of Singapore's total population (PUB 2007b). The catchment remained largely undeveloped until after Singapore achieved independence (PUB 2007b) and is currently an approximately equal mix of urban development, industrial development, and natural environment (PUB 2007a). Residential areas are concentrated on the southern edge of the catchment (PUB 2007b).

The Kranji sub-catchment is located in the northern part of the Western Catchment. It is mostly undeveloped with some rural and manufacturing industry (PUB 2007b). The Kranji Reservoir was created by an east-west dam at the estuary of the Sungei Kranji. Most of the land around the reservoir is designated as open space under current zoning regulations, with the exception of some agricultural land use and a small golf course to the west and some light industry to the east (PUB 2007b).

While the Kranji Reservoir is strong in many aspects (including beauty, ecological

uniqueness and open spaces), the Western Catchment masterplan identifies that the Kranji sub-catchment currently has low visitor rates. This is due to a combination of factors. First, the site is relatively isolated since most of the sub-catchment is undeveloped. Second, public transportation serving the area is limited. Third, there are only two entry points to the reservoir (one on either side of the dam) and poor connectivity within the site. Finally, public recreational activities are limited. Current recreational opportunities include cycling, park visits, and minor fishing areas.

A proposal for improvements to the Kranji Reservoir has been made under the Western Catchment masterplan (PUB 2007b). The proposed changes would be primarily made to the existing entrances and play to the sub-catchment's strengths to boost low visitor rates while still preserving the rich natural resources. The addition of a Kranji Reservoir Visitor Centre west of the dam will provide educational information and experiences on the wetlands and the reservoir. Minor changes to vegetation at the intake will also prime the location for bird watching and construction of a bird observation tower is planned. Also, the introduction of an electric 'eco-cruise' boat will help increase connectivity within the site. Figure 3 from the Western Catchment Master Plan shows the location of the activity and beautification projects planned for the Kranji Reservoir.

On the east side of the reservoir the existing fishing area will be improved through the addition of jetty into the reservoir, and a native fish aqua-culture zone. The western side of the reservoir has the most improvements proposed. Proposed activities include a kayak launch, a visitor's center, an eco-tour cruise, and a nature walk.

LEGEND
(Symbols not to scale)

- Proposed Boardwalk
- Proposed Park Connector (NParks)
- Existing Path
- Proposed Path
- Restricted Public Access
- Proposed Shelter
- Proposed Building
- Proposed Bird Observation Tower
- Proposed Jetty
- Proposed Eco Tour Cruise
- Proposed Wetland Walkway Along Bund with Shelters
- Proposed Wetland
- Future Additional Projects
- Eco-Waterway

Kranji Reservoir - Masterplan

Figure 3: Kranji Reservoir Proposed Improvements (PUB 2007b)

1.3   Previous Bacterial Water Quality Studies

A team from the school of Civil and Environmental Engineering at Nanyang Technical University (NTU) conducted a major study of the water quality of the Kranji Catchment and Reservoir system from May 2004 to December 2007. The final report by NTU (2008) was submitted to PUB in March 2008. The goal of this study was to determine baseline water quality and gather information to develop an integrated water quality model for the Kranji system. The study collected information using seven sampling stations throughout the catchment (marked with red dots) and seven sampling stations within the reservoir (marked with crosses), as seen in Figure 4. The northern main body of the reservoir has four stations: Station 4 (Sta 4), Station 3 (Sta 3), Station 1 (Sta 1), and Three Arm Junction (3 Arm Jun). The southern arms of the reservoir each have one station each, named after the rivers that originally ran through them: Kangkar (KK), Tengah (TG), and Peng Siang (PS).
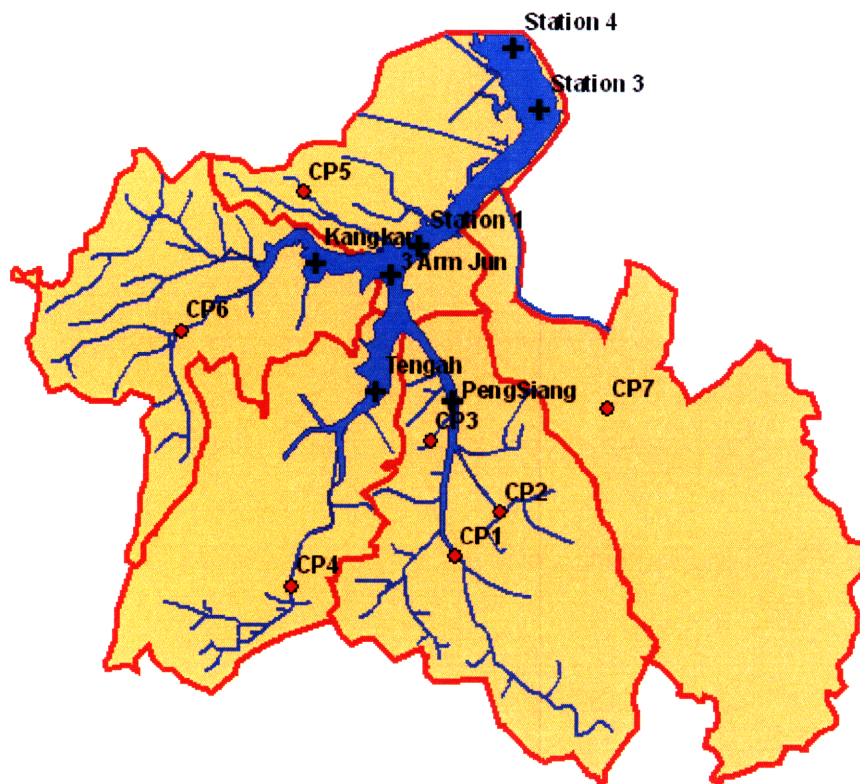
Figure 4: NTU Sampling Locations (NTU 2008)

The NTU study (2008) determined bacterial densities in the reservoir at the seven stations located in the Kranji Reservoir and identifies in Figure 4. The study measured

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Enterococci and *E. coli* as the indicator bacteria for water quality. Table 1 presents the results for *Enterococci* concentrations. Guideline concentrations for recreational waters can be expressed as a combination of a maximum value and geometric means, or as 95th percentile values (see Section 2.0 below for a more complete explanation). The USEPA uses the former method, and the Singapore government the latter. The bold and italicized values in Table 1 indicate stations that exceed USEPA (USEPA 1986) values and bold values in Table 1 indicate concentrations that exceed Singapore guidelines (SGNEA 2008).

Table 1: Kranji Reservoir *Enterococci* data (Sept 2005 to Sept 2007)(NTU 2008)

Location	Density (MPN/100ml)					Sample Size
	Minimum	Maximum	Geometric Mean	95th Percentile	Standard Deviation	
Sta 1 0m	1	*200*	15	36	60	13
Sta 3 0m	1	*100*	4	14	27	16
Sta 4 0m	1	*73*	4.6	12	23	16
Arm Jun 0m	1	*200*	19	42	69	13
Peng Siang	1	*2000*	**42**	**323**	600	16
Tengah	4.1	*220*	22	35	66	16
Kangkar	1	*200*	10	30	56	16

Note: ***Bold Italic*** values exceed USEPA guidelines, **Bold** values exceed Singapore guidelines

The results in Table 1 indicate that the 95th percentile *Enterococci* levels in the Kranji Reservoir are generally lower than Singapore guidelines for fresh water with the exception of concentrations measured at Station PS (NTU 2008). Stations 3 and 4, which are located at the north end of the reservoir, had the lowest concentrations. However, when compared to USEPA guidelines, all stations except for Station PS had acceptable geometric means, but all stations had at least one sample that exceeded the single-sample maximum.

The study of the Kranji catchment stations indicated that storm events contained higher bacterial concentrations than dry-weather flow (NTU 2008). This is to be expected, as higher bacteria levels are strongly associated with the first flush, during which a storm event washes any bacteria on the surface of adjoining land into the drainage system.

This finding was not confirmed in the reservoir because the NTU study did not take any storm-event samples from the reservoir.

1.4   Current Study

The data collected in January 2009 for this study will be combined with a statistics-based risk model to determine the risk of illness to potential recreational users of the Kranji Reservoir. The available risk models will be discussed, and an appropriate risk model will be chosen for this study. The risk model will also be used to calculate recommended mean bacteria concentrations and single-sample maximums for future use of the reservoir. The data from this study and the previous study by NTU (2008) will be combined and used to determine an appropriate area of the reservoir to be opened for recreational use. Finally, based on insights gained during this study additional studies of the Kranji Reservoir will be recommended.

2   Recreation and Risk Assessment

The link between water recreation and adverse health effects has been known since the early 1900s. Common ailments associated with water recreation include gastrointestinal symptoms, ear symptoms or infections, dermal symptoms, and respiratory complaints (Pruss 1998). There exist many documented cases of viral or bacterial outbreaks caused by contaminated water, particularly prior to the widespread use of sewage treatment plants when there are many recorded outbreaks of Salmonella-caused disease from using contaminated waters (Dufour 1984). Given the wide range of infectious agents in water and the many health problems potentially caused by recreational water contact, it is not economically feasible to test for each possible pathogen. Instead, risk equations based on bacterium that are generally indicative of the presence of pathogens (indicator bacterium) were developed to calculate guidelines for the overall risk of illness associated with recreational water use.

Calculated indicator bacterium guidelines are used in monitoring programs that sample bacterium concentrations in recreational waters on a regular basis. There are two types of monitoring programs used to protect the health of recreational water users. The first type is the method used by the USEPA and utilizes guidelines for geometric mean concentrations and single-sample maximums. Water samples are taken at regular intervals from the recreational water body and analyzed for the target indicator bacterium. If the geometric mean of the last five water samples exceeds the geometric mean guideline, or if a single-sample exceeds the single-sample maximum density, then the water body should be closed to recreation. The geometric mean guideline is a level of indicator bacterium that represents an acceptable amount of risk. Single-sample maximum densities are statistically-derived maximum concentrations of indicator bacterium present in a single sample, below which the guideline geometric mean for the water body is unlikely to be exceeded.

The second type of monitoring program is the method used by the World Health Organization (WHO), in which a water body is considered to be safe for recreation if the

95th percentile value of all samples is below the guideline levels(WHO 2003). This method measures the long-term safety of the recreational water body, but does little to protect recreational users against short-term increases in bacteria concentrations. PUB currently uses the WHO method to evaluate recreational safety at six marine beaches (SGNEA 2008).

2.1 History

2.1.1 United States Standards

The use of indicator bacteria as a way to quantify the quality of recreational waters began in the 1920s. The American Public Health Association adopted criteria for coliform bacteria in swimming pools as early as 1924, but identification of the risk from contaminated waters in an empirical way did not occur until later. Total coliform counts began to be used as an indicator of the safety of a water body in the mid-1930s, with many states adopting a standard of 1000 total coliform colony forming units (CFUs) per 100 ml. This standard was based on aesthetic impairment of waters rather than a determination of risk of illness (Dufour 1984). In the late 1940s a series of epidemiological studies using total coliform as an indicator of water quality were conducted by the United States Public Health Service (USPHS) in an attempt to determine safe bacterial levels (Dufour 1984). While the USPHS studies were the first to demonstrate a direct link between high coliform counts and increased amounts of illness, the USPHS did not have enough data to determine actual risk equations.

In 1968, a National Technical Advisory Committee to the newly created Federal Water Pollution Control Administration (FWPCA) recommended using fecal coliform instead of total coliform as the indicator bacterium of choice (Dufour 1984). The FWPCA recommended level was set at 200 fecal coliforms per 100 ml (Dufour 1984). This level was based on three factors: the fact that fecal coliform are approximately 18% of the total coliform count under average conditions, the observed health effects level from the USHPS epidemiological studies on recreational water use, and an additional factor of safety. In 1972, the United States Environmental Protection Agency (USEPA) confirmed the 200 fecal coliform standard based on research that showed reduced

numbers of Salmonella infections below that level (Dufour 1984). Despite a widespread criticism that the USPHS studies upon which the fecal coliform standard is based are inadequate, this standard continues to be used by some US states.

Recognizing a lack of studies that related the risk of infection to the amount of indicator bacteria in the water, the USEPA commissioned epidemiological studies at fresh and marine water swimming areas beginning in 1973. The resulting freshwater data from these studies were used to create the 1984 report "Health Effects Criteria for Fresh Recreational Waters" (Dufour 1984), which determined regression equations relating swimming-associated gastrointestinal symptom rates with the geometric mean *E. coli* or *Enterococci* density per 100 ml of freshwater.

In a 1986 report entitled "Ambient Water Quality Criteria for Bacteria." (USEPA 1986) the USEPA recommended acceptable indicator bacteria levels based on a historically accepted additional risk of illness equal to 8 per 1000 swimmers. The recommended geometric mean indicator bacterium concentration levels were determined using the regression equations from the 1984 "Health Effects Criteria ..." report. In terms of individual risk, the accepted additional risk means that there is an additional 0.8 percent chance above normal environmental infection rates that a swimmer will contract gastroenteritis from a single swimming event (USEPA 1986).

Table 2 summarizes the USEPA recommended criteria. Individual US state agencies have adopted these or stricter standards (USEPA 2003). The standards in Table 2 were developed on the basis of full-contact-immersion swimming, which is also referred to as primary contact recreation. Secondary contact recreation activities, such as boating, wading, and fishing, were not included in the 1984 epidemiological study. The USEPA mean indicator density guidelines are meant to apply to both primary and secondary recreational use, but states can apply for exemptions depending on local conditions. In general, many US states apply the single-sample maximum allowable density for Moderate Full Body Contact Recreation (column 5 of Table 2) as the standard for secondary recreation, and the Designated Beach Area single-sample maximum (column

4 of Table 2) as the standard for primary contact recreation. Section 3.5 contains further discussion of single-sample maximum allowable densities.

Table 2: Indicator Bacteria Density Criteria (USEPA 1986)

	Steady Sate Geometric Mean Indicator Density (Enterococci /100 ml)	Single-sample Maximum Allowable Density (Enterococci/100 ml)			
		Designated Beach Area (upper 75% C.L.)	Moderate Full Body Contact Recreation (upper 82% C.L.)	Lightly Used Full Body Contact Recreation (upper 90% C.L.)	Infrequently Used Full Body Contact Recreation (upper 95% C.L.)
Entero-cocci	33	61	78	107	151
E. coli	126	235	298	409	575

C.L. = Confidence Limit

The 1986 USEPA guideline criteria presented in Table 2 are still considered to be the United States standard for measuring risk of infection from freshwaters using *Enterococci* or *E. coli* as the indicator bacteria. However, this is likely to change in the future. There is a recognition that the current standards do not adequately account for different usages of recreational waters, and that with new methods it is possible to test for many more microbial agents directly instead of relying on indicator bacteria. In 2000, the United States Congress passed the Beaches Environmental Assessment and Coastal Health Act (BEACH Act) which directed the USEPA to update their guidelines to account for these factors. Starting in 2002, the USEPA and the CDC began series of epidemiological studies to account for a wider range of variables that influence the risk of illness from recreational water use (Yoder et al. 2008). However, the results from these studies are not yet known.

2.1.2 World Health Organization Standards

In 2003, the World Health Organization (WHO) released "Guidelines for safe recreational water environments: Volume 1 Coastal and Fresh Waters" (WHO 2003). In this omnibus guidance document the WHO proposed guidelines dealing with a wide range of issues that affect recreational waters, including drowning and injury prevention, bacterial water quality, dangerous aquatic organisms such as sharks, and more.

Chapter 4 of the WHO document discusses guideline indicator bacteria. Instead of using a geometric mean guideline like the USEPA, the WHO uses a 95th percentile method for measuring bacterial concentrations (WHO 2003). In this method, 95% of the water samples taken should be below the guideline values to be considered safe. The WHO recommends a four-tier rating scheme based on the 95th percentile value of *Enterococci* per 100 ml, summarized in Table 3.

Table 3: WHO Bacterium Guidelines (WHO 2003)

Class	95th percentile value of *Enterococci*/100 ml	Estimated risk per exposure	
		Gastrointestinal Illness	Acute febrile respiratory disease
A	≤40	<1%	<0.3%
B	41-200	1-5%	0.3-1.9%
C	201-500	5-10%	1.9-3.9%
D	>500	>10%	>3.9%

The estimated risks are not based on a defined risk equation like the USEPA standards. Instead, the guidelines are based on the results from a review of published epidemiological studies by Pruss (1998), with extra weight given to two randomized controlled trials conducted in marine waters. The emphasis on marine studies is based on a belief that the risk of illness is always lower for recreation in fresh waters than in marine waters (WHO 2003).

2.1.3 Singapore Standards

Current Singapore standards for marine and freshwaters are based on the WHO recommended guidelines (SGNEA 2008). Adopted in 2008, the goal for Singapore recreational waters is to achieve Class B waters or better. This translates to a guideline 95th percentile level of 200 *Enterococci* per 100 ml or less. Currently, only six marine beaches are monitored for water quality, and no freshwater bodies are monitored (SGNEA 2008).

2.2 Risk Assessment

Environmental risk assessment consists of three interconnected phases. The first phase is site characterization which includes the development of a site exposure model, the second is risk quantification through the use of risk models, and the third is risk

management and communication (Salhotra 2008). While the first two phases can be carried out through research and fieldwork, the third requires participation of the relevant regulatory agency.

2.2.1 Bacteriological Site Characterization

Site Exposure Model

The first step in site characterization is to determine an exposure model for current and anticipated use of the site. The exposure model identifies sources, exposure routes, and potential receptors. In the case of microbial contamination, the three primary sources are water, sediment, and surficial soil. From each of those sources, there are three possible exposure routes for pathogenic bacteria: dermal contact, inhalation, and ingestion (Haas Rose, & Gerba 1999). Potential receptors are divided into current receptors and future receptors. Each receptor group is then split further into drinking water consumers and recreational users of the reservoir. A complete exposure pathway is one in which a potential receptor is possibly exposed to pathogenic bacteria through an established exposure route; for instance a recreational user exposed to pathogens in sediment via ingestion. A complete site exposure model for Kranji Reservoir is presented in Table 4, where each highlighted square represents a complete exposure pathway.

Even though there exist complete exposure pathways for drinking water consumers, due to the extensive mixing and treatment by PUB before the drinking water reaches the consumer the microbial risk is considered to be negligible. The reservoir is currently used only for fishing from the bank, so current recreational users do not have any contact with the sediment or with the water. Drinking water consumers are exposed only through consumption of water from the reservoir.

Table 4: Site Exposure Model

Secondary Sources	Exposure Routes	Current Reservoir Use		Future Reservoir Use		
		Recreational Users	Drinking Water Consumers	Primary Contact Recreational Users	Secondary Contact Recreational Users	Drinking Water Consumers
Surficial Soil	Dermal Contact	■		■	■	
	Ingestion	■		■	■	
	Inhalation	■		■	■	
Sediment	Dermal Contact			■	■	
	Ingestion			■	■	
	Inhalation					
Water	Dermal Contact			■	■	■
	Ingestion			■	■	■
	Inhalation			■	■	■
Highlighted	squares indicate a complete exposure pathway.					

This study focuses primarily on potential risk to future recreational users of the Kranji Reservoir. The primary difference between future primary-contact and future secondary-contact recreational users is not the types of exposure, but the duration of exposure. Secondary-contact recreators are expected to have a much less exposure to the water and sediment, so the amount of pathogens to which they are exposed will be much lower.

2.2.2 Risk Quantification

Dose Calculations

The first step in conducting risk quantification is the calculation of the dose to which the potential receptors are exposed. The dose is calculated to determine the number of microorganisms to which a receptor is exposed during a single event or over multiple

exposures. Dose calculations begin with concentrations observed in the field. Since concentrations can vary significantly at a given location from field sample to field sample, it is crucial to use the geometric mean value in calculations.

The actual dose of microorganisms is calculated by estimating the amount of source medium that the receptor has been exposed to and the concentration of the pathogens in the medium. The best way to calculate the amount of source exposure is by a study of the current users. Since this is often difficult to do, standard sets of data have been compiled by various sources, such as the USEPA in their "Exposure Factors Handbook" (USEPA 1999b) or in available published reports. The dose over multiple exposures is estimated by taking the single event exposure and multiplying by the number of likely exposure events. Tables of likely exposures are also available from similar sources as those for the exposure factors. Exposure for the future recreational users of the Kranji Reservoir is tabulated in Section 4.3. The concentration of pathogens in the target medium is usually estimated through the use of an indicator bacterium.

Risk Equations

The key assumption behind any risk quantification is that there is a relationship between the dose of the contaminant and the response of the receptor that can be expressed using an equation or set of equations. Microbial risk relationships usually assume that the relationship is immediate, and that it increases with increased concentrations of microbial pathogens that are ingested. The relationship between dose and response for microbial pathogens is often not known and has been modeled as a log-linear relationship (Dufour 1984), as a logistic relationship (Fleisher 1991; Wymer & Dufour 2002), and using more complicated statistics-based models (Wiedenmann 2007). Section 3 looks at these models in more detail.

2.2.3   Risk Management and Communication

The final risk assessment step is to take action to manage the risk, and communicate the risk to potential receptors. The most common method of managing illness risk for water bodies is by setting guideline standards for bacterial concentration. These guidelines are usually expressed as an acceptable mean concentration of an indicator

bacterium, and acceptable maximum limits for single-samples. The agency responsible for managing the water body is required to take periodic water samples to ensure that the guidelines are met. If the guidelines are exceeded, the water body should be closed to use, and the closure communicated to potential users.

2.3 Indicator Organisms

Using a single indicator bacterium to represent the water quality of recreational waters has been standard practice for many years. Epidemiological studies of recreational waters usually measure multiple indicator bacteria in order to find which one represents the risk most accurately. No indicator is perfect, but often *Enterococci* is chosen as a reasonable compromise. The indicator bacteria concentrations are an important component of the risk assessment process.

2.3.1 The Ideal Indicator Organism

The choice of indicator organism is very important. According to Palmer et al. (1984) the ideal indicator organism will have specific characteristics.

- "be associated with the source of pathogens;
- be able to provide an accurate estimate of the number of
  pathogens present at the levels which pose a health risk; and
- be measurable by simple methods with considerable accuracy."
  (Palmer, Lock, & Gowda 1984)

First, requiring the indicator bacterium to be associated with the source of the pathogens is a guard against false positives. If the indicator organism reproduces naturally in the environment, then the concentrations of the indicator bacterium may indicate a problem when there is not one. *E. coli* and fecal coliforms have been shown to grow in tropical soils, so their use in tropical climates such as Singapore is problematic. As early as 1991, a study was conducted by researchers at the University of Puerto Rico that showed that tropical streams could contain levels of fecal coliforms higher than the recommended levels, yet be free from pathogens (Hernandez-Delgado, Sierra, & Toranzos 1991). *Enterococci* may also grow in tropical soils. The USEPA

recognizes this problem (USEPA 1999a), but until more epidemiological studies have been conducted in tropical waters there are no approved alternatives.

Secondly, picking an indicator organism that provides an accurate estimate of all the pathogenic organisms is essential, but very difficult. There is a wide variance in the causes of adverse health effects, and for some the cause is not always known. According to a CDC report on illness associated with recreational water use in 2005 and 2006, most common etiologic agents were parasitic (43.6% of identified cases), bacterial (28.2%) and viral (5.1%) (Yoder et al. 2008). The most common adverse health effect of gastroenteritis is usually assumed to be caused by cryptosporidium, a parasite that is usually associated with human fecal contamination. The common ailment of swimmer's rash or itch is associated with avian schistosomes, a parasite whose reproductive cycle includes snails and waterfowl, but in humans presents as a rash which usually resolves itself in approximately a week (Levesque et al. 2002). Swimmers ear (otitis externa) is associated with recreational water use, but the exact cause is not known, though there is an unconfirmed assumption that the bacteria *Pseudomonas aeruginosa* is the cause (Calderon & Mood 1982). In 2005-2006, the CDC also recorded outbreaks due to Norovirus and Shigella sonnei virus in recreational waters (Yoder et al. 2008). Given this wide range of causes and sources, it is impossible to find an indicator bacterium that can accurately estimate concentrations of all of these etiological agents. For this reason, most large scale epidemiological studies focus on one or two waterborne diseases and attempt to correlate their target indicator bacteria with the studied illness. See Section 2.3.2 below for a discussion of large scale studies and the indicator bacteria that they chose.

Finally, Palmer (1984) states the ideal indicator organism needs to be easily and accurately measurable. The biggest problem with the most common indicator organisms is not the ease of measurement, but the amount of time that it takes to obtain test results. All of the commonly used indicator organisms are bacterial in nature, and whether a direct filtration or most probable number (MPN) method is used, a minimum of 24 hours is required before results are obtained. This is changing however, as the

development of quick polymerase chain reaction (QPCR) methods makes it possible to quickly identify concentrations not only of indicator organisms but of the actual pathogens. Unfortunately QPCR remains expensive and lacks easy portability.

2.3.2 Large Scale Epidemiological Studies

Since the 1980s there have been many epidemiological studies that sought to establish the relationship between various adverse health effects and recreational water use. The studied health outcome and the indicator organisms used in six of these freshwater epidemiological studies are shown in Table 5. Section 2.3.3 and 2.3.4 will look at the Dufour (1984) and Wiedenmann (2006) studies in additional detail. The summary data of these studies was collected in two papers one by Annette Pruss (1998) and one by Denis Zmirou et al. (2003). Each paper looked at the available published epidemiological studies to determine the relative illness risks associated with recreational use of waters, as measured by the chosen indicator bacteria.

Table 5: Description of select freshwater epidemiological studies (Pruss 1998; Zmirou et al. 2003)

First Author	Year	Country	Indicator	Health Outcome	Notes
Dufour	1984	US	2,3,4	GI	Used for EPA guidelines
Ferley	1989	France	1,2,4,7,8	AGI	
Fewtrell	1992	UK	2,4,5,6	All adverse health effects	
Seyfried	1985	Canada	2,4,6,7,12	All adverse health effects	
Lightfoot	1989	Canada	2,3,6	GI	Unpublished Thesis
Van Asperen	1998	Holland	2,3,4,5,10	GI	
Wiedenmann	2006	Germany	3,4,9,11	GI	Only freshwater randomized trial. Not included in Pruss, Zmirou
1 = total coliforms, 2 = fecal coliforms, 3 = *E. coli*, 4 = fecal streptococci or *Enterococci*, 5 = enterovirus, 6 = staphylococci, 7 = *Pseudomonas aeruginosa*, 8 = Aeromonas spp, 9 = *Clostridium perfingens*, 10 = bacteriophages, 11 = coliphages, 12 = heterotrophic bacteria, GI= gastro-intestinal symptom, AGI= acute gastro-intestinal disease					

All of the freshwater studies except for Wiedenmann (2006) are prospective-cohort studies, where participants of the study were recruited from people already using the water for recreational use. Wiedenmann (2006) was the first randomized-controlled-trial that looked at freshwater recreational use risks.

All of the studies measured fecal coliforms, most likely due to historical use and ease of testing. The second most commonly used indicator bacterium was fecal streptococci/*Enterococci*. Additionally, a wide variety of other indicator bacteria was used including the most commonly regulated indicator, *E. coli*.

2.3.3 1984 Dufour Epidemiological Studies

In 1984, Dufour published an article summarizing the results of an epidemiological study commissioned by the USEPA to determine the link between water quality and gastrointestinal illness (Dufour 1984). The prospective-cohort study recruited a total of 34,598 participants from beaches at Lake Erie, Pennsylvania, and Keystone Lake, Oklahoma. Field surveys were conducted in 1979, 1980, and 1982 at the Lake Erie location, and 1979 and 1980 at Keystone Lake. The study measured rates of illness among swimmers and non-swimmers alike through the use of follow-up surveys conducted by phone 8-10 days after the participant's beach visit. Swimmers were defined as having complete exposure of the head to the water, while non-swimmers were participants who did not immerse their heads in the water. Note that non-swimmers may or may not have had contact with the water. Illness rates were measured using two definitions, gastrointestinal illness (GI) and highly-credible gastrointestinal illness (HCGI). Follow-up surveys defined GI symptoms as vomiting or diarrhea or stomachache or nausea; HCGI was defined as having vomiting or diarrhea with a fever, or stomachache/nausea with a fever (Dufour 1984). Of the indicator bacteria measured, both *E. coli* and *Enterococci* were found to have good correlation with gastrointestinal illness. Section 3.1 below describes the risk model derived from this study.

2.3.4 2006 Wiedenmann Epidemiological Studies

The purpose of the Wiedenmann et al. (2006) trials was to provide a better scientific basis for recreational freshwater standards. This was the first randomized controlled trial conducted on freshwater. There were 2,196 participants recruited prior to recreational contact, and participation was strictly controlled to achieve a representative population. The study took place at five freshwater beaches in Germany. Exposure to

water was strictly controlled, with swimmers directed to a roped off area of approximately 10 by 20 meters. They were allowed to swim for 10 minutes, and instructed to completely immerse their heads at least three times during their swimming activity. The microbial makeup of the water in the swimming zone was analyzed every 20 minutes (Wiedenmann et al. 2006). Non-swimmers were restricted to a roped off area of the sand, and unlike the USEPA (1984) studies, were not allowed any contact with the water. This makes the Wiedenmann study the most controlled of all the freshwater epidemiological studies. Illness rates were tracked through phone interviews by doctors one week and three weeks after exposure to the water. To determine risk equations based on this study, Wiedenmann defined gastroenteritis as diarrhea, or vomiting, or nausea and fever, or indigestion and fever (Wiedenmann 2007). This definition is not significantly different from the definition Dufour used for HCGI. Wiedenmann also found that *E. coli* and *Enterococci* had good correlation with rates of illness. Section 3.2 below describes the risk model derived from this study.

Measuring the risk associated with recreational water use depends on measuring the correct indicator bacterium in the correct locations. Sampling locations are picked based on complete exposure pathways to recreational users. The choice of indicator bacterium is based on historical use by regulators, by the availability of epidemiological studies that quantify the relationship between the indicator bacterium and the risk of illness, and by the availability of risk models optimized for the indicator bacterium. Final guidelines specify the indicator bacteria concentrations that represent the amount of risk regulators are willing to expose recreators.

3 Risk Models

A complete risk model for freshwater recreation is an equation or set of equations that relate recreational use to the additional probability of illness to the recreator. Two complete models for estimating the risk of gastroenteritis from freshwater recreation are a model proposed by Alfred Dufour (1984) and a model proposed by Albrecht Wiedenmann (2007). The equations derived for these models and the assumptions behind these models are discussed in the following section.

3.1 Dufour Risk Model

The model that the USEPA currently uses to estimate risk for freshwater recreation was developed from the epidemiological studies conducted at both fresh and marine water beaches and reported by Dufour (1984). From the data collected in those studies, Dufour (1984) developed a set of risk equations that link the concentration of *E. coli* or *Enterococci* in the water to the additional rate of gastrointestinal illness. The USEPA then used these risk equations to calculate the guideline geometric mean bacteria densities shown in Table 2 (USEPA 1986). While the model that was suggested by Dufour in 1984 is still the basis for the current USEPA guidelines, subsequent papers, including one co-authored by Dufour himself, have suggested major revisions to the model. This section discusses how Dufour derived the equations in the risk model, the improvements suggested to the risk model based on the same data, and problems with the Dufour model.

3.1.1 Dufour Risk Equations – A Log-Linear Model

Dufour (1984) developed his risk equations using data from the epidemiological studies discussed in Section 2.3.3. Dufour separated the data for fresh and marine waters then plotted graphs where the dependent axis was the log_{10}Bacterium concentration, and the independent axis was the difference between swimmer and non-swimmer illness rates per 1000 swimmers (Dufour 1984). Figure 5 is a plot of the original data for *Enterococci* in freshwater, along with the linear regressions found by Dufour. Two sets of data were plotted, one for general gastro-intestinal illness (GI) rates (Equation 1) and one for highly credible gastrointestinal illness (HCGI) rates (Equation 2) where GI and HCGI are

as defined in Section 2.3.3. The data used to plot the points were developed by taking the geometric mean bacterium concentrations for an entire year and the illness difference rate for that same year.
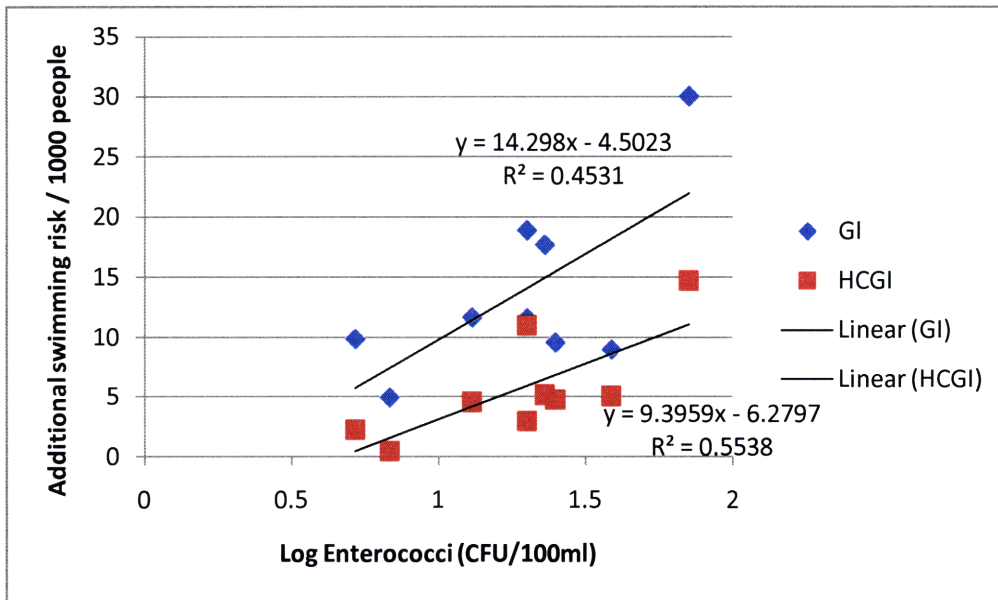
Figure 5: 1984 Linear Regressions (Data from Dufour 1984)

After plotting the data, the relationship between illness rate and bacteria indicator density was assumed to be log-linear, of the form seen in Equation 1 and 2.

$$Additional\ GI\ Risk/1,000\ people = -4.5 + 14.3 * \log(C_{en}) \qquad (1)$$

$$Additional\ HCGI\ Risk/1,000\ people = -6.3 + 9.4 * \log(C_{en}) \qquad (2)$$

Dufour rejected Equation 1 as an appropriate risk model for GI risk because the symptoms were less well defined and the correlation was weaker than that for HCGI symptoms. Equation 2 is the final risk model Dufour suggested for *Enterococci* concentrations in freshwaters, and this was the model adopted by the USEPA in 1986 (USEPA 1986).

3.1.2 USEPA Guidelines

In 1986, the USEPA used the Dufour risk equations to formulate the suggested guidelines for recreational waters seen in Section 2.1.1 and Table 2. They accomplished this by assuming an acceptable level of risk for recreational use of waters over the baseline rate of HCGI in the recreator population and then solving Equation 2 (and the corresponding equation for *E. coli*) for bacterium concentration C_{en}. This

acceptable level of risk was set at 8 additional cases of HCGI per 1000 swimmers. This is equal to an additional risk of 0.8% for each individual user per swimming event. The guideline value for *Enterococci* in freshwater was calculated to be 33 *Enterococci* per 100 ml. Figure 6 shows the risk curve generated by Equation 2 over the range of *Enterococci* concentrations found in the Kranji Reservoir. The dashed line represents the EPA guideline of 33 *Enterococci* per 100 ml and the associated additional risk of 0.8%.
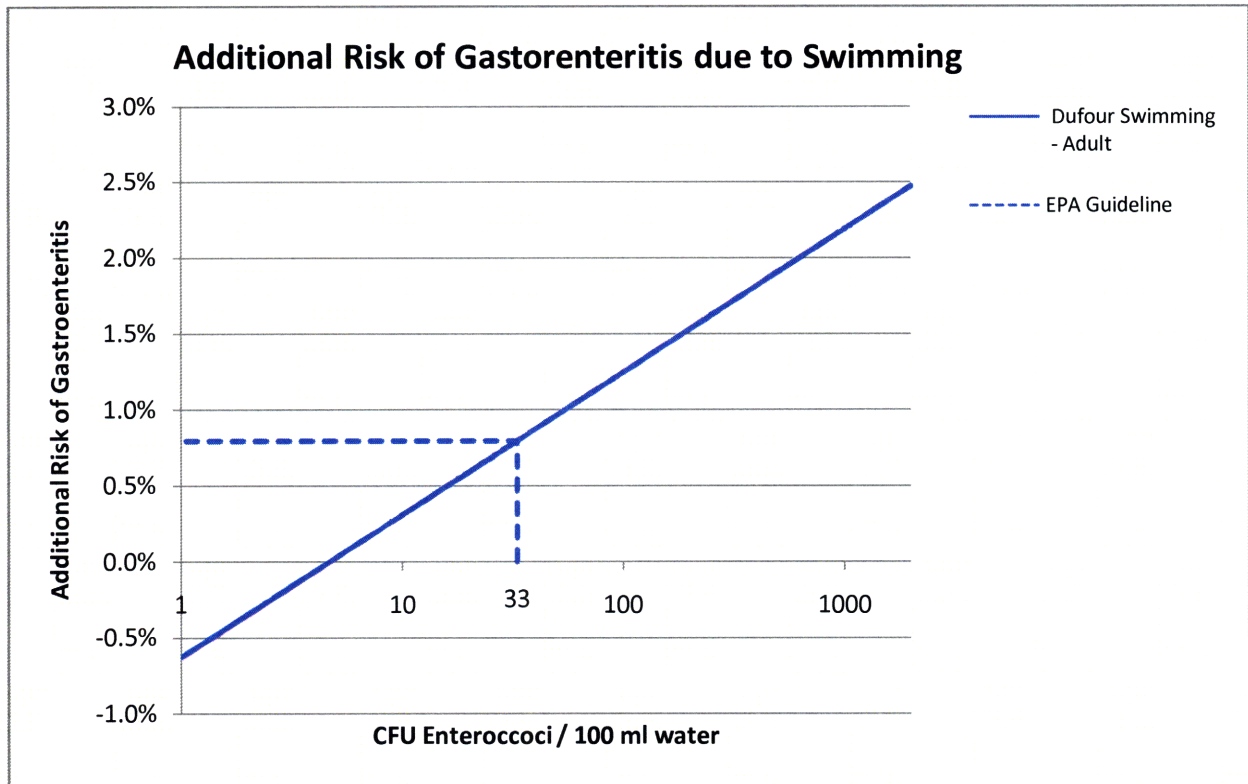
Figure 6: Dufour Risk Curve

3.1.3   Problems with the Dufour Model

Criticisms of the Dufour risk equations and the EPA guidelines derived from them generally fall into three categories. The first is a criticism that the data was improperly manipulated before deriving the risk equations. Secondly, the study that collected the data has been criticized on several counts. Finally the assumption of a log-linear model for the risk equations is a major source of criticism. The following discusses these issues in more detail.

Loss of Data

One of the most significant problems with the Dufour model is that the risk equations were calculated by using the geometric means of the bacterial concentrations over an entire year. The actual concentrations ranged over one to two orders of magnitude during each year, and the standard deviation of the bacterial density was not given in the original analysis. Since the higher concentrations probably account for a majority of the incidences of illness, using the yearly geometric mean eliminates many data points that might change the risk equations. In addition to averaging the data, the non-swimmer illness rates were pooled across all locations, which would obscure possible outbreaks at a specific location.

Prospective Cohort vs. Randomized Trials

Most epidemiological studies done on recreational waters have been prospective cohort studies, including the studies upon which Dufour (1984) based his risk model. This is a common type of epidemiological study, but it has several problems when compared to a randomized controlled epidemiological study.

Prospective-cohort study participants are recruited from people who have already used the recreational waters. Investigators usually recruit swimmers and non-swimmers (e.g. waders or sunbathers), survey them at the recreation location, and follow up with another survey a week to a month later to determine illness rates. Water quality characteristics are measured on the day that study participants are using the water, but there is no attempt to control the amount of exposure that the participants have with the water.

Randomized trials represent a more rigorous approach to an epidemiological study. In a randomized trial, participants are recruited before recreation takes place. The locations, the amount of time in the water, and the type of exposure to the water (full submersion versus partial submersion) are strictly controlled. Participants are screened beforehand for symptoms of the target illness, and follow-up surveys are often of the form of in-person interviews, or telephone and mailed surveys. Follow-up interviews are

conducted as blind studies, in which the interviewer does not know if the participant was a swimmer or a non-swimmer.

Randomized trials are a more accurate way to determine dose-response relationships since they account for more of the relevant risk factors. However, prospective-cohort studies are often used due to the expense and difficulty in designing and executing randomized trials. There has been only one published freshwater randomized trial, that of Wiedenmann (2006). Section 3.2 below will introduce the model proposed by Wiedenmann based on that study.

3.1.4 Fleisher Risk Equations - Logistic Regression Model 1

One common criticism of the risk equations developed by Dufour is that the log-linear model of the underlying epidemiological data is incorrect (Fleisher 1991; Wade et al. 2003; Wymer & Dufour 2002). In 1991, Fleisher published a paper that re-analyzed the portion of the data from the 1984 studies collected in marine water using a logistic regression model. The advantage of a logistic regression model is that it specifies the probability of illness directly and has a sinusoidal shape that corresponds with dose-response curves calculated from animal infectivity studies (Fleisher 1991). The general formula for a logistic regression model is seen in Equation 3 (Wymer & Dufour 2002).

$$P = \frac{1}{1+e^{-(\alpha+\beta x)}} \qquad (3)$$

In Equation 3, P represents the absolute probability of contracting gastrointestinal illness from recreational water use. α and β are terms that describe the shape of the risk curve and are solved for by fitting the risk curve to data from an underlying epidemiological study. x represents the $\log_{10}$ indicator bacteria concentration.

Fleisher (1991) constructed a series of models using logistic regression in attempts to find a useful risk model. One major difference in the way Fleisher (1991) and Dufour (1984) constructed their models was that Fleisher separated the data from the three locations used in the marine studies. He also did not use the non-swimmer illness rates in his equations, choosing to solve for absolute risk rather than additional risk. After

separating the data by location, Fleisher (1991) then attempted to derive generalized risk equations using that data. Fleisher came to the conclusion that the risk varied so significantly between the locations that the Dufour risk equations (Equation 2) were not useful (Fleisher 1991). He also concluded that the underlying data was not sufficient to develop a truly accurate risk model.

3.1.5 Wymer and Dufour Risk Equations -Logistic Regression Model 2

Wymer and Dufour (2002) revisited Dufour's 1984 data, and applied a logistic regression model to the data. Unlike Fleisher (1991), they decided to incorporate the background illness rate of non-swimmers into their model (P_0 in their equations). By re-arranging Equation 3, and dividing P by P_0, they derived Equation 4, which is the natural log of the risk ratio (absolute risk/baseline risk) and its relationship to $\propto$, β, and x from Equation 3.

$$\ln\left[\frac{P/(1-P)}{P_0/1-P_0}\right] = \propto + \beta x \qquad (4)$$

Using Equation 4, Wymer and Dufour (2002) then calculated values for $\propto$ and β using two different models. They concluded that by incorporating the background illness rates into the model, their results accounted for much of the discrepancy between locations that Fleisher found (Wymer & Dufour 2002). Despite this improvement, Wymer and Dufour still concluded that the exposure-response relationship found in the 1984 paper could not be extended to multiple locations (Wymer & Dufour 2002). They also concluded that it may not be possible to derive a generic risk equation that would be valid for multiple locations, since population susceptibility and pathogen ratios differ significantly over time (Wymer & Dufour 2002).

3.2   Wiedenmann Theoretical Risk Equation

Wiedenmann (2007) proposed a theoretical statistics-based equation for recreational water use risk. This risk equation is composed of several parts. The first assesses population susceptibility, the second describes the risk of infection from a single pathogen, and the third estimates the number of pathogens ingested. Equation 5 is the risk equation proposed by the Wiedenmann (2007).

$$Risk = (MR - BR) * \{1 - [1 - p(1)]^z\} \qquad (5)$$

The next three sections will examine this risk model part by part.

3.2.1 Population Susceptibility - $(MR - BR)$

The first term in Equation 5 measures the extent that a population is susceptible to the disease of interest. MR represents the maximum rate of disease in a population if everyone was exposed to the etiological agents. The largest this number can be is 1, representing a perfectly infecting pathogen. In reality, pathogen infectivity widely varies. For the pathogens that cause gastroenteritis, the maximum rate in any given population is usually less than 0.1 (10%) (Wiedenmann 2007). The second part of the population susceptibility is the base rate (BR) of the population. This is the rate that the disease naturally occurs in the population without recreational water use. For gastroenteritis this is usually between 0.01 to 0.03 (1-3%) (Pruss 1998)

3.2.2 Single Pathogen Risk - $p(1)$

The term $p(1)$ in Equation 5 represents the probability of getting sick through the ingestion of a single pathogen. This is determined by infectivity studies on human subjects. For water-related diseases, such as gastroenteritis, which are caused by more than one type of pathogen, the $p(1)$ term represents the average single-pathogen infectivity. The risk of illness from ingesting multiple pathogens, $p(z)$, is calculated by determining the probability of not getting sick $[1 - p(z)]$, where z represents the number of pathogens ingested. $[1 - p(z)]$ is equal to $[1 - p(1)]^z$. Finally, the probability of illness from ingesting z pathogens is equal to $\{1 - [1 - p(1)]^z\}$.

3.2.3 Pathogen Ingestion - z

In Equation 5, the number of pathogens ingested is represented by the single term z. Since we can't measure the number of pathogens ingested directly, z is an equation that relate pathogen concentrations to indicator organism concentrations which can be measured directly. Equation 6 is the expanded z term for the risk equation (Wiedenmann 2007).

$$z = PIR * FIO * v_{intake} \qquad (6)$$

The first two terms in Equation 6 allow the conversion from measuring pathogen concentrations directly to using an easily measured indicator bacteria. PIR is the

Pathogen/Indicator Ratio and FIO is the Fecal Indicator Organism concentration. If the pathogens of interest and the indicator organism had exactly the same die-off characteristics, then the PIR would be a simple constant that could be multiplied by the FIO concentration. However, the PIR is usually more complicated than a simple constant. Based on experimental observations, Wiedenmann suggested Equation 7 for the PIR (Wiedenmann 2007).

$$PIR = 10^q/(FIO/100\ ml),\ where\ q = a + b * \log_{10}(FIO/100\ ml) \quad (7)$$

a and b are constants that describe the shape of the risk curve and can be found by conducting a study that measures the pathogen and indicator concentrations in the target waters, or by curve-fitting the risk data from an epidemiological study. Wiedenmann chose the latter method to determine the values of a and b for his model (Wiedenmann 2007). Variations in a and b have different effects on the resulting risk curves. a primarily acts to shift the risk curve to the left or right, while b determines how quickly the risk changes between the base rate and the maximum rate.

The final variable needed to determine the rate of pathogen ingestion is the volume of contaminated material that has been ingested, v_{intake}. The intake term is expressed as a percentage of the base concentration unit. Since bacteriological water quality is expressed as the number of indicator organisms per 100 ml, the ingestion term for water is divided by 100 ml (Wiedenmann 2007). When the concentration of interest is measured in the sediment, the ingestion term is per 1 g (Donovan et al. 2008). The complete ingestion term seen in Equation 8 combines Equations 6 and 7.

$$z = v_{intake} * 10^{a+b*\log_{10} C_{EN}} \quad (8)$$

3.2.4 No-Observed-Adverse-Effect Levels (NOAELs)

A no-observed-adverse-effect level (NOAEL) is the bacterial concentration below which illness rates for recreational users are not significantly different than the background rate of illness. Wiedenmann et al. (2006) identify a NOAEL of 25 *Enterococci* per 100 ml (Wiedenmann et al. 2006) for swimming. Wiedenmann et al. (2006) suggested that this would be the appropriate regulatory level for agencies to adopt. Figure 7 shows the risk curve generated by Equation 5 over the range of concentrations found in the Kranji

Reservoir. The dashed line represents the NOAEL of 25. Although the model shows a risk of 1.1% associated with the NOAEL, this is due to the need to use a smooth equation to generate the risk curve. The additional risk at levels below the NOAEL is actually zero.
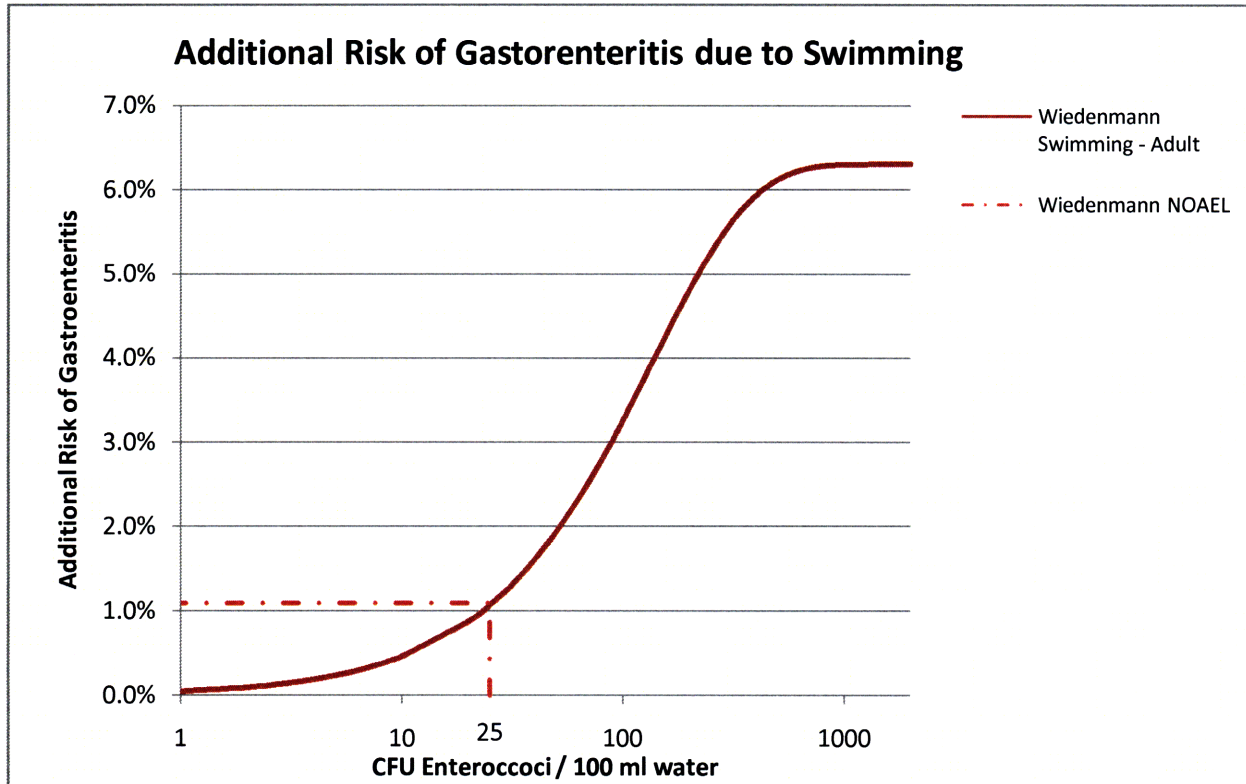
Figure 7: Wiedenmann Risk Curve

3.2.5 Problems with the Wiedenmann Model

The main problem with the Wiedenmann Model is the derivation of the PIR term described by Equation 7. The Pathogen Indicator Ratio is the weakest link in the generalized risk model as embodied by Equation 5 and 6. In the past, the PIR has been inferred from epidemiological studies, usually by assuming an ingestion rate. Wiedenmann assumes a non-constant PIR that varies with the FIO, as seen in Equation 7.

$$PIR = 10^q/(FIO/100\ ml),\ where\ q = a + b * \log_{10}(FIO/100\ ml) \quad (7)$$

Wiedenmann found the PIR for his model by assuming an ingestion rate of 30 ml for the 10-minute swimming period in his study (Wiedenmann 2007). He then calculated the a and b in Equation 7 by fitting the risk curve to the observed data and seeking the a and b values that resulted in a curve with the minimum sum of squared errors (Wiedenmann

2007). Wiedenmann's ingestion assumption of 30 ml is questionable however, since research by Dufour on swimmers in chlorinated swimming pools shows that adult swimmers ingested approximately 4 ml in a 10-minute recreational period (Dufour et al. 2006). The actual PIR is thus likely to be higher than the one calculated by Wiedenmann.

Assuming that the PIR is incorrect, there are three options for modifying Equation 7. The entire PIR could be adjusted by a constant factor, just the a term could be adjusted by some factor, or just the b term could be adjusted by some factor. Figure 8 shows the different risk curves obtained by varying the entire PIR by constant factors of 10 and 1/10, and the associated NOAELs for comparison of each curve.
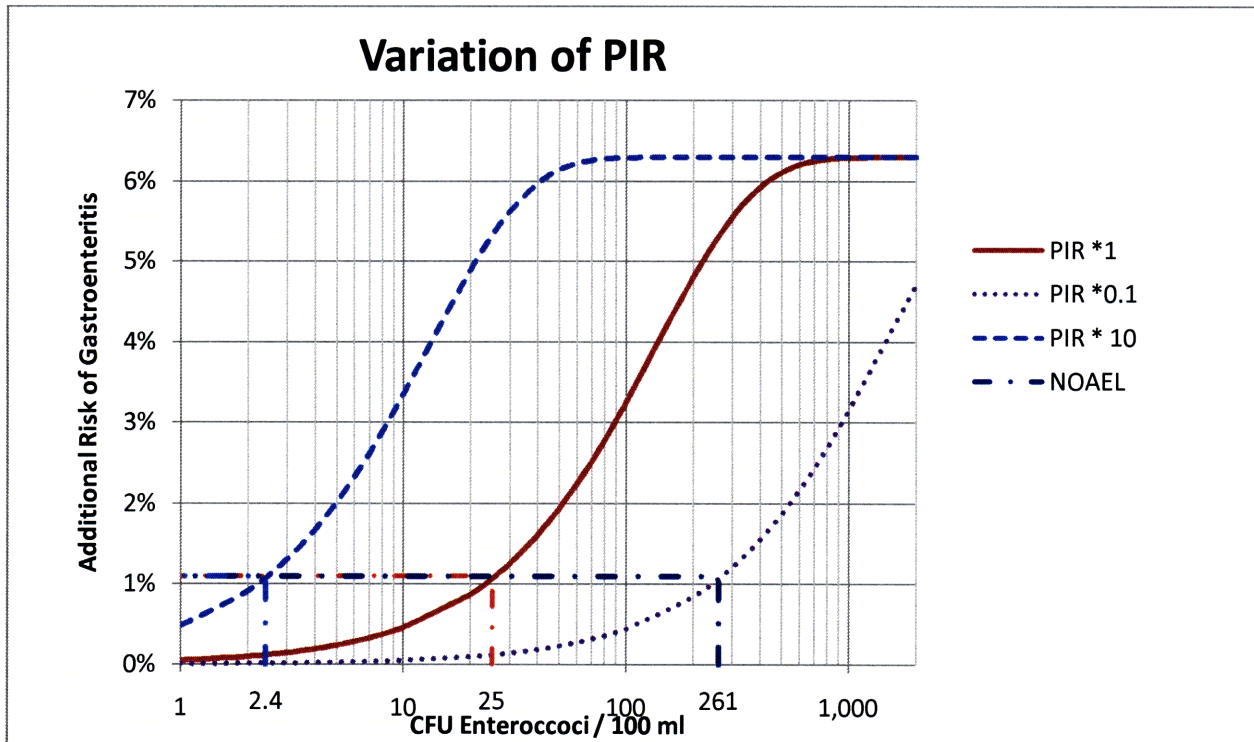
Figure 8: Variation of PIR

Adjusting the entire PIR by some factor results in approximately proportional change in the NOAEL, as can be seen in Figure 8, where multiplying the PIR by 10 decreases the NOAEL by 10 while multiplying the PIR by 1/10 increases the NOAEL by 10.

Figure 9 shows the risk curves obtained by multiplying just a in Equation 7 by a factor of 2 or 1/2. Adjusting only a in Equation 7 results in a less direct change than adjusting

the entire PIR, as can be seen in Figure 9, where multiplying a by two results in lowering the NOAEL by a factor of five, while multiplying a by ½ more than doubles the NOAEL.

Figure 10 shows the effect of multiplying just b in Equation 7 by a factor of 2 or 1/2. The effect of varying b is even more dramatic than that of a. As can be seen in figure 10, multiplying b by 2 raises the NOAEL by a factor of 5, while multiplying b by ½ lowers the NOAEL by a factor of 25.
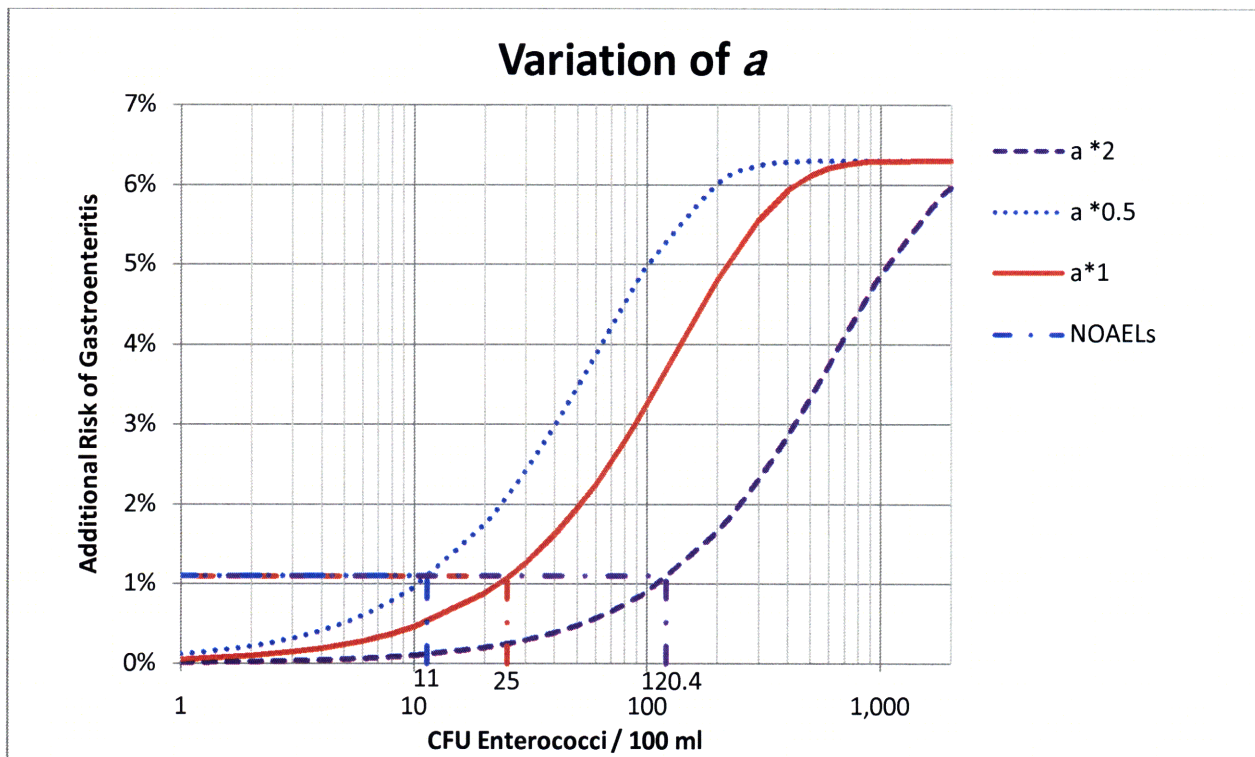
Figure 9: Variation of *a*
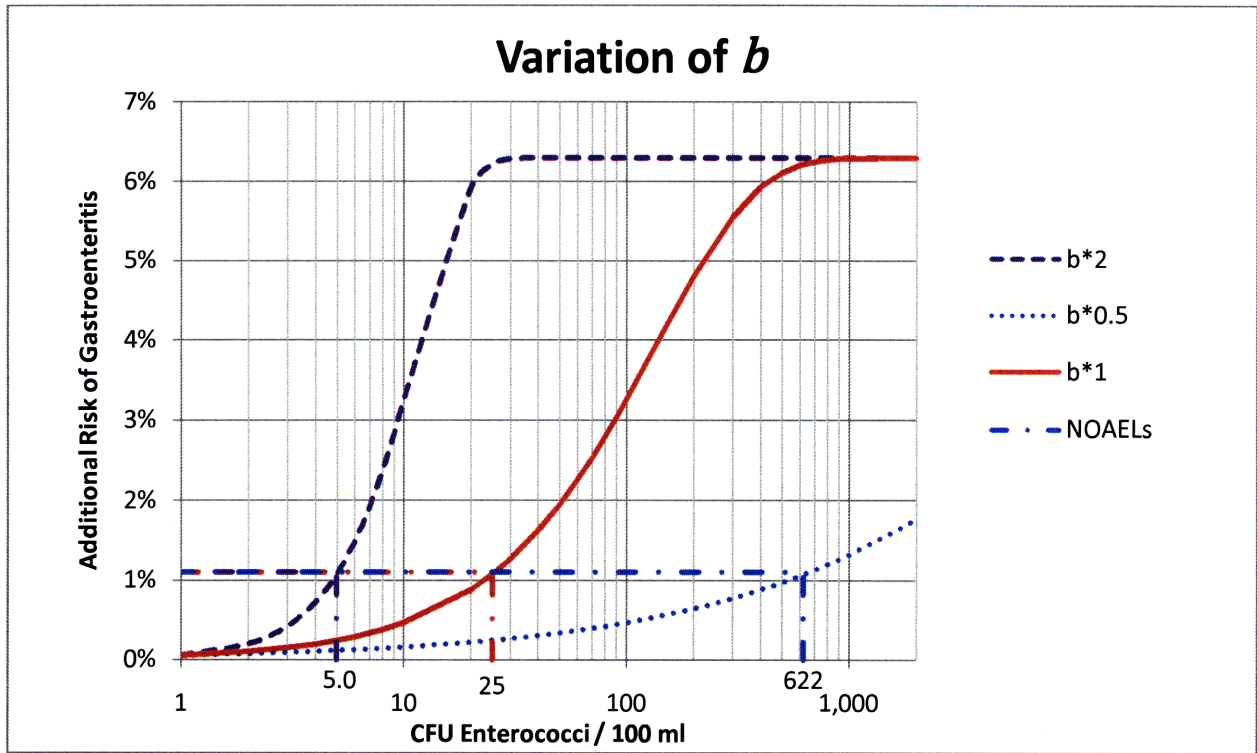
Figure 10: Variation of *b*
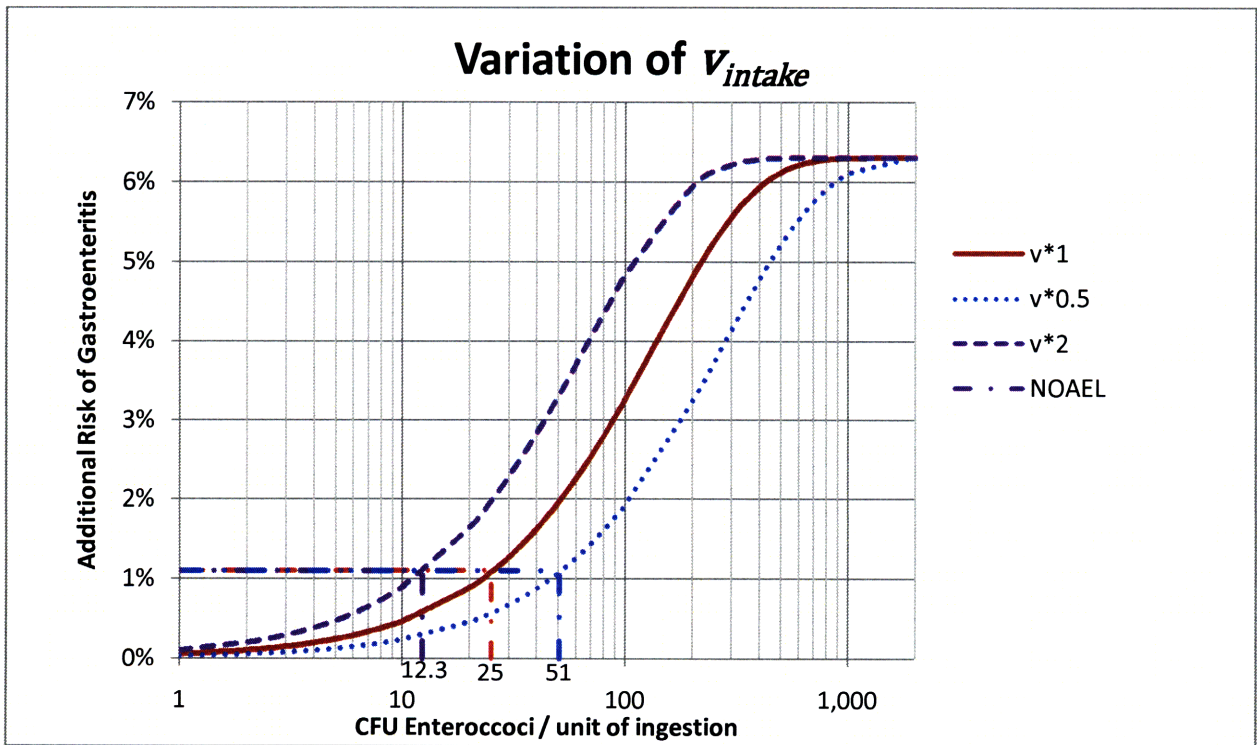
Figure 11: Variation of *v*intake

The small changes to the PIR factors in Figures 8, 9, and 10 results in large shifts of the NOAELs for each risk curve. In contrast, the risk equation is much less sensitive to changes in the ingestion term v_{intake}. Figure 11 shows the effect of multiplying v_{intake} by 2 and by 1/2. The NOAEL is approximately halved on the low end, and doubled on the high end. Compare this to changing either a or b by the same factor, in which the NOAEL is on average changed by an order of magnitude. This shows that having the correct PIR is very important in calculating risk of illness to recreational water users, since even small errors in this term translate into significant differences in the calculated NOAEL, and thus the safety of the water body.

3.3   Common Risk Assumptions

Both the Dufour (1984) and Wiedenmann (2007) risk models make three major assumptions. First, the risk models assume that the ingestion of water is similar among all participants and that differing exposures are insignificant compared to the bacterial concentration. This assumption is a necessary one, and is not normally a significant source of error, as long as the assumed ingestion rate is of the correct order of magnitude. The second assumption is that using indicator bacteria as a stand-in for the actual pathogens that cause gastroenteritis is an accurate substitution, as discussed in Section 2.3. The last major assumption is that the pathogen/indicator bacteria ratio remains relatively constant, and is similar temporally and spatially. We know that this last assumption is not correct, as a disease outbreak in the general population will result in higher PIRs in any recreational waters that receive wastewater (Wiedenmann, 2007). This would happen because the pathogenic organism concentrations would be raised by the outbreak while the indicator organisms would be unchanged. Spatially, the ratios may differ if there is preferential die-off or growth of indicator organisms in the natural media, such as E. coli and Enterococci growth in tropical soils (Hernandez-Delgado et al. 1991). Differing ratios will result in the actual risk being lower or higher – as shown in Section 3.2.5 for the Wiedenmann equation. The pathogen indicator ratio assumption is one of the least studied variables, even though it is key to all risk models based on using indicator bacteria to measure risk.

3.4   Dufour vs. Wiedenmann Risk Models

The Dufour model and the Wiedenmann model differ in several respects. First, the Dufour model was based on a study that did little to control for the variables in recreational use. One of the major variables is how much water is ingested during a swimming event. The amount of water ingested is related to the amount of time spent swimming, and the age of the recreator. The Dufour study did not attempt to control for this variable at all, while the Wiedenmann study strictly controlled for it by restricting participants to ten minutes in the water with at least three head immersions.

The Dufour model also did very little to model the pathogen/indicator relationship. The Dufour study consolidated much of the data collected into yearly averages and the log-linear model Dufour chose has only two variables to model all the different factors that influence the risk. In contrast, the Wiedenmann model controlled for all of the variables, so that the only parts of the risk equation that had to be derived from the epidemiological study were the PIR and the range of risks as represented by MR and BR. The MR and BR are easy to determine, since they are the bounds of the data collected during the epidemiological study. Section 3.2.5 discusses the problems with the PIR in the Wiedenmann model, but it is at least possible to correct for this in the future. In addition, both the Dufour model and the Wiedenmann model may overestimate the risk in tropical waters since *E. coli* and *Enterococci* can grow in tropical soils, thereby causing a PIR that may be much lower in tropical climates than in temperate climates (Hernandez-Delgado et al. 1991). There is a lack of data for tropical climates, since there have not been any recreational freshwater epidemiological studies conducted in a tropical climate (Zmirou et al. 2003).

Figure 12 shows the risk curves generated from the Dufour model and the Wiedenmann model plotted together. The dashed lines represent the guideline values for bacterial density in recreational freshwaters.
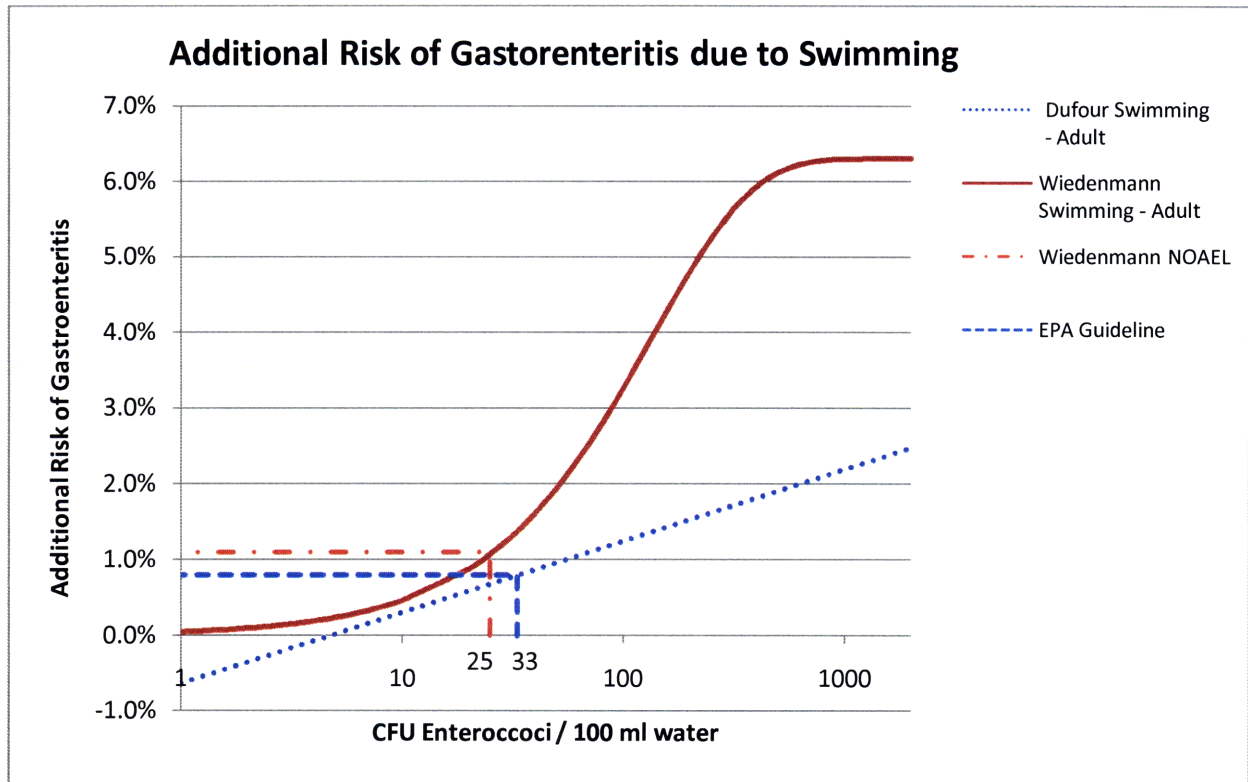
Figure 12: Dufour vs. Wiedenmann Risk Curves

The two models come closest to agreeing in the zone of concentrations where the risk is lowest, but they disagree on how quickly the risk rises with higher bacterial concentrations. The Dufour model calculates a lower risk than the Wiedenmann model over the entire range of likely bacterial concentrations. This lower calculated risk is most likely incorrect since the Wiedenmann study showed the maximum rate of illness occurs before extremely high bacterial concentrations are reached. One possible reason that the Dufour model does not show this is because of the averaging of the study data over one-year periods.

Overall, the Wiedenmann model is superior to the Dufour model. The underlying data used to generate the model is from a much more accurately designed study, where many of the factors Dufour ignored were accounted for. Also, the Wiedenmann model is much more flexible, since it explicitly accounts for different ingestion rates and PIRs. With further research, the Wiedenmann model can be fully customized for any location and potential population. The Wiedenmann model is what will be used in this study to calculate risk to recreational users of the Kranji Reservoir.

3.5 Single-Sample Maximum Allowable Densities

Single-sample maximum allowable densities are an important part of any water sampling program. One of the problems when determining whether a water body is safe for recreation is the amount of time it takes to determine the mean concentration. Because of temporal and spatial variations in bacteria density, a single sample may be higher than the allowable mean without indicating excess risk. To avoid unnecessary recreational water closings, it is important to have single sample maximums (SSM) as part of the water quality guidelines. Exceeding the SSM indicates that the likely mean indicator density is higher than the acceptable risk level, and that the recreational water should be closed.

3.5.1 SSMs in the United States

The USEPA-calculated SSMs are used in many states in the US (USEPA 2003). The different confidence levels in Table 2 were chosen by USEPA (1986) based on judgment as to the allowable risk in letting the geometric mean be higher than allowed levels. The lower confidence limits result in a lower SSM, which represents a more conservative approach to risk. A higher confidence limit results in a higher SSM, which means there is a higher chance that the geometric mean exceeds guidelines.

Ideally, the SSMs should be calculated for each recreational water-body. The USEPA recommends this water-body-specific approach in an attempt to compensate for the generalized nature of the risk equations(USEPA 1986). By using data that must be collected anyway, the SSMs can be adjusted at each water body; however in practice this is not often done. USEPA (2003) lists the standards that each US state has adopted for their recreational waters and the SSMs if the state has adopted any. None of the states have adopted SSMs that have been adjusted for the different characteristics of the recreational water bodies in the state. The most likely reason for this is the large number of recreational water bodies that are regulated, and the effort that it would take to customize regulations for each water body. Another possible reason is that the USEPA standards were developed for temperate water bodies, so for much of the U.S. the SSMs may not change much, though further study is needed to determine if this is true.

3.5.2 Calculating SSMs

To customize the SSMs for each water-body, it is necessary to determine the statistical distribution that describes bacterial concentrations in the water. Bacterial concentrations in most natural water bodies exhibit a log-normal distribution. To test the type of distribution that the Kranji Reservoir exhibits, the measured *Enterococci* concentrations in water were checked by two statistical tests using the USEPA ProUCL software (USEPA 2007). The bacteria concentration data for the Kranji Reservoir is well fit by a log-normal distribution. Details of the distribution-fit test are included in Section 4.6.

The SSM for a given water body is the one-sided upper confidence limit (UCL) for the confidence level chosen. There are two methods that the USEPA recommends to calculate the UCLs for a given log-normal distribution. The first is using Lands method (USEPA 2002), where the SSM is calculated using the log-standard-deviation and the one-sided H-statistic as seen in Equation 9 (USEPA 2002).

$$UCL = e^{(\overline{\ln x}+0.5s^2+\frac{sH_{1-\alpha}}{\sqrt{n-1}})} \qquad (9)$$

Where $\bar{x}$ is the mean concentration associated with the risk level chosen (i.e. 33 *Enterococci* per 100 ml from Table 2), s is the standard deviation of the transformed data, n is the number of samples used to calculate s, and $H_{1-\alpha}$ is the H statistic for the confidence levels chosen. The H-statistic is found in tables based on the standard deviation and the number of samples. In USEPA (1986), the authors of the document appear to use this method to calculate the SSMs. Figure 13 is taken from the USEPA document, and shows the equation used by the EPA (USEPA 1986).

$$(4) \quad \text{Single sample limit} = \text{antilog}_{10} \left[\begin{array}{ll} (\log_{10} \text{ indicator geometric} & + \text{ Factor determined} \times (\log_{10} \text{ standard} \\ \text{mean density/100 ml}) & \text{from areas under} \quad \text{deviation)} \\ & \text{the Normal prob-} \\ & \text{ability curve for} \\ & \text{the assumed level} \\ & \text{of probability} \end{array} \right]$$

Figure 13: SSM calculation method (USEPA 1986)

The 1986 USEPA document does not specify how the authors calculate the "Factor" multiplied by the standard deviation, but Lands method was published in 1975, and it is not unreasonable to assume that the authors used the H-statistic.

A more recently proposed method for calculating the UCL is through use of the Chebyshev Inequality (CI) method (USEPA 2002). The CI method uses the variance to calculate the minimum-variance unbiased estimator (MVUE) for the standard deviation. Equation 10 gives the MVUE standard deviation (USEPA 2002).

$$\sigma_\mu^2 = \exp(2\ln X) \left(\left(g_n (s_{\ln X}^2 / 2) \right)^2 - g_n \left(\frac{n-2}{n-1} s_{\ln X}^2 \right) \right) \tag{10}$$

Where g_n is found in available tables. The one-sided upper confidence limit on the chosen mean is calculated using Equation 11 (USEPA 2002).

$$UCL_{1-\alpha} = \hat{\mu}_{LN} + \sqrt{\left(\frac{1}{\alpha} - 1 \right) \sigma_\mu^2} \tag{11}$$

Where α is the chosen confidence limit, and $\hat{\mu}_{LN}$ is the log of the mean concentration associated with the risk level chosen (i.e. 33 *Enterococci* per 100 ml).

Land's Method versus Chebyshev's Inequality Method

The CI method was proposed to deal with sites that have small sample sizes with large skew or standard deviations. In cases where there are few samples upon which to base the mean, then Land's method may indicate an unacceptably risky UCL. Since the CI method is significantly more conservative than Land's method, the CI UCL will always be lower than the Land UCL. In USEPA (2002), Exhibit 7 lists combinations of standard deviation and sample size for which the CI method should be used. If the SSMs for

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Kranji were based only on the data collected during January 2009, then the CI method would be recommended. However, when combining the data collected in January 2009 with the data collected during the previous NTU study (Hwa et al. 2008), a much more complete picture of the variation in the reservoir can be calculated, and with a total sample size of 135 samples, the Land method is most appropriate.

4 Data Collection and Analysis

Water and sediment samples were collected during January 2009 and analyzed for
Enterococci concentrations. Water and sediment ingestion rates were collected for
possible recreational activities. The ingestion rates and sample information were used
to analyze risk using the Wiedenmann (2007) model. The data collected during January
2009 was combined with the data previously collected by NTU (2008) and analyzed
using statistical methods to calculate recommended indicator bacterium guidelines and
single sample maximums.

4.1 Field Sample Collection

Field sampling occurred over the week of January 19 through January 23. 2009.
Samples were taken from the main body of the reservoir in the north and from the three
arms that feed into the reservoir in the south. Figure 15 shows the locations in the
reservoir where samples were taken.
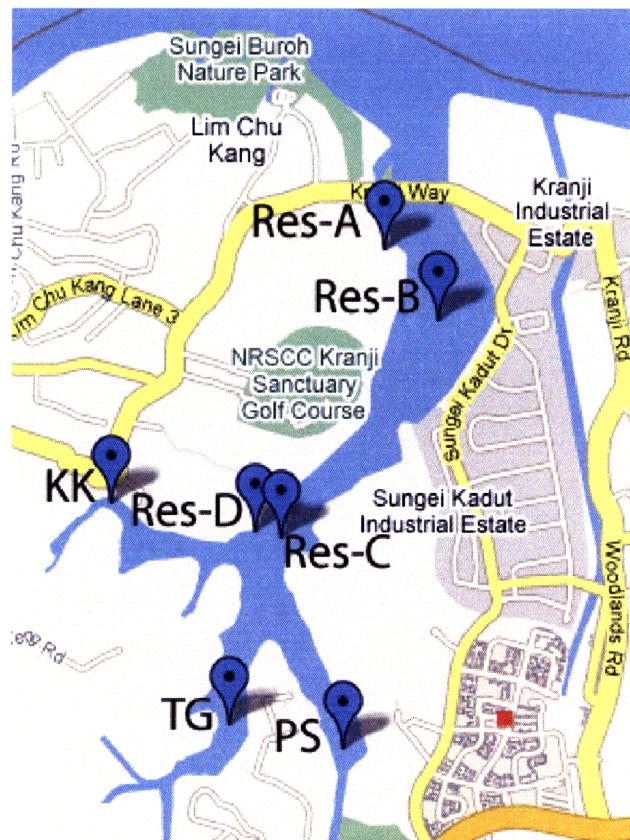
Figure 14: Reservoir Sampling Locations (Google Maps 2009)

Sample locations Res-A through Res-D were chosen based on the completed exposure pathways in Table 6 and on the locations used in the reservoir study by NTU (2008). Res-A is where the boat launch and visitors center proposed by the Western Catchment Masterplan (PUB 2007b) will be located, as seen in Figure 3. Sediment samples as well as water samples were taken at this point since recreational users will have contact with the sediment at this location. Res-B and Res-C in the main body of the reservoir are in the same areas as Station 3 and Station 1 from the previous reservoir study by NTU (2008), as seen in Figure 4. Since users are not likely to have contact with the bottom sediment in the center of the reservoir, only water samples were taken at these locations. Res-D is located next to a proposed pavilion and dock as seen in Figure 3, therefore both water and sediment samples were taken at this location. Sampling locations TG, KK, and PS are located in the three arms of the reservoir, and duplicate the sampling locations of the same name from the NTU study (Figure 4).

4.1.1 Water

Water samples were collected from a boat provided by PUB in either Whirl-Pak sample collection bags or clean sterile 1-liter containers. Samples were collected by removing the seal, and then placing the mouth of the container approximately 10 cm beneath the surface of the water until the container was almost full. The pre-labeled container was then sealed and placed on ice in a cooler in the boat. Samples were kept on ice until they were analyzed in the lab.

4.1.2 Sediment

Sediment samples were collected from the bottom of the reservoir by using a Kajak-Brinkhurst core sampler provided by Nanyang Technical University. Due to lack of space in the boat, samples were composited in the tube of the sampler by dropping it three times in each sampling area. The drift of the boat ensured that there was spatial separation between each drop of the probe. The composite sample was then removed from the sampling tube and placed in a sterile 600 ml container. The pre-labeled container was then sealed and placed into a cooler. Samples were kept on ice until analyzed at the laboratory.

4.2   Laboratory Analysis

To determine the fecal indicator organism concentrations for risk quantification, samples were analyzed for *Enterococci* and *E. coli* concentrations. Samples were analyzed using both a direct filtration method (Hach 2008) and a MPN method using IDEXX Quanti-Trays and growth media (IDEXX 2008a). Water samples were diluted using de-ionized water and sterilized equipment.

Sediment preparation presented an additional challenge. The USEPA does not provide a method for testing of sediment for indicator bacteria. The method used for sediment preparation was taken from the United States Geological Service Techniques of Water-Resources Investigations Book 9, commonly called the USGS Field Manual (Myers et al. 2007). Chapter 7 "Fecal Indicator Bacteria" of the field manual provides a method for calculating the indicator bacteria concentration in sediment. Section 7.1.3.B of the manual provides a five step method for processing of the bed sediments which was used for this study and paraphrased below.

1. Prepare for processing by labeling the following sterilized containers; two 500-ml sterile bottles for eluting and collection of supernatant, and a dish for dry weight analysis.
2. Composite the samples (The sediment samples were composited in the field for this study).
3. Prepare an aliquot of the sample for dry-weight analysis.
   a. Record the tare weight of a clean dry heat-tolerant dish. Ceramic drying dishes were used for dry weight analysis.
   b. Place approximately 25 g of the composited sample into the drying dish. Record the new weight.
   c. Place in an oven at 105° C. Dry until a constant weight is obtained. Samples were dried for 24 hours before being re-weighed.
4. Elute bacteria from the composited sample/
   a. Place 20 to 30 g of sample in the elution bottle. Add 100 ml of phosphate-buffered water with magnesium chloride per 10 g of sample.

b. Label the bottle with the time that it should be removed from the wrist-action shaker.

c. Place the bottle on a wrist-action shaker. When the bottle was placed on the shaker, a kitchen timer was started for 45 minutes.

d. After 45 minutes, remove the bottle from the wrist-action shaker. Let it rest for 30 seconds, and then pour off the supernatant into a new, labeled, sterile bottle.

5. Analyze the supernatant using the selected bacterial analysis method.

The supernatant extracts of the soil samples were analyzed for *Enterococci* concentration.

4.2.1 *Enterococci*

Analysis of *Enterococci* concentrations was performed using IDEXX Enterolert media and Quanti-Tray/2000 MPN trays. The IDEXX provided Quanti-Tray enumeration procedure for the Enterolert test kit was followed (IDEXX 2008a). A sample of 100 ml of reservoir water and the contents of one Enterolert packet were placed in a sterile jar with a screw-cap lid. The jar was sealed, and then shaken until no granules of media were visible. The sample was then poured into a Quanti-Tray/2000 MPN tray, sealed, and labeled with the sample identifier, date, and time. The sealed trays were placed in an incubator set at 41° Celsius. Samples were removed and read 24 to 28 hours later. Samples were read in a light-box with a 365-nm UV light, and the number of positive large and small wells was recorded on the sample sheet. The most probable number of colony forming units (CFU) per 100 ml was then read from the IDEXX-provided MPN table (IDEXX 2008b).

A source of error was introduced into the study at this point as the trays were not read properly. There are 49 possible large wells, composed of 48 square wells and 1 large rectangular well, as can be seen in Figure 16. At the time the research was conducted it was not understood that the large rectangular well should be counted. The possible error from not counting the large rectangular well is on average 10%, with a range of 3%

to 33%. The error only occurs if the large rectangular well was positive and not counted. It is unknown how many sample counts were affected.
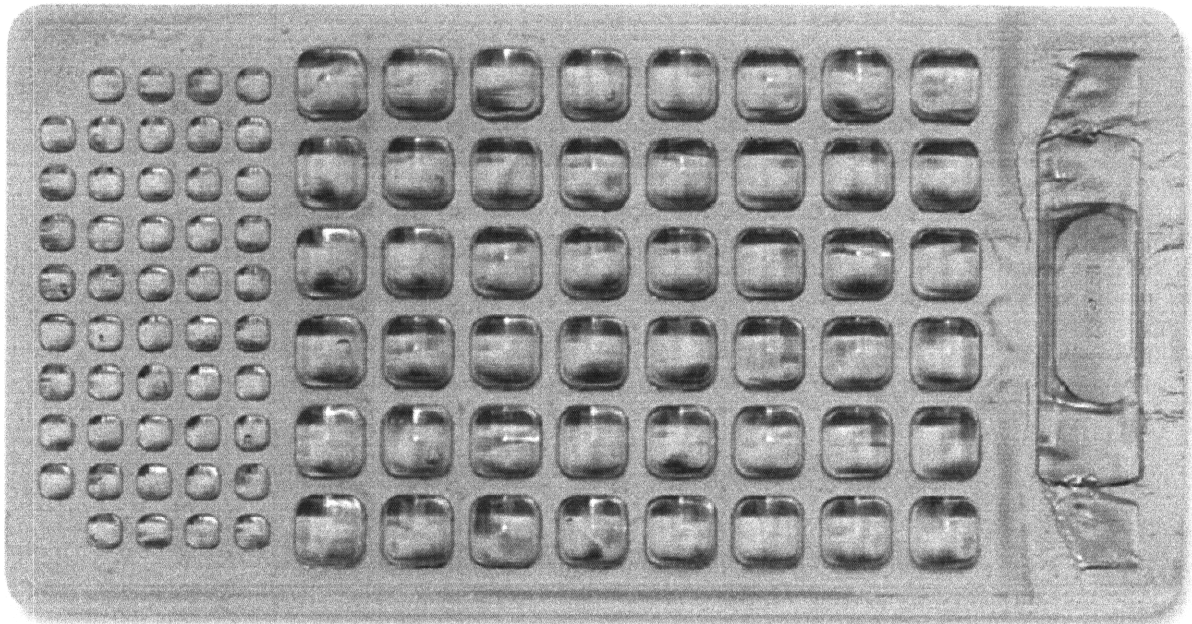
Figure 15: Quanti-Tray/2000 (IDEXX 2008b)

4.2.2 *E. coli* and Total Coliform

Samples were tested for *E. coli* and total coliform using direct filtration and incubation on Hach m-ColiBlue24 Broth (Hach 2008). In the laboratory, 100 ml reservoir water samples were diluted to 1:100, 1:10, and 1:1 and vacuum filtered. The filter paper was placed on a labeled petri dish that contained a pad wetted with the growth media. The petri dishes were placed in an incubator at 35° C for 24 hours, before being removed and read. Due to the high turbidity of the waters, no useable results were obtained at the 1:1 dilution level. Since there were no *E. coli* colonies at the higher dilution levels, the only conclusion could be that there were less than 10 *E. coli* colonies in these 100-ml samples. Because of the problems with this method, *E. coli* and total coliform were only tested for on the samples collected on 01/19/09 and 01/20/09, and discontinued for the rest of the sampling period.

4.3 Dose Calculations

After determining the concentration of bacteria in the field, the next step in conducting a risk assessment is the calculation of the dose to which the potential receptors are

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exposed. Exposure parameters for water and sediment ingestion were gathered from available sources. Exposure values for kayaking, a key planned recreational use of the reservoir, are not available in published literature, so an estimate for kayaking exposure was made based on personal interviews.

4.3.1 Water Ingestion

Exposure rates for swimming are calculated using an average water ingestion rate during swimming and a mean swimming duration. According to Dufour (2006) the average amount of water swallowed during a 45-minute swimming period is 16 ml for adults, and 37 ml for children under the age of 18 (Dufour et al. 2006). The USEPA recommends a mean swimming duration of 60 minutes per event (USEPA 1999b). Combining these factors, the ingestion rate per swimming event is 20 ml per event for adults and 50 ml per event for children.

Exposure rates for kayaking were calculated using the ingestion rates for swimming and adjusting them for the relative amount of contact with the water. Kevin Horner and Daniel Smith are kayak instructors at Charles River Canoe and Kayak in Boston, Massachusetts and were interviewed on April 11, 2009 about their kayaking patterns. According to Mr. Horner and Mr. Smith the average recreational kayaking session is approximately 2 hours and during that period their head was usually immersed 1 to 3 times. They usually enter their kayaks from docks so there is no contact with sediment. Based on these interviews I assume an ingestion rate approximately half that of swimming. This results in a per-event ingestion rate of 10 ml for adults and 25 ml for children. This is a rough estimate, and any guidelines derived from this estimate should be confirmed by an ingestion study of kayakers.

4.3.2 Sediment Ingestion

There are no soil and sediment ingestion studies that focus only on recreational ingestion. Table 4-23 in the USEPA Exposure Factors Handbook (USEPA 1999b) recommends a value for soil ingestion of 100 mg/day for children under the age of 6, with an upper percentile value of 400 mg/day, and 50 mg/day for adults, with no upper percentile value given (USEPA 1999b). The only guidance that was found on sediment

ingestion during recreation is from the State of Virginia. Guidance for the State of Virginia (VADEQ 2008) calculates sediment ingestion rates for recreational contact by assuming that the rate of soil ingestion is constant through 16 waking hours of the day, and that the average recreational event lasts for 2 hours. This gives a fraction of daily sediment ingestion from recreation of 0.125 (VADEQ 2008). Multiplying this fraction by the USEPA-recommended values for daily soil ingestion, the total ingestion rate for children under the age of 6 is 12.5 mg/day of recreation, with an upper percentile ingestion rate of 50 mg/day. The total ingestion rate for adults is 6.25 mg/day with no upper percentile value given. Table 6 summarizes the per-event ingestion rates for both water and sediment.

Table 6: Ingestion Rates

Activity	Ingestion Rate Per-Event
Swimming, Adults	20 ml water
Swimming, Non-Adults	50 ml water
Kayaking, Adults	10 ml water
Kayaking, Non-Adults	25 ml water
Wading, Adult	6.25 mg water
Wading, Child under 6	12.5 mg sediment, mean child 50 mg sediment, 95th upper percentile child

4.4 Complete Risk Equation

To calculate the risk associated with recreational use of the Kranji Reservoir values for all variables in Equation 5 and 7 need to be provided. Table 7 lists the variables and associated values for these equations.

Table 7: Risk Equation Variables

Variable	Value	Source
MR	0.091	Wiedenmann 2007
BR	0.028	Wiedenmann 2007
$p(1)$	0.17	Wiedenmann 2007
a	-0.67	Wiedenmann 2007
b	0.98	Wiedenmann 2007
C_{EN}	1-2,000	Measured, NTU 2008
v_{intake}	0.006 - 1	Table 6

Variables $MR, BR, p(1), a,$ and b were provided by Wiedenmann (2007) from literature review and the freshwater randomized controlled trial that he conducted. The *Enterococci* concentrations C_{EN} were measured in the Kranji Reservoir, and the value v_{intake} comes from literature reviews and personal interviews as discussed above and shown in Table 6. Equation 12 is the simplified risk equation with all but the final two variable terms inserted.

$$Risk = (0.063) * \left\{ 1 - [0.83]^{10^\wedge((-0.67+0.98*log_{10}(FIO))*v_{intake})} \right\} \tag{12}$$

FIO concentrations are input from the measured values in the reservoir, and the appropriate v_{intake} is input from Table 6.

4.5 Guideline Geometric Means

Guideline geometric means for the three PUB proposed levels of recreational activity are based on the NOAEL determined in the epidemiological study conducted by Wiedenmann et al. (2006). The NOAEL for swimming given by Wiedenmann et al. (2006) is 25 *Enterococci* per 100 ml. This NOAEL corresponds to a specific number of ingested pathogens, z in the risk equation (Equation 5).

$$Risk = (MR - BR) * \{1 - [1 - p(1)]^z\} \tag{5}$$

Equation 8 represents the expanded form of the ingestion term z. Equation 8 has two variables, C_{EN} and v_{intake}, and two constants a and b. The constants a and b are provided by Wiedenmann (2007) based on epidemiological studies. Table 8 shows the values Wiedenmann (2007) used to calculate the pathogen ingestion term z associated with the no-observed-adverse-effects level found by Wiedenmann et al. (2006).

$$z = v_{intake} * 10^{a+b*log_{10} C_{EN}} \tag{8}$$

Table 8: NOAEL Ingestion Terms

Variable	Value used by Wiedenmann (2007)
a	-0.67
b	0.98
C_{EN}	25
v_{intake}	0.3

Using the values in Table 8 from Wiedenmann (2007) in Equation 8 and solving for z, the number of pathogens ingested is 1.5 at the NOAEL found by Wiedenmann et al. (2006). To calculate the NOAEL for activities that have a different v_{intake} than the intake assumed by Wiedenmann (2007) for swimming, Equation 8 is rearranged to solve for the *Enterococci* concentration C_{EN}, holding z constant at 1.5. Equation 13 is the new equation, where the dependent variable is v_{intake}, and the independent variable is the *Enterococci* concentration which represents a NOAEL for each activity.

$$\log_{10} C_{EN,NOAEL} = 1.02 * \log_{10} \frac{1.5}{v_{intake}} + 0.68 \qquad (13)$$

NOAELs for the different proposed activities in the Kranji Reservoir are calculated by setting the value of v_{intake} equal to the ingestion rates in Table 6 associated with each activity, then solving Equation 13 for $C_{EN,NOAEL}$. It is possible to calculate NOAELs for both children and adults. However, due to the variability in child immune systems and rates of ingestion, guidelines are usually set based on adult NOAELs.

4.6 Single-Sample Maximum Allowable Densities

Single-sample maximums were calculated using Land's (1975) method as described in Section 3.3. Equation 7 has three inputs, the guideline mean, the log-normal standard deviation, and the one-sided H-statistic. The guideline means are the NOAEL values calculated using Equation 13. The standard deviation of the bacterial concentrations in the reservoir was calculated using the data provided by NTU (2008) and the data from the January 2009 sampling period. The one-sided H-statistic is interpolated for the given standard deviation and degree of freedom using published tables (Land, 1975). The guideline means were calculated as in Section 4.5.

Before calculating SSMs, the combined data was checked for outliers and log-normality. Statistical analysis was performed using the ProUCL software provided by the USEPA (USEPA 2007). The data for the reservoir was aggregated and input into the software. The ProUCL software calculated three potential outliers at the 1% significance level. These values were discarded, and the remaining data was analyzed. Appendix B contains all the data used for statistical analysis, and the outliers are italicized. Table 9

lists the standard deviation S, and the associated H-statistics for the degrees of freedom available.

Table 9: H-Statistics(Land, 1975; USEPA, 2007)

Degrees of Freedom: 131					
	One-Sided (Upper) Confidence Levels				
S	0.05	0.1	0.75	0.9	0.95
1.4	-2.1	-1.7	0.7	2.0	2.6

Single-sample maximums for each activity level were calculated at the 75% level for the guideline means. The H-statistics for the 0.05, 0.1, 0.9, and 0.95 confidence levels in Table 9 are given as references for the four-point Lagrangian interpolation used to calculate the 75% level (Land 1975). The appropriate upper confidence limits were then calculated using Equation 9.

5   Results

5.1   Sampled Values and Current Risk

Over the five-day sampling period, 35 water samples and 10 sediment samples were
collected. Of those samples, 29 water samples and 10 sediment samples were
analyzed for *Enterococci*, and 14 water samples were analyzed for *E. coli*. Due to the
high turbidity of the reservoir water samples, valid *E. coli* counts were not obtained.
Enterococci analysis for sample locations TG, PS, and KK did not begin until 01/21/09,
as only *E. coli* analysis was originally planned for those locations. Appendix A lists the
raw results from the January sampling period. Table 10 lists the sampling locations, the
bacterial concentration per sample, and the mean bacterial concentration over the five-
day period.

Table 10: *Enterococci* analysis results

Date	01/19/09	01/20/09	01/21/09	01/22/09	01/23/09	Geometric Mean
IDENTIFIER	MPN Colony Forming Units / 100 ml					
Res-A	19.7	2	3.1	11.5	4.1	5.7
Res-B	4.1	9.4	10.9	5.2	19.8	8.5
Res-C	17.2	6.3	20.2	19.5	13.2	14.1
Res-D	12.8	10.9	41.4	18.7	10.9	16.4
TG	NA	NA	20.6	47.4	24.6	28.9
PS	NA	NA	67.6	23	31.8	36.7
KK	NA	NA	11.5	13.5	11.9	12.3
	MPN Colony Forming Units / gram sediment					
Res-A Sed	458	282	761	324	509	438
Res-D Sed	4430	2180	12600	3770	4790	4660

During the sampling period, the overall geometric mean for the entire reservoir and the
sediment samples were 13 CFU / 100 ml and 1400 CFU / 1 g respectively. The risk
posed to potential recreational users if the reservoir remained at the sampled
concentrations is summarized in Table 11.

Table 11: Additional Risk of Gastroenteritis Associated with Kranji Water Quality - January 2009

Activity	Age	Relevant mean Concentration	Wiedenmann Risk
Swimming	Adult	13.3 CFU /100 ml water	0.6%
	Non-Adult <18		1.4%
Kayaking	Adult		0.3%
	Non-Adult <18		0.7%
Wading	Adult	1400 CFU /g sediment	1.7%
	Mean Child < 6		2.9%
	95% Child < 6		5.8%

Mean water concentrations are below the NOAEL found by Wiedenmann (2006), meaning that the reservoir is safe for swimming and kayaking. However, the mean sediment concentration was significantly higher than the calculated NOAEL for sediment. A review of the bacterial concentrations in Table 10 shows that the primary drivers of the high geometric sediment mean are the samples taken at location Res-D. Section 6.1.2 discusses the potential cause of the high sediment concentrations at that location.

Since the water quality varies significantly with time, it can be useful to look at the possible range of risks associated with the Kranji Reservoir, and the risk curves produced by the two different concentration-response models. Figure 16 shows the risk due to swimming in waters with different concentrations of *Enterococci*. The solid lines represent the adult risk curves for the different types of recreational contact. The units for swimming and kayaking are *Enterococci* per 100 ml of water, and the units for wading are *Enterococci* per gram of sediment. The shape of the curves is governed by Equation 13 in Section 4.5. The curves for kayaking and wading are shifted only by their respective ingestion rates from Table 6. The dashed lines represent the calculated NOAELs that are discussed in Section 5.2.
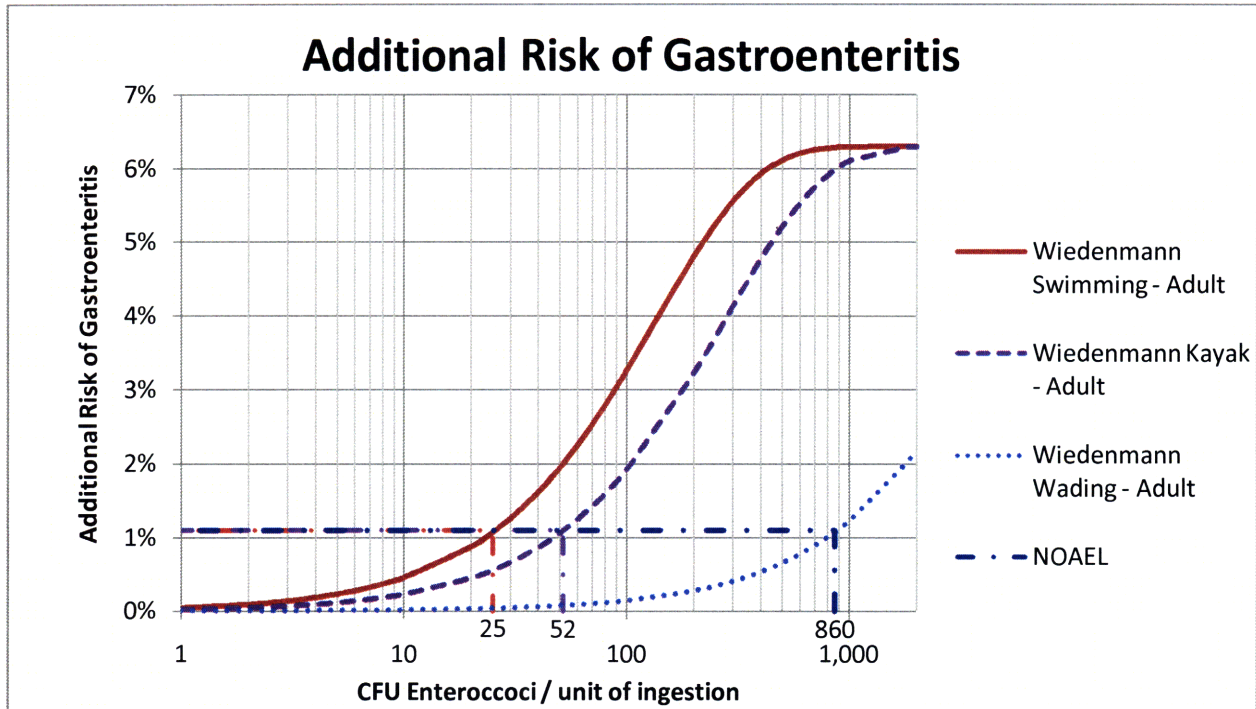
Figure 16: Additional Risk of Gastroenteritis

The risk curves for kayaking and swimming are very similar, which is to be expected since their ingestion rates vary by only a factor of two. At any given concentration, the risk due to kayaking is approximately half that of swimming, except for higher concentrations when the maximum rate is reached. The risk curve for wading is spread much further out, recognizing that the ingestion of sediment is much lower than that of water. The risk curve for sediment is somewhat misleading however, since the concentration of bacteria per gram of sediment is usually much higher than the concentration of bacteria per 100 ml of water. It is important to note that the geometric mean water concentration during the January 2009 sampling period was below the NOAEL for swimming and kayaking, but that the geometric mean concentration for sediment was not.

5.2 Guidelines and Single Sample Maximums

The guidelines for different activities are presented in Table 12. Guideline geometric means were calculated using Equation 13 from Section 4.5. The SSMs were calculated using Equation 9 as discussed in Sections 3.5.2 and 4.6.

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Table 12: Guideline Geometric Means and SSMs

Activity	January Geometric Mean	Guideline Geometric Mean (*Enterococci* CFU/100 ml)	75% SSM
Swimming - Adult	13.3 *Enterococci* CFU/100ml	25	73
Kayaking - Adult		51	150
Activity	**January Geometric Mean**	**Guideline Geometric Mean (*Enterococci* CFU/g)**	**75% SSM**
Wading - Adult	1400 *Enterococci* CFU/g	860	2500

The guideline geometric means for swimming and kayaking are applicable to the water in the area of the reservoir that is made available for those recreational activities. If PUB chooses to restrict recreational access to a subsection of the reservoir as recommended in Section 6.1.3 below, the guideline would apply to only the recreational area. The sediment geometric mean guidelines need only apply to the areas of the reservoir where recreators are likely to have contact with sediment. These areas would include the shore near docks and any wading areas. However, this guideline would not need to apply to non-near-shore sediment since recreators would be unlikely to have contact with sediment at these locations.

Final guidelines adopted by PUB should be based on the type of activities that PUB decides to allow and the area of the reservoir PUB opens to recreation. For example, if PUB allows only kayaking, and entering and exiting the reservoir is allowed only from a floating dock, then the target concentrations would be a geometric mean of 51 *Enterococci* CFU per 100 ml water, with no single sample greater than 150 *Enterococci* CFU per 100 ml water and no guideline concentrations would be needed for sediment. However, if the kayakers entered and exited the reservoir directly from the shoreline, then a geometric mean guideline of 860 *Enterococci* CFU per g of sediment with associated SSM of 2,500 *Enterococci* CFU per g of sediment would be appropriate.

If the guideline concentrations were exceeded, then the risk of illness is unacceptably high, and the Kranji Reservoir would need to be closed for recreation until additional

sampling showed that *Enterococci* levels were below the guidelines. During the January sampling period reported in this thesis, water *Enterococci* concentrations did not exceed the calculated geometric mean guidelines or SSMs for any type of activity. The Res-A sediment samples were also below guideline values, but the Res-D sediment samples were significantly greater than both the guideline *Enterococci* mean concentration and SSM for sediment.

6 Conclusions

The goal of the Public Utilities Board is to use the Kranji Reservoir for recreational purposes. To that end, it is necessary to determine appropriate guideline levels for bacterial water quality, single sample maximums for the sampling programs, and the area of the reservoir that is to be used for recreation. Current fresh and marine water indicator bacterium standards in Singapore are set at 200 *Enterococci* per 100 ml, but there are no freshwater beaches that are open for recreation or monitored for quality at this time. Suggested guidelines for use at Singapore freshwater bodies are based on potential exposure to water and sediment as well as the statistical distribution of reservoir bacterial concentrations.

Based on the goals of PUB for the Kranji Reservoir, and by the analysis of water quality data provided by NTU (2008) and data measured during January 2009, portions of the Kranji Reservoir can be opened to use of the public for primary contact recreation. Geometric mean water and sediment quality guidelines from Sections 6.1.1 and 6.1.2 are recommended as interim standards and a restricted recreational use area is recommended in Section 6.1.3.

6.1 Suggested Guidelines

The choice of a restricted area for recreation in the Kranji Reservoir is based on maximizing the recreational use of the Kranji while meeting the recommended water quality guidelines.

6.1.1 Water Quality Guidelines and SSMs

The recommended geometric mean *Enterococci* concentration for primary contact recreation is 25 *Enterococci* per 100 ml. This represents a level that should result in no additional cases of gastroenteritis among recreational swimmers, and is equal to the no-observed-adverse-effects level found by Wiedenmann (Wiedenmann et al. 2006). This level is attainable for the reservoir recreational area recommended in Section 6.1.3. During the January sampling period the geometric mean for the recommended area of the reservoir in Figure 18 was 10 *Enterococci* per 100 ml. The overall geometric mean

using all available sampling data is 7.3 *Enterococci* per 100 ml for the recommended recreational area.

The single-sample maximum associated with the recommended guideline mean is 73 *Enterococci* per 100 ml. This represents a 75% upper confidence limit that the mean is below the guideline value. The SSM was not exceeded during the January sampling period, and was only exceeded three times in the historical data for the recommended recreational area of the reservoir.

6.1.2 Sediment Quality Guidelines and SSMs

The recommended geometric mean *Enterococci* concentration for sediment is 860 *Enterococci* per gram. This level was exceeded by the geometric mean of all sediment samples taken from the Kranji during the January sampling period. This level was not exceeded by the sediment samples taken at Res-A (Figure 14). However, sediment *Enterococci* concentrations from the Res-D location were larger than the Res-A concentrations by an average of 5 times. One possible reason for the much higher levels at Res-D is that it is located very close to a chicken farm located on the western shore of the reservoir. More testing is needed to determine the variation of bacterial levels in the sediment. It is possible that sediment levels near another chicken farm located on the land between sampling location TG and PS are also elevated, but this area is less important since there are no recreational facilities proposed in this area. Until more research has been done on the sediment variation, contact with bottom sediment through wading and entrances/exits to the reservoir should be restricted to the dashed shoreline area, or to floating docks in the rest of the reservoir.

6.1.3 Recreational Area

In order to provide for the safety of recreational users, the recreational use of the reservoir should initially be restricted to the designated recreational area in Figure 18. Additional areas of the reservoir should be opened to recreation if sampling demonstrates that bacterial levels in that area are in line with the main body of the reservoir. The non-recreational area has historically presented significantly worse water

quality than the recommended recreational area. When comparing the water quality data in the two areas, the recreational area exceeded recommended swimming single-sample maximums only three times out of 65 samples, while the non-recreational area exceeded recommended single-sample maximums twelve times out of 70 samples.
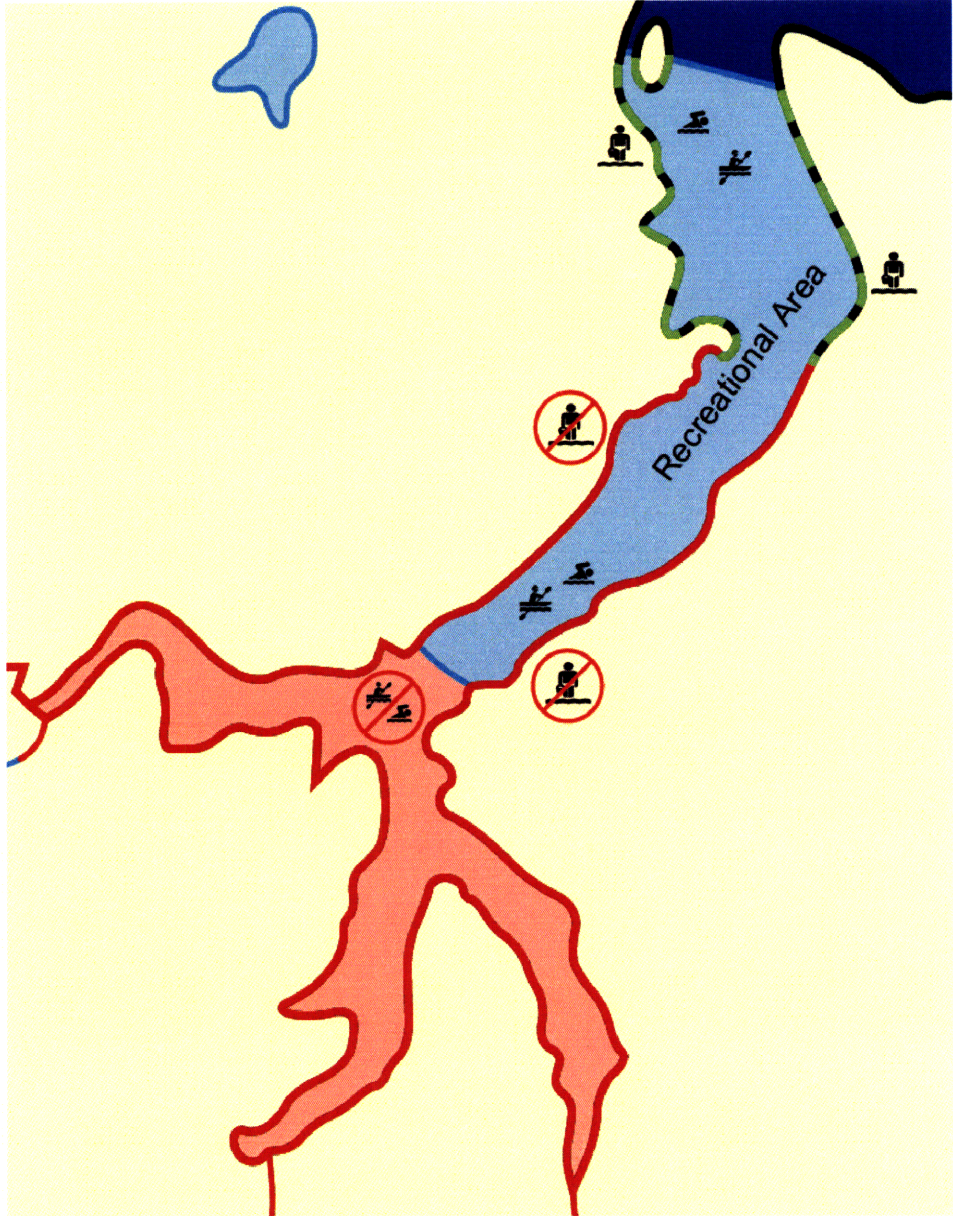

Figure 17: Recommended Recreational Areas

By restricting recreation to the recommended area, the likelihood of the reservoir exceeding the recommended guideline values is much reduced, resulting in fewer water closures. In addition, wading and entrance/exits to the water should initially be restricted to the dashed areas of shoreline, unless floating docks are used. Shoreline

areas with a solid line represent locations where the sediment quality is possibly above guideline means. The safe shoreline areas represent a conservative estimate. More shoreline can be opened for use as additional sediment testing identifies safe areas.

6.1.4 Sampling Program

Water and sediment samples should be collected and analyzed for *Enterococci* concentration at least weekly to ensure the safety of recreational water users. Samples should be taken at the southern end of the allowed recreational area in the Kranji Reservoir because the highest bacteria concentrations have been measured in the southern sections of the reservoir. If the geometric mean or single-sample-maximum guidelines are exceeded, then the Kranji Reservoir should be closed to water recreation until additional sampling shows that the indicator bacterium concentrations have returned to safe levels.

6.2 Point Source Control

An obvious step to improve water quality in the Kranji Reservoir would be to institute controls on point sources into the reservoir. An example of a possible point source that is close to planned recreational areas is the chicken farm on the western shore near Res-D. The high sediment levels at Res-D may be attributable to the settling of bacteria-laden particles from the chicken farm. The chicken farm currently has a sedimentation basin to treat its discharge, but it has not been properly maintained. By requiring the farm to make improvements to the sedimentation basin such that it removes a significant amount of settleable particles, the sediment quality in the reservoir may improve. Additional point sources are likely to be identified with further study.

6.3 Further Study

Two studies are recommended to fully characterize the risk to recreational users of the Kranji Reservoir. The first recommended study is a DNA-based analysis of the Pathogen/Indicator Ratio of the Kranji Reservoir. The second recommended study is to determine the risk levels in the reservoir associated with storm events.

6.3.1 Pathogen Indicator Ratio

New methods of DNA analysis using quick polymerase chain reactions (QPCR) now make it possible to measure concentrations of different pathogens in the water directly. It is possible for a relatively short study to be performed on the Kranji Reservoir to determine the PIR directly, rather than through inference from other studies. A PIR obtained for the Kranji Reservoir could also be used for other tropical freshwater bodies.

6.3.2 Storm Event Risk

Currently all water quality data for the Kranji Reservoir has been collected during dry-weather flow. The previous study by NTU (2008) only sampled the reservoir during dry weather, and the January 2009 sampling period took place during an exceptionally dry period. There had been no storm events in the two weeks prior to the sampling period, and there were no storm events during the sampling period. A study of the reservoir water quality after a storm event should be conducted to ensure that the safety of recreational users is not jeopardized. NTU (2008) showed that storm flows from the catchment had significantly higher bacteria densities than dry weather flow, so there is an assumption that the reservoir will experience elevated bacterial counts after a storm. A study that examines how quickly the reservoir returns to safe levels after a storm should be conducted.

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Appendix A - Raw Results

Water Sample Results

Date	Sample Name	Lat	Long	Sub-Catchment	# Large Wells	# Small Wells	MPN Enterococci / 100 ml
19/01/09	26.1-44.2	26.112	44.28	Res A	14	3	19.7
20/01/09	26.1-44.2	26.112	44.28	Res A	1	1	2
21/01/09	26.1-44.2	26.112	44.28	Res A	3	0	3.1
22/01/09	26.1-44.2	26.111	42.278	Res A	5	6	11.5
23/01/09	26.1-44.2	26.111	42.278	Res A	3	1	4.1
19/01/09	25.9-44.5	25.957	44.51	Res B	4	0	4.1
20/01/09	25.7-44.5	25.766	22.549	Res B	5	0	5.2
21/01/09	25.7-44.5	25.766	22.549	Res B	9	9	19.8
22/01/09	25.9-44.5	25.94	44.534	Res B	5	4	9.4
23/01/09	25.9-44.5	25.94	44.534	Res B	9	1	10.9
19/01/09	24.7-43.7	24.748	43.731	Res C	7	9	17.2
20/01/09	24.7-43.7	24.72	43.76	Res C	5	1	6.3
21/01/09	24.7-43.7	24.726	43.754	Res C	11	7	20.2
22/01/09	24.7-43.7	24.755	43.727	Res C	13	4	19.5
23/01/09	24.7-43.7	24.755	43.727	Res C	10	2	13.2
19/01/09	24.7-43.5	24.792	43.534	Res D	7	5	12.8
20/01/09	24.7-43.5	24.73	43.5	Res D	9	1	10.9
21/01/09	24.7-43.5	24.73	43.5	Res D	21	11	41.4
22/01/09	24.7-43.5	24.732	43.504	Res D	15	1	18.7
23/01/09	24.7-43.5	24.732	43.504	Res D	9	1	10.9
21/01/09	23.7-43.4	23.746	43.499	TG	13	5	20.6
22/01/09	23.7-43.4	23.753	43.497	TG	26	8	47.4
23/01/09	23.7-43.4	23.753	43.497	TG	19	1	24.6
21/01/09	24.8-42.8	24.804	42.825	KK	5	6	11.5
22/01/09	24.8-42.8	24.8	42.822	KK	4	9	13.5
23/01/09	24.8-42.8	24.8	42.822	KK	8	3	11.9
21/01/09	23.9-44.0	23.926	44.005	PS	33	9	67.6
22/01/09	23.9-44.0	23.903	44.035	PS	13	7	23
23/01/09	23.9-44.0	23.903	44.035	PS	21	4	31.8

Sediment Sample Results

Date	Sample Name	Lat	Long	Sub-Catchment	# Large Wells	# Small Wells	MPN Enterococci / 100 ml	Proportional Dry Wt	Wt Sediment Tested (g)	MPN/gram
19/01/09	26.1-44.2-S	26.112	44.28	Res A-S	11	2	14.5	0.33	33	458.3
20/01/09	26.1-44.2-S	26.112	44.28	Res A-S	5	6	11.5	0.43	31.63	281.7
21/01/09	26.1-44.2-S	26.112	44.28	Res A-S	15	8	27.2	0.38	29.84	760.6
22/01/09	26.1-44.2-S	26.111	42.278	Res A-S	9	0	9.8	0.32	30.79	324.4
23/01/09	26.1-44.2-S	26.111	42.278	Res A-S	9	11	22	0.45	29.28	508.5
19/01/09	24.7-43.5-S	24.792	43.534	Res D-S	38	22	119.4	0.28	29.37	4,426.7
20/01/09	24.7-43.5-S	24.73	43.5	Res D-S	18	48	87.8	0.42	29.92	2,183.1
21/01/09	24.7-43.5-S	24.73	43.5	Res D-S	48	22	298.7	0.25	30.08	12,633.9
22/01/09	24.7-43.5-S	24.732	43.504	Res D-S	23	42	93.8	0.26	30.16	3,768.6
23/01/09	24.7-43.5-S	24.732	43.504	Res D-S	44	10	125.9	0.28	29.81	4,792.7

Appendix B – All Water Data for Statistical Analysis

IDENTIFIER	MPN/100ml	IDENTIFIER	MPN/100ml	IDENTIFIER	MPN/100ml
Res-A-01/23/09	4.1	ST 3-06/05/07	2	KK-04/23/07	15
Res-A-01/22/09	11.5	ST 3-07/09/07	10	KK-05/21/07	7.5
Res-A-01/21/09	3.1	ST 3-08/20/07	107.1	KK-06/05/07	15.3
Res-A-01/20/09	2	ST 4-09/15/05	2	KK-07/09/07	134
Res-A-01/19/09	19.7	ST 4-09/29/05	5.1	KK-08/20/07	54.4
Res-B-01/23/09	19.8	ST 4-10/12/05	5.1	PS-01/23/09	31.8
Res-B-01/22/09	5.2	ST 4-11/16/05	1	PS-01/22/09	23
Res-B-01/21/09	10.9	ST 4-06/19/06	5.1	PS-01/21/09	67.6
Res-B-01/20/09	9.4	ST 4-09/04/06	3	PS-09/15/05	38.4
Res-B-01/19/09	4.1	ST 4-10/02/06	11.1	*PS-09/29/05**	*770.1*
Res-C-01/23/09	13.2	ST 4-12/18/06	11.1	PS-10/12/05	7.4
Res-C-01/22/09	19.5	ST 4-01/22/07	10	PS-11/16/05	8.4
Res-C-01/21/09	20.2	ST 4-02/05/07	4.1	PS-07/26/06	12.1
Res-C-01/20/09	6.3	ST 4-03/19/07	1	PS-09/04/06	16.9
Res-C-01/19/09	17.2	ST 4-04/23/07	2	PS-10/02/06	9.9
Res-D-01/23/09	10.9	ST 4-05/21/07	1	PS-11/16/06	11.1
Res-D-01/22/09	18.7	ST 4-06/05/07	1	PS-12/18/06	200.5
Res-D-01/21/09	41.4	ST 4-07/09/07	73	PS-01/22/07	84
Res-D-01/20/09	10.9	ST 4-08/20/07	67	PS-02/05/07	25.6
Res-D-01/19/09	12.8	JUNC-09/15/05	4.1	*PS-04/23/07**	*1986.3*
ST 1-09/15/05	4.1	JUNC-07/26/06	5.2	PS-05/21/07	1
ST 1-07/26/06	2	JUNC-09/04/06	2	*PS-06/05/07**	*1553.1*
ST 1-09/04/06	4.1	JUNC-10/02/06	1	PS-07/09/07	10
ST 1-10/02/06	1	JUNC-12/18/06	200.5	PS-08/20/07	177.5
ST 1-12/18/06	200.5	JUNC-01/22/07	20	TG-01/23/09	24.6
ST 1-01/22/07	73	JUNC-02/05/07	39.1	TG-01/22/09	47.4
ST 1-02/05/07	18.5	JUNC-03/19/07	22.2	TG-01/21/09	20.6
ST 1-03/19/07	20.7	JUNC-04/23/07	53.5	TG-09/15/05	13.4
ST 1-04/23/07	25.9	JUNC-05/21/07	12.7	TG-09/29/05	9.8
ST 1-05/21/07	3	JUNC-06/05/07	31.7	TG-10/12/05	14.3
ST 1-06/05/07	23.9	JUNC-07/09/07	199	TG-11/16/05	8.5
ST 1-07/09/07	63	JUNC-08/20/07	62.2	TG-07/26/06	4.1
ST 1-08/20/07	122.3	KK-01/23/09	11.9	TG-09/04/06	22.6
ST 3-09/15/05	1	KK-01/22/09	13.5	TG-10/02/06	8.7
ST 3-09/29/05	4.1	KK-01/21/09	11.5	TG-11/16/06	8.7
ST 3-10/12/05	3	KK-09/15/05	14.5	TG-12/18/06	200.5
ST 3-11/16/05	4.1	KK-09/29/05	1	TG-01/22/07	10
ST 3-06/19/06	4.1	KK-10/12/05	3.1	TG-02/05/07	24.3
ST 3-07/26/06	3.1	KK-11/16/05	1	TG-04/23/07	218.7
ST 3-09/04/06	4.1	KK-07/26/06	8.4	TG-05/21/07	49.7
ST 3-10/02/06	2	KK-09/04/06	2	TG-06/05/07	43.9
ST 3-11/16/06	1	KK-10/02/06	7.5	TG-07/09/07	41
ST 3-12/18/06	34.4	KK-11/16/06	6.4	TG-08/20/07	22.5
ST 3-02/05/07	6.2	KK-12/18/06	200.5	* *Outlier – removed*	
ST 3-04/23/07	2	KK-01/22/07	20	*before final analysis.*	
ST 3-05/21/07	1	KK-02/05/07	6.2		