water

MDPI

Article

Health Risks to Communities and Athletes Associated with Swimming, Wading, and Sailing in Water Bodies of Brazil's Guanabara Bay Basin

Rachel Sklar [1], Alexander E. Chabrelie [2], Renato S. Carreira [3], Patrick L. Gurian [4,*] and Jade Mitchell [2]

[1] Program for Reproductive Health and Environment, University of California, San Francisco, CA 94143, USA; rachel.sklar@ucsf.edu
[2] Department of Biosystems Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA; chabreli@msu.edu (A.E.C.); jade@msu.edu (J.M.)
[3] Department of Chemistry, Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro 22451-900, Brazil; carreira@puc-rio.br
[4] Department of Civil, Drexel University, Philadelphia, PA 19104, USA
* Correspondence: pgurian@drexel.edu

Abstract: Guanabara Bay has been known to be polluted with trash and sewage from the surrounding areas, but health risks from recreational contact with water in the basin have not been well characterized. In this paper, fecal indicator bacteria (FIB) monitoring data are used to predict risks in three different exposure scenarios: (1) bathing in freshwater rivers that discharge into Guanabara Bay, (2) wading in these freshwater rivers, and (3) sailing in Guanabara Bay. Concentrations of indicator bacteria in river samples were measured directly, and concentrations of indicator bacteria in bay samples were sourced from publicly available government data sets. Ratios between pathogens and fecal indicator concentrations were used to estimate risks for five selected pathogens based on the indicator concentrations. The median risk of disease estimated from *E. coli* indicator concentrations was 1.0, 9.9×10^{-1} and 8.2×10^{-4} for the swimming, wading, and sailing exposure pathways, respectively. Risks estimates based on concentrations of the enterococci indicator bacteria in the sailing exposure scenario were comparable, at 3.4×10^{-4}. The sum of total risk estimated from the five selected pathogens was 5.9×10^{-1}, 3.6×10^{-1}, and 1.0×10^{-3} for the swimming, wading, and sailing exposure pathways, respectively. Estimated risks of swimming and wading in the rivers far exceeded risks associated with U.S. recreational contact standards, while estimated risks for sailing in the bay were well below these risk guidelines. The 95th percentile of the sailing risk was estimated to exceed the U.S. recreational contact risk level. This paper exemplifies an approach to conducting quantitative microbial risk assessments when only fecal indicator bacteria data are available. Context-specific data on the relevant exposure routes, exposure frequency, and site-specific indicator: pathogen ratios were lacking, which ultimately led to uncertainty in the model. This study is intended to provide a framework for estimating GI risk based on fecal indicator concentrations while acknowledging that the substantial variation in indicator:pathogen ratios make the results of such efforts uncertain.

Keywords: microbial risk assessment; indicator organism; dose response; recreational contact

Citation: Sklar, R.; Chabrelie, A.E.; Carreira, R.S.; Gurian, P.L.; Mitchell, J. Health Risks to Communities and Athletes Associated with Swimming, Wading, and Sailing in Water Bodies of Brazil's Guanabara Bay Basin. *Water* **2023**, *15*, 2509. https://doi.org/10.3390/w15142509

Academic Editor: Dimitrios E. Alexakis

Received: 1 May 2023
Revised: 20 June 2023
Accepted: 26 June 2023
Published: 9 July 2023

1. Background

The poor water quality of Guanabara Bay and the surrounding rivers has been a known health hazard for the surrounding communities. Over eight million people live in the Guanabara Bay basin area, and almost half of them live in neighborhoods lacking sewage treatment facilities [1]. The sewage from these neighborhoods flows directly into the ocean, making recreational contact with the bay and its rivers a potential source of hazards which can cause gastrointestinal illness (GI). GI is recognized as one of the most

common illnesses associated with contact with contaminated recreational water sources. Thus, GI is the disease outcome that is the focus of this risk assessment [2].

The August 2016 Summer Olympics took place in Rio de Janeiro and elicited a wide range of public concerns for athletes who were scheduled to compete in waters with reportedly high levels of contamination. Despite the Brazilian government's promise to capture and treat 80% of the sewage that flows into Guanabara Bay as part of Rio's winning Olympic bid, the project was not completed before the Olympic events due to financial and logistical constraints.

Pathogen-specific risk assessments are necessary to protect vulnerable populations from exposure to the fecal pathogens. However, collecting primary pathogen data is often not possible due to resource constraints. Instead, indicator bacteria are used as a pathogen proxy, and established ratios between indicators and pathogens are used to estimate the pathogen concentration in environmental samples.

Fecal indicator bacteria including fecal coliforms, *E. coli*, and enterococci have long been used as the basis for water quality standards [3]. However, linking indicator bacteria concentrations to quantitative health risk estimates remains a challenge. Indicator-based estimates of risk will always be subject to uncertainty, given variable shedding rates, survival rates in different environmental matrices, fate and transport characteristics, and the specific mixture of different pathogens and indicators [4].

Appropriate quantification of the uncertainty associated with indicator-based risk estimates is necessary in making informed risk-management decisions. This is especially true when using an indicator such as total coliforms which, compared to other indicators, are generally seen as a less specific indicator of fecal pollution [3]. Thus, many western countries set recreational water quality standards based on other indicators, namely *E. coli* and enterococci [5–8]. The role of total coliforms in United States Environmental Protection Agency (USEPA) drinking water standards is limited to a presumptive test. *E. coli* analysis is used as a confirmatory test [9].

In Brazil, both fecal coliforms and *E. coli* are used as indicators for freshwater, in addition to enterococci for saltwater [10]. The Brazilian National Environmental Council (CONAMA) recommends that fecal coliforms, *E. coli*, and enterococci be used as indicators for recreational contact standards. Upper limits have been established of 1000 CFU/100 mL for fecal coliforms, 800 CFU/100 mL for *E. coli*, and 100 CFU/100 mL for enterococci which apply to fresh, brackish, and salt water [10–12]. Concentrations must be below these values for five consecutive days in order for the water to be classified as satisfactory.

U.S. recreational water quality standards are more stringent. The USEPA has set standards for *E. coli* in freshwater that correspond to two different risk targets [6]. The risk target of 36 illnesses per 1000 users corresponds to a statistical upper bound (90th percentile) of 410 CFU/100 mL, and the risk target of 32 illnesses per 1000 users corresponds to a statistical upper bound of 320/100 mL. U.S. requirements for geometric means for *E. coli* are <126 and <120 CFU/100 mL (for risks of 36 and 32 illnesses per 1000 users, respectively), but the upper bounds are considered most comparable to the Brazilian requirement that five samples be below the specified values. In the U.S. standard, enterococci, rather than *E. coli*, is used as the primary indicator in the marine water context. Statistical upper-bound values of 130/100 mL (for a risk of 36 illnesses per 1000 users) and 110/100 mL (for a risk of 32 illnesses per 1000) are set for enterococci, comparable to the Brazilian values [6]. U.S. requirements for geometric means for enterococci are <35 and <30 CFU/100 mL (for risks of 36 and 32 illnesses per 1000 users, respectively).

A Quantitative Microbial Risk Assessment (QMRA) for GI has been conducted in this paper based on concentrations of these two fecal indicator bacteria. Two exposed populations are considered: (1) residents of communities living along the freshwater rivers to Guanabara Bay, who lack sanitation infrastructure, and (2) athletes that competed in 2016 Olympic events that took place in Guanabara Bay [13–15].

To characterize water quality in the freshwater rivers, primary data on concentrations of *E. coli* from freshwater rivers that drain into Guanabara Bay were collected. The rivers serve as a potential source of exposure to the inhabitants of the watershed but are not regularly monitored by the Brazilian government. To characterize water quality in the bay itself, data on the concentrations of fecal coliform and enterococci were sourced from public reports released by the Brazilian State Institute of the Environment (INEA) [16].

2. Methods

2.1. Study Area

Guanabara Bay (Figure 1) lies along the northern coast of Rio de Janeiro, the second largest city in Brazil and home to one of largest urban populations in the world [17]. The Guanabara Bay drainage basin receives most liquid effluents produced from the greater metropolitan area of Rio de Janeiro, one of the most industrialized coastal areas of Brazil. Of the 11.8 million inhabitants in the Rio de Janeiro City metropolitan area, 70% are located within the Guanabara Bay drainage basin [18].

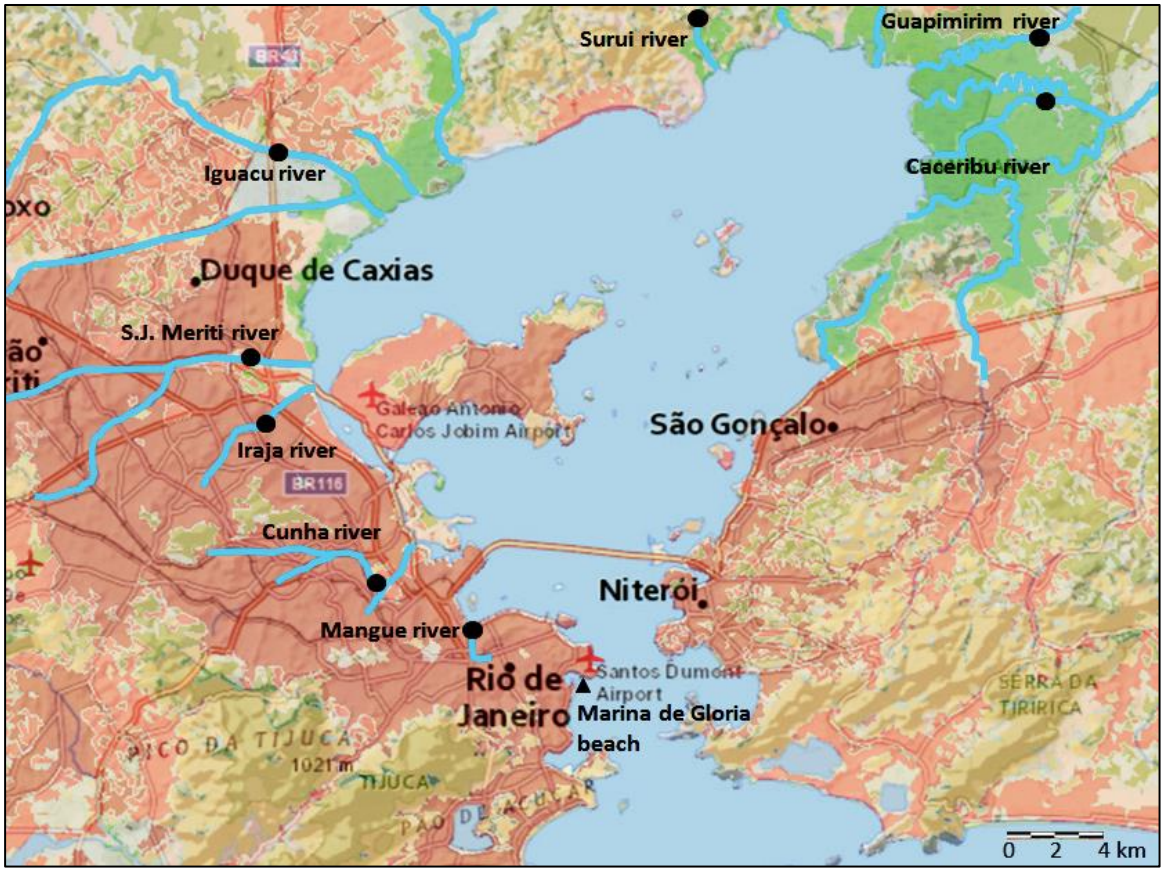

Figure 1. Guanabara Bay and its major input rivers with the sampling locations. Black circles represent the locations of sampling in freshwater rivers. A triangle represents the saltwater marina from samples of the bay that were taken.

Numerous efforts leading up to the 2016 summer Olympics that were geared at cleaning up the bay included testing and monitoring the bay waters for levels of fecal coliforms and enterococci. No testing for any specific pathogen that can cause diarrheal disease was performed as part of the pre-Olympics safety measures [1].

2.2. Population and Exposure Scenarios

Two exposed populations were considered in this risk assessment: (1) individuals in communities surrounding Guanabara Bay who are in contact with freshwater streams

contaminated with wastewater from settlements lacking wastewater treatment infrastructure, and (2) athletes competing in sailing events in Guanabara Bay which receives these contaminated freshwater inflows. Current governmental monitoring is focused on indicator organism concentrations in Guanabara Bay rather than on exposures in outlying communities where residents may contact polluted waters before they are diluted by the receiving waters in the bay [19].

Three exposure scenarios across the two different exposure groups were explored. The first exposure group included individuals living in communities along rivers draining into Guanabara Bay, and the exposure scenarios explored were: (1) swimming in and (2) wading in the freshwater rivers sampled in this study. The second exposure group included Olympic sailors, and the exposure scenario considered was the sailing event that took place in the Guanabara Bay saltwater bodies. Distinct indicators were used to model pathogen exposure to water from the freshwater rivers and the saltwater bay, as there are inherent differences in the survival and proliferation of different types of indicator bacteria in fresh versus saltwater sources [20].

2.3. Microorganisms Considered

To evaluate the risk of GI, this risk assessment considered a number of pathogens to represent bacteria, viruses, protozoa, and helminths: *Campylobacter* and *Salmonella* were selected to represent bacteria; Rotavirus to represent viruses; *Cryptosporidium* to represent protozoa; and *Ascaris* to represent helminths. These organisms were selected on the basis of their inclusion as excreta-related pathogens in WHO guidelines for drinking water and wastewater [21], and the availability of established dose-response curves [22].

The three indicators that were measured in the freshwater river samples and saltwater bay samples include fecal coliform, *E. coli*, and enterococci. These indicator organisms are linked to disease risk either through explicit indicator pathogen ratios or implicitly when a dose–response function is applied to an indicator organism. In the latter case, the indicator is not causing the disease but rather co-occurs with pathogens.

2.4. Exposure Route Assumptions

Exposure to the water sampled from the rivers was assumed to occur via two exposure routes: (1) swimming or (2) wading in surface waters. The choice of these exposure routes was informed by other studies that report on exposure to microbial risks in the context of high population density, low income and poor sanitation [23–25]. Exposure to the saltwater in the bay was assumed to occur by ingestion during sailing events.

2.5. Indicator Concentrations

Freshwater rivers were sampled for *E. coli* as part of this study, while fecal coliform concentrations of the bay were sourced from INEA reports [16]. Freshwater sampling for *E. coli* was conducted by Pontifícia Universidade Católica de Rio de Janeiro for three months to represent three seasons (September 2014, January 2015 and April 2015). A total of 24 water samples were collected at eight rivers in the Guanabara Bay basin (indicated by black circles in Figure 1), namely: Caceribu, Guapimirim, Suruí, Iguaçu, SJ Meriti, Irajá, Cunha channel and Mangue channel. These were chosen to represent both eastern and western sides of the bay. Samples were collected from bridges using sterilized four-liter amber glass bottles mounted on a metallic support. An aliquot of 50 mL was subsampled in the field and stored chilled in the dark. Samples were analyzed for *E. coli* by the Fluorcult (Merck, Darmstadt, Germany) LMX Broth modified method for detection and quantified using the classic method of multiple tubes.

Fecal coliform and enterococci sampling from saltwater in Guanabara Bay was conducted by INEA at five sites within the bay. The analysis utilized the data collected from Marina de Gloria (black triangle in Figure 1), as this is the area of the bay where sailing races were held during the 2016 Olympics [15]. *E. coli* values were not available for Guanabara Bay.

2.6. Estimation of Pathogen Dose from Indicator Data

Freshwater river data: Concentrations of *E. coli* were measured in the freshwater rivers, and an *E. coli*:pathogen ratio, $R_{EC:Pathogen}$, was used to estimate the concentration of pathogens with known dose–response curves [26–28].

Saltwater bay data: Fecal coliform and enterococci concentrations from bay water samples were published by INEA. Because no fecal coliform:pathogen ratios are reported in the literature, a fecal coliform:*E. coli* ratio, $R_{FC:E.\ coli}$ [29], followed by the *E. coli*:pathogen ratio, $R_{EC:Pathogen}$, specific to each pathogen, were used to estimate the concentration of pathogens.

The following equations represent the concentration of pathogens in the freshwater and saltwater sources sampled:

$$C_{Pathogen\ saltwater} = C_{FC} \times R_{FC:EC} \times R_{EC:Pathogen} \quad (1)$$

where $C_{Pathogen}$ represents the concentration of pathogens in MPN/mL (for *Salmonella*, *Campylobacter*, rotavirus, *Cryptosporidium*, Ascaris), C_{FC} represents the measured concentration of fecal coliform in MPN/mL, $R_{FC:EC}$ represents the fecal coliform:*E. coli* ratio used for saltwater, and $R_{EC:Pathogen}$ represents the *E. coli*:pathogen ratio specific for each pathogen.

The exposure dose of each organism was calculated as follows:

$$D = C_{Pathogen} \times V_{ingestion} \times t_{event} \quad (2)$$

where D is the exposure dose per event for each pathogen or indicator bacteria, $C_{Pathogen}$ is the concentration of pathogens in the rivers or bay, $V_{ingestion}$ is the volume of water ingested, either per event (for the swimming and wading scenarios) or per hour (for the sailing scenario), and t is the exposure time for the sailing scenario, or the duration of the sailing competition event. Table 1 summarizes the parameters used in the risk assessment model.

Table 1. Model Parameter Summary.

Parameter	Description	Units	Distribution	References
$C_{EC\ fresh}$	Concentration of *E. coli* in freshwater (from 8 rivers)	*E. coli*/100 mL	Lognormal $\mu = 3.6 \times 10^9$ $\sigma = 1.2 \times 10^{11}$	Field measurement (this study)
$C_{FC\ salt}$ $C_{ent\ salt}$	Concentration of fecal coliforms and enterococci in saltwater bay, at Marina de Gloria	fecal coliform/100 mL	Lognormal distribution $\mu = 3.2 \times 10^3$ $\sigma = 1.0 \times 10^4$	INEA (2016) [17]
$R_{FC:EC\ salt}$	*E. coli*/fecal coliform ratio in saltwater	--	Point-value $\mu = 3.0 \times 10^{-1}$	Fattal (1987) [29]
$R_{EC:Pathogen}$	*E. coli:*pathogen ratios	--	Lognormal distribution $\mu_{EC:Campylobacter} = 10^5$ $\mu_{EC:Salmonella} = 10^5$ $\mu_{EC:}$rotavirus $= 10^5$ $\mu_{EC:Cryptosporidium} = 10^5$ $\mu_{EC:}$Ascaris $= 10^6$	Ratios used are from Labite (2010) [27], These agree with the upper bounds provided by Mara (2007) [26] for rotavirus, Cryptosporidium, and Campylobacter. They also agree with the general ratios for virus, bacteria, and protozoa provided by Howard (2007) [28].
$V_{Ingestion\ Swimming}$	Mean volume of water ingested during Swimming	mL/event	Gamma distribution $r = 0.45$ $\lambda = 60$	Schets (2011) [30]

Table 1. *Cont.*

Parameter	Description	Units	Distribution	References
$V_{Ingestion\ Wading}$	Mean volume of water ingested during Wading	mL/event	Point value μ = 3.2 mL	Dorevitch (2011) [31] Westrell (2004) [32] Labite (2010) [27]
$V_{Ingestion\ Sailing}$	Mean volume of water ingested during Sailing	mL/hour	Point value μ = 3.9 mL	Dorevitch (2011) [31]
$t_{\ Sailing}$	Duration of the sailing event (time)	hours	Point value μ = 0.5 h	Aquece Rio (2015) [33]

2.7. Dose–Response Model

Dose–response relationships for *E. coli*, enterococci, and five criteria pathogens were used to estimate the risk of disease given the dose determined by the exposure assessment. Dose–response relationships for the reference pathogens and for *E. coli* in freshwater were defined from previous studies (Table 2). A mechanistic dose–response model for *E. coli* in saltwater was not available in the literature. However, data relating *E. coli* exposure from saltwater recreation to health outcomes have been published from studies done in the US [34,35]. These studies applied a regression analysis to the epidemiological study data for both fresh and marine waters, resulting in a log-linear model representing the relationship between *E. coli* and the risk of GI. More recently, a non-threshold, mechanistically-based model, the exponential model, has been developed to evaluate the best fit dose–response model for the same data, using maximum likelihood estimation methods [36]. These later dose–response fitting methods were fit for the freshwater context only. In this study, an exponential model was fit to the saltwater data from [34] using maximum likelihood estimation methods.

An exponential dose–response model was used to estimate the risk of *Cryptosporidium* and *Ascaris* infection, while a beta-Poisson model was used for *Campylobacter*, *Salmonella*, and rotavirus. The general equations for the exponential and beta-Poisson dose–response models are given by the following equations:

$$\text{Exponential}: \ P[inf] = 1 - e^{(-k \times d)} \tag{3}$$

$$\text{Beta-Poisson}: \ P[inf] = 1 - \left[1 + d \times \left(\frac{2^{\frac{1}{\alpha}} - 1}{N_{50}}\right)\right]^{-\alpha} \tag{4}$$

where $P[inf]$ is the probability of becoming infected by ingesting d number of organisms, N_{50} is the median infection dose representing the number of organisms that will infect 50% of the exposed population, α is a shape parameter with no biological meaning, and k is the probability of a pathogen surviving, reaching, and infecting at an appropriate site in the host cell.

The dose–response models for the criteria pathogens were derived from studies of direct inoculation or ingestion of pathogens. In contrast, the dose–response models for indicator *E. coli* were derived from epidemiological studies of recreational water users. Evidence from the literature suggests that the dose–response for indicator *E. coli* differs between fresh and saltwater exposure mediums, as *E. coli* persistence has been shown to be inversely related to salinity [37]. Thus, two distinct dose–response models were selected and used for estimating the risks from *E. coli* in freshwater versus saltwater.

Table 2. Dose–response Parameters.

Microorganisms	Parameters	Model Form	References
E. coli (saltwater)	$k = 1.4 \times 10^{-4}$	Exponential	Derived here based on data from Cabelli et al. (1982) [34]
E. coli (freshwater)	$k = 5.1 \times 10^{-5}$	Exponential	Sunger (2013) [35]
Enterococci (saltwater)	$k = 1.8 \times 10^{-4}$	Exponential	Sunger (2015) [36]
Campylobacter	$\alpha = 1.6 \times 10^{-1}$ $N_{50} = 8.9 \times 10^{2}$ $PDI = 0.3$	beta-Poisson	Haas (2014) [22]
Salmonella	$\alpha = 3.1 \times 10^{-1}$ $N_{50} = 2.4 \times 10^{4}$ $PDI = 3.0 \times 10^{-1}$	beta-Poisson	Haas (2014) [22]
Rotavirus	$\alpha = 2.5 \times 10^{-1}$ $N_{50} = 6.2$ $PDI = 5.0 \times 10^{-1}$	beta-Poisson	Haas (2014) [22]
Cryptosporidium	$k = 4.2 \times 10^{-3}$ $PDI = 7.0 \times 10^{-1}$	Exponential	Teunis (1996) [38]
Ascaris	$k = 3.9 \times 10^{-2}$ $PDI = 3.9 \times 10^{-1}$	Exponential	Navarro (2009) [39]

Several dose–response models relating *E. coli* in freshwater and disease can be found in the literature [30,40–43]. These models are either linear, exponential, beta-Poisson or Weibull Gamma-type dose–response models. In this study, linear models are excluded, because they lack a mechanistic basis [30]. Furthermore, the aforementioned studies are based on a specific pathogenic *E. coli* strain which can differ with the dose–response in the Guanabara bay context where *E. coli* serve primarily as indicators of pathogens.

2.8. Disease Risk Estimation

The risk of disease to an individual per swimming, wading, or sailing event was calculated as the product of the risk of infection and the probability of disease, given infection (*PDI*). The resulting probability of disease is given by the following equation:

$$P[disease] = P[inf] \times PDI \quad (5)$$

Uncertainty ranges in input parameters were described with probability distributions, and these probability distributions were used in a Monte Carlo simulation to develop a probability distribution of risk [22]. Tables 1 and 2 summarize the statistical distributions and input variables used to characterize uncertainty in the model parameters.

The measured concentrations of *E. coli* in river sources and fecal coliform in the bay were fit to lognormal distributions, as fecal indicators in recreational waters have been shown to be lognormally distributed [43]. A distribution of *E. coli*:pathogen ratios was built using a lognormal distribution reported in the literature [26–28]. The volume ingested during swimming was estimated by sampling from a lognormal distribution reported by USEPA-based exposure assessments for swimmers in freshwater [6]. The parameters for the ingestion volumes of adult males were used as a conservative estimate for the population [30]. The volume ingested during wading was estimated as a point value representing the mean of reported volumes ingested during wading and splashing in a swimming pool study [31], wading at a wetland inlet [32], and playing near open drainage channels [27]. The volume ingested during sailing was based on sampling from a published distribution of accidental ingestion during canoeing [31]. Mean volumes ingested during canoeing have been reportedly higher than other water-based recreational activities and serve as a conservative estimate for accidental ingestion during sailing. A point value for

the average sailing time was used based on the predicted times for all sailing races reported by the test event program [33].

A Monte Carlo simulation model was constructed, in which parameters were populated by a distribution or point value, using the mc2d package in R (R-3.2.4 version, R Foundation for Statistical Computing, Vienna, Austria) [44]. For each pathogen considered in a given exposure scenario 10,000 simulations were carried out to estimate the risk of infection and disease.

3. Results

3.1. Concentration of Indicator Organisms in Environmental Samples

The concentration of fecal indicator concentrations differed between fresh and saltwater samples, both in magnitude and range (Table 3, Figure 2). Measured concentrations of *E. coli* in rivers (median $C_{EC\ fresh} = 1.6 \times 10^6$ MPN/100 mL) were much higher than the reported fecal coliform and enterococci concentrations in the bay (median $C_{FC\ salt} = 7.90 \times 10^2$ and median $C_{ent} = 7.9 \times 10^1$ MPN/100 mL). This difference cannot simply be attributed to the normal concentration variability between *E. coli* and enterococci, since the recognized gap between those two indicators is generally one order of magnitude or less [45]. *E. coli* concentrations in the river show much greater variability (SD of $C_{EC\ fresh} = 2 \times 10^{11}$) than fecal coliform (SD of $C_{FC\ salt} = 4 \times 10^3$) and enterococci (SD of $C_{ent} = 9 \times 10^2$) concentrations measured in the bay.

Table 3. Summary of indicator organism concentrations in freshwater and saltwater sources in the Guanabara Bay basin (MPN/100 mL).

Organisms–Water Type (Source)	N	Percentiles (MPN/100 mL)			Geometric Mean	Standard Deviation
		5th	50th	95th		
E. coli—freshwater (measured here)	24	4.3×10^3	1.6×10^6	1.74×10^{10}	3.20×10^6	2.20×10^{11}
Fecal coliform—bay (INEA 2016 [17])	12	9.9×10^1	7.9×10^2	9.72×10^3	8.61×10^2	4.24×10^3
ENT—Saltwater (INEA 2016 [17])	11	1.0×10^1	7.9×10^2	2.26×10^3	7.7×10^1	9.1×10^1

A comparison of indicator concentrations with their corresponding Brazilian regulation limits show that for freshwater exposure scenarios including swimming and wading, limits are greatly exceeded, while for the saltwater sailing exposure, indicator concentrations are around or slightly above the limit (Figure 2). Specifically, in the swimming and wading pathways, only 4% of the 24 samples comply with CONAMA's limit of 800 MPN/100 mL, whereas a minimum 80% of samples are required by the law to have "satisfactory" water quality.

Results of the saltwater sampling campaign conducted by INEA show that two of the 11 samples were far above the Brazilian enterococci limit of 400 MPN/100 mL (with values of 2729 and 1782 MPN/100 mL) while the other eight values were below this limit. Within the same sample set reported by INEA, 73% of the 11 data points were below the threshold limit of 2.5×10^3 MPN/100 mL for fecal coliform, below the government's requirement of an 80% minimum sample number to be below the threshold limit.

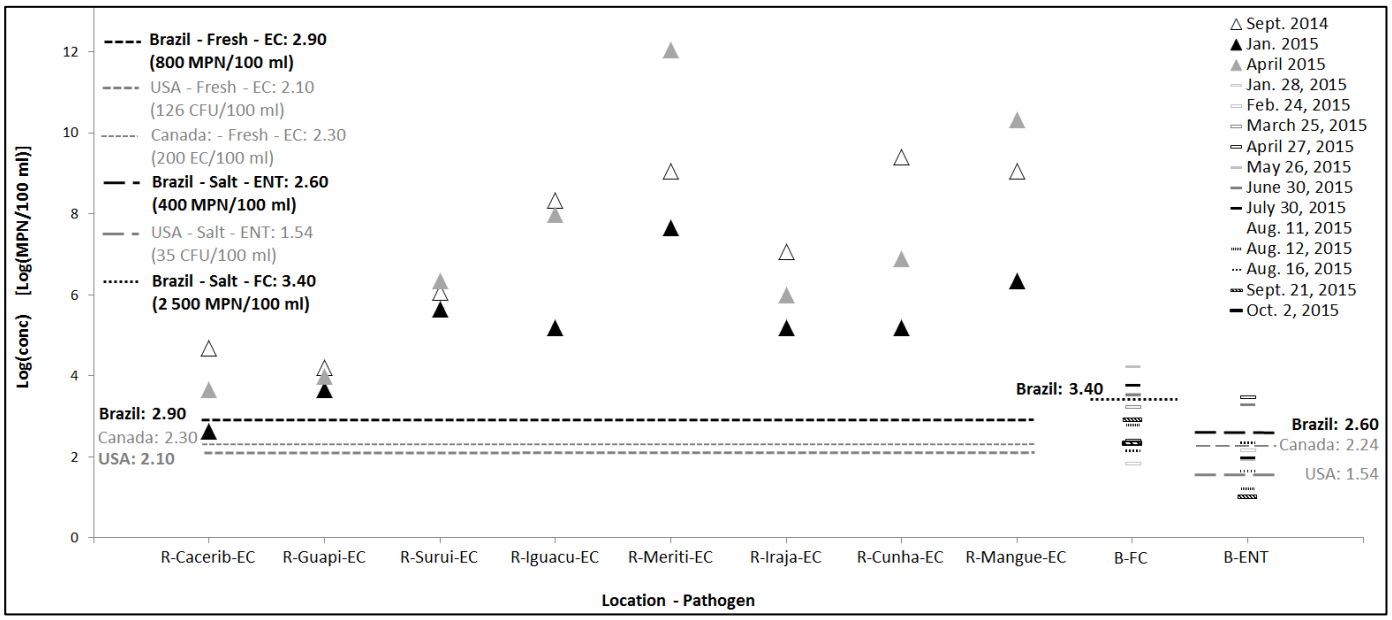

Figure 2. Scatter plot of measured indicator concentrations in river and bay samples from the Guanabara Bay basin compared to international regulatory limits in Brazil, Canada, and the United States. Triangles indicate concentrations of indicators measured in samples from eight freshwater rivers (R) and single dashes show concentrations for the Marina de Gloria area of the saltwater bay (B). The lines represent standards for various countries for freshwater and saltwater as applicable for *E. coli* (EC), fecal coliform (FC), and enterococci (ENT).

3.2. Risk Estimation

Estimated risks in the freshwater exposure pathways were three orders of magnitude higher than the estimated risks in the sailing exposure in the bay (Table 4). Median probabilities of disease based on *E. coli* exposure were higher in the river-exposure scenarios ($P[disease]_{swim} = 1$ and $P[disease]_{wade} = 9.9 \times 10^{-1}$) than in the bay-exposure scenario (median $P[disease]_{sail} = 8.2 \times 10^{-4}$). Risk estimates in the saltwater scenario were found to be comparable using the two different indicators, *E. coli* ($P[disease]_{E.\ coli} = 8.2 \times 10^{-4}$) and enterococci ($P[disease]_{enterococci} = 3 \times 10^{-4}$). As the saltwater *E. coli* concentrations were derived using a fecal coliform-to-*E. coli* ratio, $R_{FC:EC}$, the similarity in risk results obtained using *E.coli* and enterococci suggests that the ratio used is appropriate, as it led to an equivalent risk estimation for both of these indicators.

Table 4. Summary of risk of illness, per exposure scenario and organism.

	Exposure Scenarios (Water Type, Location)								
	Swimming (Fresh Water, Rivers)			**Wading (Fresh Water, Rivers)**			**Sailing (Saltwater, Bay)**		
	Risk Percentiles			**Risk Percentiles**			**Risk Percentiles**		
Organisms	**5th**	**50th**	**95th**	**5th**	**50th**	**95th**	**5th**	**50th**	**95th**
E. coli	1.9×10^{-2}	1.00	1.00	4.7×10^{-4}	9.9×10^{-1}	1.0	1.5×10^{-5}	8.2×10^{-4}	3.6×10^{-2}
Enterococci	-	-	-	-	-	-	8.0×10^{-6}	3.4×10^{-4}	1.1×10^{-2}
$\sum$ Organisms	1.8×10^{-1}	5.9×10^{-1}	8.8×10^{-1}	1.1×10^{-2}	3.6×10^{-1}	7.5×10^{-1}	4.2×10^{-5}	1.0×10^{-3}	3.5×10^{-2}
Campylobacter	2.3×10^{-5}	6.9×10^{-2}	2.4×10^{-1}	5.6×10^{-7}	5.5×10^{-3}	2.0×10^{-1}	6.2×10^{-9}	3.5×10^{-7}	1.5×10^{-5}
Salmonella	1.9×10^{-7}	1.5×10^{-3}	2.4×10^{-1}	3.8×10^{-9}	3.9×10^{-5}	1.2×10^{-1}	3.3×10^{-11}	1.8×10^{-9}	8.0×10^{-8}
Rotavirus	1.4×10^{-3}	3.5×10^{-1}	4.9×10^{-1}	3.1×10^{-5}	1.4×10^{-1}	4.6×10^{-1}	2.9×10^{-7}	1.8×10^{-5}	8.3×10^{-4}
Cryptosporidium	1.4×10^{-6}	1.2×10^{-2}	7.0×10^{-1}	3.0×10^{-8}	2.8×10^{-4}	6.9×10^{-1}	3.1×10^{-10}	1.7×10^{-8}	7.1×10^{-7}
Ascaris	6.8×10^{-6}	6.7×10^{-2}	3.9×10^{-1}	1.6×10^{-7}	1.6×10^{-3}	3.9×10^{-1}	1.6×10^{-9}	9.2×10^{-8}	3.8×10^{-6}

The risk estimate derived from the sum of specific pathogens had a lower magnitude than the risk derived from indicators. This could be due to the omission of other pathogens that contribute to risk from the model derived here. For example, norovirus, adenovirus, and enterovirus are all common causes of GI that were not included in the risk assessment conducted here and may account for some of the differences between the indicator-based risk assessment and the sum of the specific pathogen risks.

4. Discussion

The output of the risk model constructed here showed exceedances of recreational contact guidelines for freshwater rivers flowing to Guanabara Bay. Disease risk in the sailing context was estimated to be on the order of 1 in 10,000, which is considered acceptable for GI illness due to recreational water contact by U.S. recreational contact standards, which have target-risk levels ranging from 3.2 to 3.6 in 100.

The analysis here does not explore the scenario of swimming in Guanabara Bay, a scenario that would have higher risks than sailing due to the greater amount of water that is ingested. It is also notable that the upper bound on risk is roughly four in 100, which does exceed U.S. targets for allowable recreational water contact risk. There is great variability in indicator concentrations, likely related to tidal flows in and out of Guanabara Bay. In the future, the acceptable risks for exposure scenarios such as the Olympic sailing event need to be evaluated on a case-by-case basis, as they are highly subject to the environmental conditions prevailing on a given day. Strategies such as scheduling events during a rising tide, when presumably cleaner ocean water is flowing through the area in which events are to be held, may help reduce risk for future events of a similar nature.

Compared to existing standards, the fecal coliform and enterococci concentrations measured in saltwater at the Marina de Gloria in Guanabara Bay are either close to the limits (for Brazil and US), far above (for Europe, Australia and WHO), or below the national limits (Canada), while the values reported of the *E. coli* concentrations measured in freshwater rivers around Guanabara Bay are far above the Brazilian and all other reference limits explored in this study. This observation highlights the exceptional risk that local populations face due to contaminated surface waters. As a non-quantitative retrospective validation, there were no widely publicized reports of athletes in sailing events contracting GI during the Rio Olympics of 2016.

5. Limitations

This paper has a number of important conceptual and technical limitations that are worth discussing. For one, total risk was calculated as the sum of the risks from selected pathogens in the model and compared to the risk magnitude of the indicators. However, the total risk resulting from the select pathogens included in the model (*Campylobacter*, *Salmonella*, rotavirus, *Cryptosporidium*, *Ascaris*) may be underestimated due to inexhaustive collection of pathogens that were chosen for this risk assessment. Additional pathogens such as bacteria-like *Shigella* spp., *Vibrio cholera*, viruses such as noroviruses and adenoviruses, or protozoan-like *Giardia lamblia*, are endemic in Brazil and could be present in the water and contribute to the risk of gastrointestinal disease.

We made a major assumption that the transmission routes and behavior of the populations in this study could be modeled using data from other country contexts. We were not able to conduct a field survey in Brazil to determine the potential exposure pathways that were the most relevant for the exposed population. Rather, exposure activities were selected from those reported in similar contexts, informal settlements in Accra, Ghana, and Uganda [25].

Further assumptions were made regarding the dose–response parameters used and assumptions on the growth, removal, and inactivation of pathogens in this specific context. The majority of the dose–response relationships that were used in this paper come from either epidemiological experiments or human feeding trials that were conducted in the USA. The *Cryptosporidium* dose–response curve used was derived from data from an

epidemiology study that was conducted in Mexico. In reality, the susceptibility of humans to the pathogens explored in this study may differ depending on individual susceptibility, which may vary by culture, age, current health status, and previous exposures. Because the dose–response relationships employed here originated in many cases from studies done on healthy American volunteers, we hypothesize that the response and risk may be underestimated for populations living in informal settlements near Guanabara Bay.

Another limitation was the use of the same *E. coli*:pathogen ratio distribution for all seasons. Evidence has suggested that this ratio may vary by season. Thus, there is a need for further research to determine the seasonal variation of the pathogen concentrations and derive specific distributions of ratios for each season.

Finally, we used ratios between indicator bacteria and specific pathogens based on limited experimental data from different country contexts. The ratio of *E. coli* to fecal indicators used in the saltwater was derived from studies conducted in Israel. The ratios used for comparing *E. coli* to pathogens were derived from datasets collated from nine studies of raw sewage in Australia, Netherlands, USA, Scotland, England, and New Zealand, and Brazil [28]. The diversity of pathogens and their concentrations in raw sewage have been shown to depend on fecal input and the health status of the contributing populations [46]. Both of these factors may differ by population or country context. As such, the ratios from the studies cited may be a good estimate of the relative concentrations of the organisms within the industrialized world, but it is likely that the ratio in a developing country like Brazil is lower than what was assumed in this study.

6. Conclusions

The work done in this paper exemplifies a risk assessment conducted in a low-resource setting with environmental data limited to concentrations of fecal indicator organisms. Context-specific data on the relevant exposure routes, exposure frequency, and site-specific indicator:pathogen ratios was also lacking. Overall, the use of surrogate data from different contexts to construct model parameter distributions resulted in a model with high uncertainty. Future research should focus on exploring field-based sampling methods to create context-specific pathogen:indicator ratios and reduce the uncertainty of context. Further development of field-based exposure assessments which make use of behavioral observations and personal sampling methods are necessary to refine our understanding of exposure dose and reduce the uncertainty associated with assuming exposure routes and pathways that are derived from other studies. Despite its limitations, this study highlights the risks of exposure to water contaminated with wastewater, particularly in the freshwater rivers draining to Guanabara Bay and, hence, the need for proper wastewater infrastructure.

Author Contributions: Conceptualization, P.L.G. and J.M.; Methodology, P.L.G., R.S., A.E.C. and R.S.C.; Formal Analysis, R.S. and A.E.C.; Validation P.L.G.; Manuscript preparation, R.S.; Manuscript editing and review P.L.G., R.S.C. and J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by the Indo-U.S. 21st Century Knowledge Program.

Data Availability Statement: Data are available upon request to the corresponding author.

Acknowledgments: Katharine Hammond provided valuable comments on a draft which was a chapter in Rachel Sklar's dissertation [47]. Luiza A.A. Costa and Denise M.M. Pessoa assisted with sampling and analysis. Two anonymous reviewers provided detailed comments that improved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fistarol, G.O.; Coutinho, F.H.; Moreira, A.P.B.; Venas, T.; Cánovas, A.; De Paula, S.E.M.; Coutinho, R.; De Moura, R.L.; Valentin, J.L.; Tenenbaum, D.R.; et al. Environmental and sanitary conditions of Guanabara Bay, Rio de Janeiro. *Front. Microbiol.* **2015**, *6*, 1232. [CrossRef] [PubMed]
2. CDC Healthy Swimming—Diarrheal Illness. Available online: http://www.cdc.gov/healthywater/swimming/swimmers/rwi/diarrheal-illness.html (accessed on 21 June 2016).
3. Dufour, A.; Cabelli, V.; Ericksen, T.; Ballantine, R.; Goldberg, M.; Fox, W. Ambient Water Quality Criteria for Bacteria. In *Bacteriological Ambient Water Quality Criteria for Marine and Fresh Recreational Waters*; United States Environmental Protection Agency: Washington, DC, USA, 1986.
4. Korajkic, A.; McMinn, B.R.; Harwood, V.J. Relationships between Microbial Indicators and Pathogens in Recreational Water Settings. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2842. [CrossRef]
5. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Off. J. Eur. Union* **2006**, *64*, 37–51.
6. EPA. *2012 Recreational Water Quality Criteria*; United States Environmental Protection Agency: Washington, DC, USA, 2012.
7. Federal-Provincial-Territorial Working Group on Recreational Water Quality (Canada); Health Canada. *Guidelines for Canadian Recreational Water Quality*; Water, Air and Climate Change Bureau Healthy Environments and Consumer Safety Branch; Health Canada: Ottawa, ON, Canada, 2012.
8. WHO. Guidelines for Safe Recreational Water Environments. Available online: http://www.who.int/water_sanitation_health/publications/srwe1/en/ (accessed on 30 September 2020).
9. EPA. *Total Coliform Rule: A Quick Reference Guide*; United States Environmental Protection Agency: Washington, DC, USA, 2013.
10. CONAMA. *Current CONAMA Resolutions Published between September 1984 and January 2012*; Conselho Nacional do Meio Ambiente: Brasilia, Brasil, 2012.
11. CONAMA. *CONAMA Resolution No 274/2000*; Conselho Nacional do Meio Ambiente: Brasilia, Brasil, 2000; Volume 53.
12. CONAMA. *CONAMA Resolution No 357/2005*; Conselho Nacional do Meio Ambiente: Brasilia, Brasil, 2005.
13. Ford, B. *Rio Olympic Games—The Promise Rio Couldn't Keep*; ESPN: Bristol, CT, USA, 2016.
14. Balch, O. Funding Problems Hit Plan to Clean Rio's Polluted Waterways Ahead of Olympics. Guardian Sustainable Business. *The Guardian*, 1 February 2016. Available online: https://www.theguardian.com/sustainable-business/2016/feb/01/funding-problems-hit-plan-clean-rios-polluted-waterways-olympics (accessed on 8 July 2023).
15. Fidalgo, M. Rio's "Bay of All Delights": The Polluted Waters of the 2016 Olympics. *Vice*, 2 March 2016.
16. INEA. *Boletim de Qualidade das Aguas da Baia de Guanabara (No 02/16). Area de Provas Olimpicas—2016 (Bacteriologia)*; INEA: Rio De Janeiro, Brazil, 2016.
17. Demographia. *World Urban Areas & Population Projections*; Demographia: Belleville, IL, USA, 2015.
18. SEMADS. *Bacias Hidrográficas e Rios Fluminenses: Síntese Informativa por Macroregião Ambiental*; Secretaria de Estado de Meio Ambiente e Desenvolvimento Sustentável: Rio de Janeiro, Brazil, 2001; ISBN 8587206109.
19. Ministério da Saúde. *Guia de Vigilância Epidemiológica*; Ministério da Saúde: Brasilia, Brazil, 2009; ISBN 9788533416321.
20. Evison, L.M. Comparative Studies on the Survival of Indicator Organisms and Pathogens in Fresh and Sea Water. *Water Sci. Technol.* **1988**, *20*, 309–315. [CrossRef]
21. World Health Organization. *Guidelines for Drinking-Water Quality: First Addendum to the Third Edition*; Volume 1: Recommendations; WHO: Geneva, Switzerland, 2006; ISBN 978-92-4-154696-6.
22. Haas, C.N.; Rose, J.B.; Gerba, C.P. *Quantitative Microbial Risk Assessment*, 2nd ed.; John Wiley & Sons: New York, NY, USA, 2014; ISBN 978-1-118-14529-6.
23. Katukiza, A.Y.; Ronteltap, M.; Niwagaba, C.B.; Foppen, J.W.A.; Kansiime, F.; Lens, P.N.L. Sustainable sanitation technology options for urban slums. *Biotechnol. Adv.* **2012**, *30*, 964–978. [CrossRef]
24. Kundu, A.; Poma, H.R.; Jenkins, M.W.; Rajal, V.B.; Wuertz, S. QMRA of intestinal nematode infection via multimedia exposure pathways. In Proceedings of the7th International Congress on Environmental Modelling and Software, San Diego, CA, USA, 6 June 2014.
25. Shuval, H.; Lampert, Y.; Fattal, B. Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Sci. Technol.* **1997**, *35*, 15–20. [CrossRef]
26. Mara, D.D.; Sleigh, P.A.; Blumenthal, U.J.; Carr, R.M. Health risks in wastewater irrigation: Comparing estimates from quantitative microbial risk analyses and epidemiological studies. *J. Water Health* **2007**, *5*, 39–50. [CrossRef]
27. Labite, H.; Lunani, I.; Van Der Steen, P.; Vairavamoorthy, K.; Drechsel, P.; Lens, P. Quantitative microbial risk analysis to evaluate health effects of interventions in the urban water system of Accra, Ghana. *J. Water Health* **2010**, *8*, 417–430. [CrossRef]
28. Howard, G.; Ahmed, F.M.; Teunis, P.; Gaifur Mahmud, S.; Davison, A.; Deere, D. Disease burden estimation to support policy decision-making and research prioritization for arsenic mitigation. *J. Water Health* **2007**, *5*, 67–79. [CrossRef] [PubMed]
29. Fattal, B.; Peleg-olevsky, E.; Agursky, T.; Shuvsl, P.H.I. The association between seawater pollution as measured by bacterial indicators and morbidity among bathers at Mediterranean bathing beaches of Israel. *Chemosphere* **1987**, *16*, 565–570. [CrossRef]
30. Schets, F.M.; Schijven, J.F.; De Roda Husman, A.M. Exposure assessment for swimmers in bathing waters and swimming pools. *Water Res.* **2011**, *45*, 2392–2400. [CrossRef]

31. Dorevitch, S.; Panthi, S.; Huang, Y.; Li, H.; Michalek, A.M.; Pratap, P.; Wroblewski, M.; Liu, L.; Scheff, P.A.; Li, A. Water ingestion during water recreation. *Water Res.* **2011**, *45*, 2020–2028. [CrossRef]
32. Westrell, T.; Schönning, C.; Stenström, T.A.; Ashbolt, N.J. QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Sci. Technol.* **2004**, *50*, 23–30. [CrossRef]
33. Aquece-Rio. *International Sailing Regatta 2015*; Notice of Race; Aquece-Rio: Rio de Janeiro, Brazil, 2015.
34. Dufour, A.P. *Health Effects Criteria for Fresh Recreational Waters*; United States Environmental Protection Agency: Washington, DC, USA, 1984.
35. Cabelli, V.J.; Dufour, A.P.; McCABE, L.J.; Levin, M.A. Swimming-Associated Gastroenteritis and Water Quality. *Am. J. Epidemiol.* **1982**, *115*, 606–616. [CrossRef]
36. Sunger, N. Quantitative Health Risk Assessment of Recreational Water Users in Philadelphia. Ph.D. Thesis, Department of Civil, Architectural, and Environmental Engineering, Drexel University, Philadelphia, PA, USA, 2013.
37. Anderson, I.C.; Rhodes, M.; Kator, H. Sublethal stress in *Escherichia coli*: A function of salinity. *Appl. Environ. Microbiol.* **1979**, *38*, 1147–1152. [CrossRef] [PubMed]
38. Teunis, P.F.M.; Van der Heijden, O.G.; Van der Giessen, J.W.B.; Havelaar, A.H. *The Dose-Response Relation in Human Volunteers for Gastro-Intestinal Pathogens*; Rijksinstituut voor Volksgezondheid en Milieu: Bilthoven, The Netherlands, 1996.
39. Navarro, I.; Jiménez, B.; Cifuentes, E.; Lucario, S. Application of Helminth ova infection dose curve to estimate the risks associated with biosolid application on soil. *J. Water Health* **2009**, *7*, 31–44. [CrossRef] [PubMed]
40. Coster, T.S.; Wolf, M.K.; Hall, E.R.; Cassels, F.J.; Taylor, D.N.; Liu, C.T.; Trespalacios, F.C.; DeLorimier, A.; Angleberger, D.R.; McQueen, C.E. Immune response, ciprofloxacin activity, and gender differences after human experimental challenge by two strains of enterotoxigenic *Escherichia coli*. *Infect. Immun.* **2007**, *75*, 252–259. [CrossRef]
41. Levine, M.M.; Rennels, M.B.; Cisneros, L.; Hughes, T.P.; Nalin, D.R.; Young, C.R. Lack of person-to-person transmission of enterotoxigenic *Escherichia coli* despite close contact. *Am. J. Epidemiol.* **1980**, *111*, 347–355. [CrossRef]
42. Rendtorff, R.C. The experimental transmission of human intestinal protozoan parasites. *Am. J. Hyg.* **1954**, *9*, 196–208.
43. López-Pila, J.M.; Szewzyk, R. Estimating the infection risk in recreational waters from the faecal indicator concentration and from the ratio between pathogens and indicators. *Water Res.* **2000**, *34*, 4195–4200. [CrossRef]
44. Pouillot, R.; Delignette-Muller, M.L. Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages. *Int. J. Food Microbiol.* **2010**, *142*, 330–340. [CrossRef] [PubMed]
45. WHO. *WHO Guidelines for Drinking-Water Quality*, 3rd ed.; Volume 1—Recommendations; WHO: Geneva, Switzerland, 2008; Volume 38.
46. Hamilton, A.J.; Stagnitti, F.; Xiong, X.; Kreidl, S.L.; Benke, K.K.; Maher, P. Wastewater Irrigation: The State of Play. *Vadose Zone J.* **2007**, *6*, 823–840. [CrossRef]
47. Sklar, R.S. Microbial Health Risks to Sanitation Workers in Low-Resource Settings: Incorporation of Field, Molecular, and Modeling Approaches. Ph.D. Thesis, UC Berkeley, Berkeley, CA, USA, 2020. Available online: https://escholarship.org/uc/item/11t4t1qs (accessed on 17 May 2023).