Modeling Iloilo River Water Quality

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The analysis of covariance model (ANCOVA) with heterogeneous variance first-order autoregressive error covariance structure (ARH1) was used to model the differences in fecal streptococci concentration in Iloilo River over time with fixed site and seasonal effects as primary factors of interest, and water temperature, pH, dissolved oxygen, and salinity as covariates. The restricted maximum likelihood estimation (REML) procedure was used to derive the parameter estimates and the Kenward-Roger adjustment in the degrees of freedom was used to better approximate the distributions of the test statistics. The effect of season was highly significant (p = 0.0019). The site effect was significant at the 0.0539 level. The effects of water surface temperature and pH were significant at the 0.0655 and 0.0828 level, respectively. The effects of dissolved oxygen and salinity were not significant. Although the coefficient of determination was modest, the result of the study is useful in characterizing the dynamics of Iloilo River bacteriological system which contributes to an improved understanding of the Iloilo River water quality.

Keywords: analysis of covariance (ANCOVA), heterogeneous variance first-order autoregressive error covariance structure (ARH1), restricted maximum likelihood estimation (REML), fecal indicator bacteria (FIB), fecal streptococcus

1. Introduction

Fecal indicator bacteria (FIB) that are commonly found in human and animal feces such as total coliforms, fecal coliforms, *Escherichia coli* (*E. coli*), fecal streptococci, and enterococci are used as indicators of possible river contamination because their presence in rivers suggests that pathogenic microorganisms might also be present that can contaminate water and result in illness or death (Parajuli et al., 2009; USEPA 2015). FIB is typically used to evaluate river water quality because of the following reasons: (1) it is not practical to evaluate water for hundreds of different pathogens that might be present; (2) it is normally present in high number in feces of human and warm-blooded animals; (3) it is higher in number, survives longer, and easier to detect and quantify in laboratory than pathogens; and, (4) it is used for almost a century as indicators of the bacteriological safety of water (Paule et al., 2014).

Fecal pollution is associated with the presence of FIB concentrations above certain thresholds. The US Environmental Protection Agency (USEPA) recommended water quality criteria of fresh water fecal contamination based on *E. coli* was a geometric mean of 126 colony forming units (CFU) per 100 milliliters (mL) and a statistical threshold value (STV) of 410 CFU/100mL. The STV approximates the 90th percentile of the water quality distribution and is intended to be a value that should not be exceeded by more than 10 percent of the samples taken (USEPA, 2012). In the Philippines, the water quality criteria for total coliform in class C fresh waters is 5000 most probable number (MPN)/100mL (DENR Administrative Order No. 34, 1990). Fecal pollution may result either from point sources such as effluent discharges from wastewater treatment plants, on-site septic systems, and nonpoint sources such as domestic and wild animal manure, and stormwater runoff (Ibekwe et al., 2011; David and Haggard, 2011; Paule et al., 2014; USEPA, 2015).

The Iloilo River, classified as a class C water system by the Department of Environment and Natural Resources (DENR) with an approximate length of 11 km and runs the length of densely populated and economically important areas in the city of Iloilo, is an important landmark in the province of Iloilo, Philippines (DENR Administrative Order No. 34, 1990). The river has no watershed of its own and the bulk of water entering it comes from its two major tributaries (Calajunan and Dungon Creek), from agricultural lands and the inundation from the Iloilo Strait (Taquiso et al., 2008). Moreover, along its banks are infrastructures such as oil depots, commercial food establishments, hotels, schools, hospitals and clusters of households that contribute to the river through drainage into it. With great potentials as a tourist attraction, source of livelihood and a host to a transport system, this water system is viewed to boost the economy of the city.

The potential uses of the river, however, are not maximized due to concerns of its sanitary quality. The lax implementation of environmental laws, such as the Clean Water Act of 2004 (RA 9275) and Ecological Solid Waste Management Act of 2000 (RA 9003), contributed to the high level of pollution that made the river unsafe for both marine life and humans, especially for swimming or bathing. The high level of coliform content was attributed to domestic and commercial establishments without proper waste treatment facilities and end up dumping all the waste into the river (Yap, 2011). It has been estimated that the Iloilo River receives one million cubic meter of wastewater daily, making it a virtual septic tank of more than 100 establishments, including the Iloilo Provincial Capitol. Records of the City Environment and Natural Resources Office (CENRO) showed that 200 drainage pipes from 13 hotels and seven hospitals discharge untreated wastewater into the river (Nepomoceno, 2011).

Laboratory-based monitoring of the water and sediment microbial load is essential in policy making and the formulation of action plan development of the river water system, but they are tedious, expensive and time consuming. Statistical models have also been extensively employed in many water quality management design and assessment in addition to laboratory-based assessment (Mirbagheri et al., 2009; Ani et al., 2010). In many studies, statistical models had been used to predict water quality conditions by correlating bacteria concentration with precipitation or other easily measurable surrogate explanatory variables, and thus, reducing dependence on water quality sampling (Christensen et al., 2000; Christensen et al., 2002; Eleria and Vogel, 2005; David and Haggard, 2011; Ibekwe et al., 2011).

Most statistical models developed in previous researches (Table 1) on bacteria concentration used multivariate regression (Ferguson et al., 1996; Christensen et al., 2000; Clark and Norris, 2000; Christensen, 2001; Christensen et al., 2002; Rasmussen and Ziegler, 2003; Eleria and Vogel, 2005; David and Haggard, 2011; Gonzalez et al., 2012; Paule et al., 2014) and analysis of variance models (Ibekwe et al., 2011). The FIB used as response variables in these studies were log transformed (Ferguson et al., 1996; Christensen et al., 2000; Christensen, 2001; Christensen et al., 2002; Rasmussen and Ziegler, 2003; Eleria and Vogel, 2005; Ibekwe et al., 2011; David and Haggard, 2011; Paule et al., 2014). The explanatory variables considered in these models included meteorologic variables such as rainfall and season, hydrologic variables such as streamflow and waterflow, and easily measurable physicochemical water quality parameters such as temperature, pH, dissolved oxygen, salinity, specific conductance, and turbidity. Goodness-of-fit statistics obtained from these models were not overwhelming with R^2 or Adjusted R^2 values ranging from 0.11 to 0.85.

The present study investigated the use of statistical models to evaluate and quantify variations in bacterial measurements as well as examine the effects of geographical and meteorological factors and easily measurable physicochemical parameters, such as water temperature, pH, dissolved oxygen, and salinity on FIB concentration of Iloilo River over time. Specifically, the models tested for the effects of sampling locations (site effect), climatic conditions (season effect), and the different easily measurable physicochemical parameters, such as water temperature, pH, dissolved oxygen, and salinity, on FIB concentration. The model building process also included the examination of temporal trends and spatial patterns to further understand the dynamics of FIB in Iloilo River. The result of the study is useful in understanding the dynamics of Iloilo River bacteriological system which can be used for effective bacteriological monitoring and water quality assessment that will eventually lead to an improved understanding of Iloilo River water quality.

Table 1. Previous Research on Modeling of Fecal Indicator Bacteria

Citation	Statistical Model/Tool	Response	Explanatory	Goodness-of-Fit Statistic
Ferguson et al. (1996)	Regression	(Log) Fecal coliform	Rainfall, sewage overflows, site	0.80 (R2)
		(Log) Fecal Streptococcus	Rainfall, sewage overflow	0.71 (R2)
Christensen et al. (2000)	Regression	(Log) Fecal coliform	Turbidity and time (month)	0.55 to 0.60 (Adj R2)
Clark and Norris (2000)	Spearman Rank Correlation Coefficient	Fecal coliform and water quality parameters, such as discharge, specific conductance, pH, temperature, and dissolved oxygen were correlated		0.012 to 0.775 (Spearman rank correl coeff)
Christensen (2001)	Regression	(Log) Fecal coliform	water temperature, turbidity	0.661 (R2)
Christensen et al. (2002)	Regression	(Log) Fecal coliform	Regression was site specific and includes at least one predictor from (turbidity, season (day of year), discharge, specific conductance)	0.591 to 0.620 (R2)
Rasmussen and Ziegler (2003)	Regression	(Log) Fecal coliform	Turbidity	0.16 to 0.79 (R2)
Eleria and Vogel (2005)	Regression	(Log) Fecal coliform	Antecedent rainfall, lag-1 bacteria	0.54 (Adj R2)
Ibekwe et al. (2011)	ANCOVA	(Log)Total Coliform	Site, waterflow, pH	0.84 (R2)
		(Log) Fecal Coliform	Site	0.79 (R2)
		(Log) E. coli	Site, turbidity, temp	0.85 (R2)
		(Log) Enterococci	Site, turbidity	0.79 (R2)
David and Haggard (2011)	Regression	(Log) Fecal coliform	Regression was site specific and includes at least one predictor from (instantaneous discharge, temp, specific conductance, pH, dissolved oxygen)	0.13 to 0.75 (R2)
		(Log) E. coli		0.11 to 0.75 (R2)
		(Log) Fecal Strepto		0.11 to 0.84 (R2)
Mwakalobo et al. (2013)	Pearson Correlation Coefficient	Total coliform, fecal coliform, and enterococci were correlated with rainfall, water temperature, pH ,and salinity		0.038 to 0.516 (Correlation Coefficient in absolute value)
Paule et al. (2014)	Regression	(Log) E. coli and (Log) Fecal Streptococcus	Antecedent dry days, average rainfall intensity, runoff duration	0.419 to 0.789 (R2)

2. Method

The data set analyzed in this study was taken from the two-year (March 2008 through February 2010) bacteriological survey of the Iloilo River that assessed the sanitary condition of the river and determined the common sources of contamination (Padilla and Sinoben, 2012). Data represented monthly sampling of the water and bottom sediment of the five sampling areas (Table 2), chosen according to contamination exposure and economic significance, along the length of the river, namely: Forbes Bridge, Dungon Creek, IBRD Bridge, Carpenter’s Bridge, and So-oc Bridge.

Table 2. Sampling Location and Site Description

Sampling Site	Geographic Positioning System (GPS) Location	Land Use
Forbes Bridge	10° 42’ 20.66 “ N 122° 34’ 4.06 “ E	Commercial
Dungon Creek	10° 43’ 26.62 “ N 122° 32’ 56.67 “ E	Commercial/Residential
IBRD Bridge	10° 42’ 6.07 “ N 122° 33’ 14.06 “ E	Commercial
Carpenter’s Bridge	10° 41’ 59.83 “ N 122° 32’ 33.49 “ E	Commercial
So-oc Bridge	10° 41’ 33 “ N 122° 31’ 4.20 “ E	Residential/Agricultural

The location of Iloilo City in the Philippines as well as the different sampling sites in Iloilo River is shown in the following map (Figure 1). Iloilo City is located at the southern portion of the Iloilo province (10° 45’ N, 122° 33’ E), which in turn is centrally located in the Philippines. The different sampling sites were followed from those used by the Department of Environment and Natural Resources Environmental Management Bureau Region VI (DENR-EMB6).

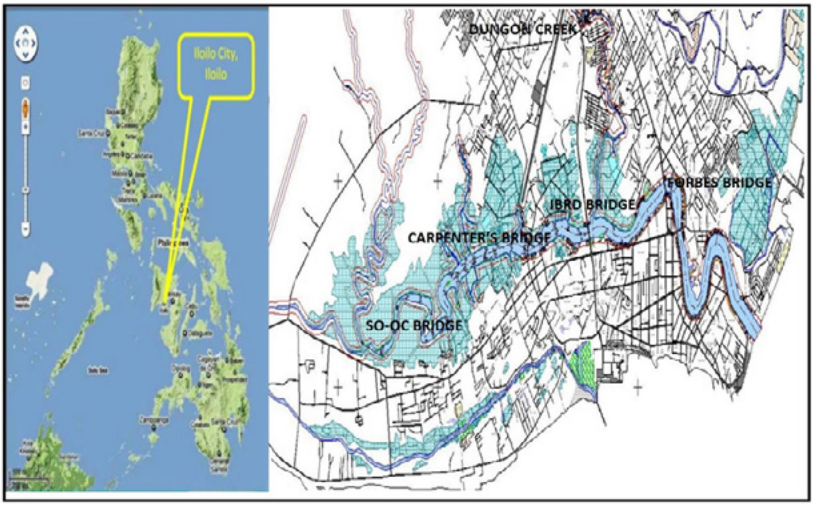

Figure 1. Iloilo River and the Different Sampling Sites (Iloilo City, Philippines)

Water and bottom sediment samples were brought to UPV Biological Sciences for processing to determine their bacteriological content. Bacteriological parameters such as heterotrophic plate count (HPC), total coliform count (TCC), fecal coliform count (FCC) and fecal streptococci count (FSC) were determined monthly for the whole duration of the project. Physicochemical parameters essential to the proliferation of microorganisms (pH, temperature, salinity and dissolved oxygen) were also monitored in each sampling area.

Statistical analysis

The primary factors of interest in this study were the geographical locations of the sampling sites and the temporal conditions surrounding the sampling locations based on climatic seasonality. These factors were believed to influence variations in bacterial density (Christensen, 2000; Christensen et al., 2002; Eleria and Vogel, 2005; Ibekwe et al., 2011). The analysis of covariance (ANCOVA) model (Neter et al., 1996; Montgomery, 2001) using site and season effects as primary factors of interest was the statistical model used to analyze differences in bacteria concentration. Four physicochemical parameters measured at each sampling site, namely: temperature of the water surface, pH, dissolved oxygen, and salinity were included as covariates in the subsequent statistical analyses. Variations in each of these water quality parameters were known to affect bacteria concentrations (Clark and Norris, 2000; Christensen, 2001; David and Haggard, 2011; Ibekwe et al., 2011; Gonzalez et al., 2012).

The following analysis of covariance (ANCOVA) model was used to explain differences in bacteria concentrations (Neter et al., 1996; Montgomery, 2001):

$$\log(y_{ij}) = \mu + \tau_i + \delta_k + \beta_1 pH_{ij} + \beta_2 SL_{ij} + \beta_3 DO_{ij} + \beta_4 TP_{ij} + \varepsilon_{ij} \tag{1}$$

where y_{ij} represents the average bacteria count at the i^{th} site during the j^{th} sampling period, the τ_i parameters quantify the 5 distinct site effects, the δ_k parameters measure the two (temporally dependent) seasonal climatic conditions, the four β parameters represent the four water quality covariate effects (water temperature, pH, dissolved oxygen, and salinity), and the ε_{ij} error terms are assumed to be normally distributed but possibly temporally correlated. Bacterial data were log-transformed to reduce the skewness in the residual error distribution and induce approximate normality.

Different error covariance structures were examined for modeling the ε_{ij} residual error distributions to account for the temporal nature of the sampling design. In addition to the default assumption that the errors were independently and identically distributed (IID), three temporal error covariance structures were specified and estimated using restricted maximum likelihood (REML) estimation techniques (McCulloch and Searle, 2001). An autoregressive model of order 1 (AR1) defined as:

$$
\begin{aligned}
&Var(\varepsilon_{ij}) = \sigma^2 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{ik}) = \rho^{|j-k|}\ \sigma^2 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{lk}) = 0 \ \forall\ \mathrm{i} \neq l
\end{aligned}
\tag{2}
$$

was used to test the presence of temporal trend in the residual errors. In addition, site-specific compound symmetric (CS) covariance structure was also estimated using REML techniques and was also used to test for temporal correlation in the residual errors. The site-specific CS structure was defined as:

$$
\begin{aligned}
&Var(\varepsilon_{ij}) = \sigma^2 + \sigma_1 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{ik}) = \sigma_1 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{lk}) = 0 \ \forall\ \mathrm{i} \neq l
\end{aligned}
\tag{3}
$$

Heterogeneous variance first-order autoregressive (ARH (1)) error covariance structure was also investigated. This error covariance structure estimated a separate variance for each month within each site. The ARH(1) autocorrelation parameter ρ represented the correlation between pairs of measurements made on the same site one time interval (one month) apart. The site-specific ARH(1) error covariance structure was the following:

$$
\begin{aligned}
&Var(\varepsilon_{ij}) = \sigma_j^2 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{ik}) = \rho^{|j-k|}\ \sigma_j\ \sigma_k \forall\ j \neq k,\ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{lk}) = 0 \ \forall\ \mathrm{i} \neq l
\end{aligned}
\tag{4}
$$

A spatial error covariance structure (Spatial-Power) was also tested to account for a continuous time in the covariances among errors, such that the time intervals between measurements would be more or less unique in each site. The Spatial-Power error covariance structure was given as:

$$
\begin{aligned}
&Var(\varepsilon_{ij}) = \sigma^2 \ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{ik}) = \rho^{d_{jk}}\ \sigma^2 \forall\ j \neq k,\ \forall\ i \\
&Cov(\varepsilon_{ij},\varepsilon_{lk}) = 0 \ \forall\ \mathrm{i} \neq l
\end{aligned}
\tag{5}
$$

where the distance metric used (d_{jk}) was a continuous time between measurements, specifically the distance in time between periods j and k, and ρ is the correlation parameter that describes the linear association between the errors measured one unit (one month) apart. When the time intervals are integers, the Spatial-Power error covariance structure reduces to the first-order autoregressive model AR(1).

The ANCOVA model (1) was estimated under each of the five residual error assumptions and the appropriate model that best fits the data was chosen using the likelihood ratio tests (LRT) and fit statistics, such as the Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (BIC) (Littell et al., 1996; Raudenbush and Bryk, 2002; Schabenberger, 2005).

For testing the significance of temporal and spatial correlations, the ANCOVA model was re-estimated as a linear mixed model using the REML estimation techniques (McCulloch and Searle, 2001). The F-tests and t-tests for testing the significance of parameter estimates and/or contrasts of interest were adjusted using the Kenward-Roger procedure to provide better approximations to the distributions of the test statistics (Kenward and Roger, 1997; Moser and Macchiavelli, 2002). The model building process as well as the analysis for this study was performed using the GLM and MIXED procedures in the SAS STAT software package, version 9.1 (SAS, 2004).

3. Results

Data were encoded in SPSS and eventually converted into a permanent SAS dataset. Frequency tables were generated and summary measures were produced to check for outlier and influential observations. Spurious outliers were verified and corrected before exploratory techniques were applied on the data.

The results of the current data (Table 3) indicated that the sanitary quality of the Iloilo River was very low based on the Philippine DENR-EMB standard (5,000 MPN/100mL for total coliform) for class C fresh waters (DENR Administrative Order No. 34, 1990). The overall mean total coliform concentration (19,398.41 MPN/100mL) was way beyond the 5,000MPN/100mL cut-off and this scenario was the same across the different sampling sites and seasons. The threshold for total coliform was exceeded 88.2% (75/85) of the time in the sample. Furthermore, based on the FC/FS ratio (Gerba et al., 1999), a significant portion of bacterial contaminants (44.7%; FC/FS >4.0) were of purely human origin and that only 10.6% (FC/FS < 0.7) were of purely animal origin.

Table 3. Summary Statistics for Bacteria Concentration (MPN/100mL) and Mean Bacteria Concentration by Site and Season

	Total Coliform	Fecal Coliform	Fecal Streptococcus
Site			
Forbes Bridge	18,376.47	10,747.29	3,122.12
Dungon Creek	21,780.29	12,136.18	5,723.06
IBRD Bridge	19,310.59	9,661.47	5,055.35
Carpenter's Bridge	18,671.18	11,540.29	4,135.24
So-oc Bridge	18,853.53	9,576.18	4,307.71
Season			
Dry	21,062.78	13,209.73	6,413.8
Rainy	17,526.00	7,945.15	2,280.45
Overall			
Min	485	33	30
Max	24,000	24,000	24,000
Mean	19,398.41	10,732.28	4,468.69
Median	24,000	11,000	1,500
Std Dev	7,414.54	9,955.45	6,368.02

Monitoring of some physicochemical parameters in all sampling areas (Table 4) revealed that pH, temperature, and salinity were within the acceptable range set by DENR-EMB for class C water systems except for the mean dissolved oxygen (DO) range which was lower than the standard of 5 mg/L.

Table 4. Summary Statistics for Physicochemical Properties and Mean Physicochemical Property by Site and Season

	Temp	pH	DO	Salinity
Site				
Forbes Bridge	28.77	7.71	3.29	23.88
Dungon Creek	29.06	7.60	2.55	18.50
IBRD Bridge	29.24	7.70	2.96	19.32
Carpenter's Bridge	29.03	7.67	3.27	17.29
So-oc Bridge	29.00	7.68	4.04	8.23
Season				
Dry	28.66	7.74	3.20	22.57
Rainy	29.42	7.60	3.24	11.69
Overall				
Min	24.6	7	0.30	0
Max	31.95	8.48	7.53	32
Mean	29.02	7.67	3.22	17.45
Median	29.4	7.64	2.85	18.5
Std Dev	1.46	0.29	1.42	9.87

Bacteria concentrations (total coliform count, fecal coliform count, fecal streptococci count) were log-transformed to reduce the influence of a few large bacterial readings. Exploratory techniques such as the use of scatterplots, boxplots, stem-and-leaf display, and histogram were used as preliminary steps in exploring the data.

The basic univariate summary statistics for the FIB measurements are displayed in Table 5. Initial results revealed that log-transformed total coliform count (TCC) and log-transformed fecal coliform count (FCC) exhibited extreme skewness (skewed to the left) while the log-transformed fecal streptococci count (FSC) showed near symmetry and approximate normal distribution (Figure 2). The skewness observed in two sets of bacterial data was due to the limitation of the method used in determining bacteria concentration. Analytical error for the determination of fecal indicator bacteria, particularly fecal coliform bacteria, can be as high as 50% (American Public Health Association, American Water Works Association, and Water Environment Federation 1992; Christensen, 2001). In the present dataset, bacteria densities in water samples exceeding 24,000 MPN/100mL were all set at 24,000 MPN/100mL (Padilla and Sinoben, 2012). Consequently, only the log-transformed fecal streptococci count was used as the response variable in the subsequent statistical analyses. In addition, only monthly assessments on water surface from October 2008 through February 2010 with complete covariate readings were used throughout the analysis.

Table 5. Univariate Summary Statistics for FIB Measurements (N=85)

Characteristic	Log TC	Log FC	Log FS
Mean	4.21	3.55	3.11
Median	4.38	4.04	3.18
Minimum	2.69	1.52	1.48
Maximum	4.38	4.38	4.38
Std Dev	0.34	0.86	0.78

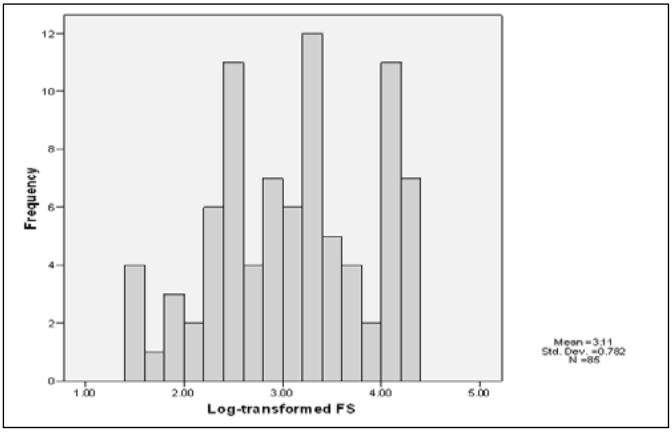

Figure 2. Histogram of the Log-transformed FSC

Figure 3 displays how the mean log-transformed FSC fluctuated over time. The graph shows the highest peak in January 2009 and lowest trough in July 2009. The graph usually have peaks during dry season (December through May) and troughs during rainy season (June through November).

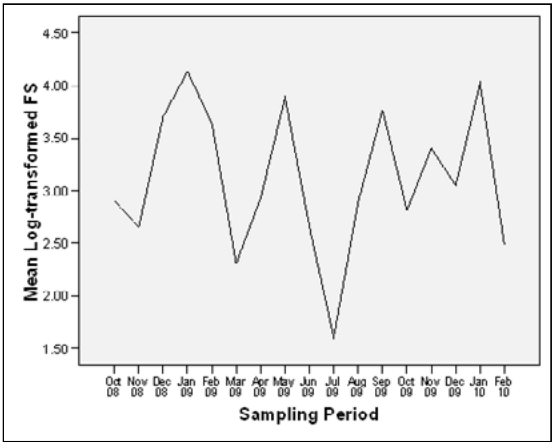

Figure 3. Mean Log-transformed FSC Over Time

The mean log-transformed FSC for each season across sites in Figure 4 shows that the average log-transformed FSC might marginally differ across the different sampling sites and that the average log-transformed FSC were consistently higher during dry season than rainy season.

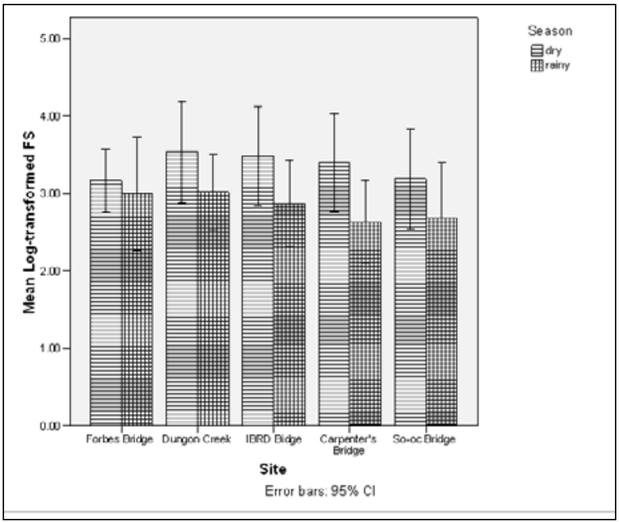

Figure 4. Mean Log-transformed FSC for Each Season Across Sites

Identification of suitable error covariance structure

The summary statistics for the ANCOVA model with normally, independently, and identically distributed errors on the log-transformed FSC is shown in Table 6. The results revealed that the proposed model was highly significant (F statistic = 2.39, p = 0.019) which indicated that the model appropriately fits the data. Investigation of the residuals revealed that the normality assumption (Shapiro-Wilk statistic = 0.983, p = 0.349) was satisfied. The R-squared value suggested that the model can substantially explain about 22% of the total variation in log FSC.

Table 6. ANCOVA Model Summary Statistics and Shapiro-Wilk Test

Statistic	Value
F statistic	2.39
F p-value	0.019
R-squared	0.223
MSE	0.532
S-W statistic	0.983
S-W p-value	0.349

The REML -2log-likelihood (-2LL) scores for the ANCOVA models estimated using all the five residual error covariance structures are shown in Table 7. Since the IID error covariance structure is nested within the autoregressive order 1 (AR1), compound symmetric (CS), first-order heterogeneous autoregressive (ARH1), and spatial-power (Spatial(Power)) error covariance structures, formal likelihood ratio tests could be computed by directly differencing the -2LL scores (Littell et al., 1996; Schabenberger, 2005). The results for these tests, along with Akaike's Information Criterion (AIC) (Akaike, 1974) and Schwarz Bayesian Criterion (BIC) are also shown. The result of the likelihood ratio tests suggested that the ARH1 error covariance structure was the best fitting model (Chi-square value was statistically significant, p = 0.0336) among all error covariance structures considered. The choice of the best model was further justified since it had the lowest BIC score. Thus, the data exhibited temporal correlation structure, did not exhibit spatial correlation, and that the first-order heterogeneous autoregressive (ARH1) error covariance structure should be adopted when estimating the ANCOVA model.

Table 7. REML -2LL, AIC and BIC Scores for Various Error Covariance Structures

Covariance Structure	-2ResLL	Difference	DF	Chi-square p-value	AIC Score	BIC Score
IID	202.6				204.6	206.9
CS	202.6	0	1	1	206.6	205.8
AR1	200.1	2.5	1	0.11385	204.1	203.4
ARH(1)	173.5	29.1	17	0.0336	209.5	202.5
Spatial (Power)	200.1	2.5	1	0.11385	204.1	203.4

The ANCOVA model was re-estimated using the restricted maximum likelihood estimation method (REML), with Kenward-Roger adjustment in the degrees of freedom for better approximation of the distribution of the test statistic, and using the heterogeneous first-order autoregressive error covariance structure. The resulting variances of the log-transformed FSC at each assessment period, within each site, truly reflected the observed non-constant variability of fecal streptococci count over time. The error temporal correlation estimate (ARH1 correlation estimate, ρ = - 0.26, p = 0.07) explains the observed fluctuation in bacteria concentration over time. The result of the test of fixed effects in the ANCOVA model using the ARH1 error covariance structure is shown in Table 8. The seasonal effect was highly significant (p = 0.0019), the site effect was marginally significant (p = 0.0539), temperature and pH were significant at the 0.0655 and 0.0828 level, respectively, and dissolved oxygen and salinity were not significant (p = 0.5796 and p = 0.3356, respectively).

Table 8. Test of Fixed Effects using ARH(1) Error Covariance Structure

Factor or Covariate	F-statistic	p-value
Site	2.67	0.0539
Season	11.79	0.0019
Temperature	3.63	0.0655
pH	3.40	0.0828
Dissolved Oxygen	0.31	0.5796
Salinity	0.95	0.3356

The solution for fixed effects in the ANCOVA model using the ARH1 error covariance structure showed that the water temperature had a negative marginally significant effect on mean (log) FSC (estimate = - 0.1173, t = - 1.90, p = 0.0655), above and beyond the effect of other variables in the model. This means that water surface temperature had a marginally significant positive effect on mean FSC ($10^{-0.1173} = 0.7633$), holding other variables in the model constant. Water pH also had a moderate positive effect on mean (log) FSC (estimate = 0.5944, t =1.84, p = 0.0828), after controlling for other variables in the model.

The results of the method of contrast shown in Table 9 revealed that the mean (log) FSC was significantly higher during the dry season than the rainy season (F=11.79, p = 0.0019). The result further revealed that the mean (log) FSC in site 2 (Dungon Creek) was higher than site 1 (Forbes Bridge), site 4 (Carpenter's Bridge), and site 5 (So-oc Bridge) at the 0.0547, 0.0607, and 0.0107 significance level, respectively. In addition, the mean (log) FSC in site 3 (IBRD Bridge) was higher than site 5 (So-oc Bridge) at the 0.0521 significance level.

Table 9. Contrast Results using ARH(1) Error Covariance Structure

Contrast	F-statistic	p-value
Season 1 vs Season 2	11.79	0.0019
Site 2 vs Site 1	4.01	0.0547
Site 2 vs Site 4	3.89	0.0607
Site 2 vs Site 5	7.39	0.0107
Site 3 vs Site 5	4.06	0.0521

4. Conclusion

This study examined and evaluated the water quality data of Iloilo River over time (October 2008 through February 2010). The ANCOVA model was used to investigate the effects of sampling sites, season, and the different easily measurable physicochemical parameters on mean (log) fecal streptococci count (FSC) over time.

The data indicated that the sanitary quality of the Iloilo River during the said period did not pass the Philippine DENR-EMB standard of 5,000 MPN/100mL for class C fresh waters (Padilla and Sinoben, 2012; DENR Administrative Order No. 34, 1990). The overall mean total coliform concentration was almost four (4) times (19,398.41 MPN/100mL) the threshold value and that this water quality criterion was exceeded 88.2% (75/85) of the time in the sample. Furthermore, bacterial contaminants that were of purely human origin were more than four times (44.7%) those of purely animal origin (10.6%).

The site effect was significant at the 0.0539 level. The mean (log) FSC in site 2 (Dungon Creek) was higher than site 1 (Forbes Bridge), site 4 (Carpenter's Bridge), and site 5 (So-oc Bridge) at the 0.0547, 0.0607, and 0.0107 significance level, respectively. In addition, the mean (log) FSC in site 3 (IBRD Bridge) was higher than site 5 (So-oc Bridge) at the 0.0521 significance level. The high fecal streptococci concentration in Dungon Creek is because the area that surrounds it is characterized by a higher population density in contrast with other sampling sites. The land near Dungon Creek is combination of commercial and residential zones including socialized housing where domestic waste coming from nearby residential areas and untreated waste water from commercial establishments contribute to river contamination. Fecal streptococci concentration in IBRD Bridge is also high because it is near the mouth of Dungon Creek. On the other hand, the area near So-oc Bridge is predominantly agricultural composed mainly of fishponds. This result is consistent with previous studies (DiDonato et al., 2009; Ibekwe et al., 2011) documenting the effect of increased urbanization on fecal pollution. This finding provided further support and helped establish the link between elevated coliform concentrations and increased urbanization.

The effect of season was highly significant ($p = 0.0019$). Fecal streptococci concentration was significantly higher during the dry season than the rainy season. Because of the flushing effect mechanism associated with bacteria transport, bacteria concentration in streams tends to vary seasonally (Christensen, 2002; Eleria and Vogel, 2005). Bacteria densities and streamflow are related because runoff from a watershed may transport sediments to streams, and since runoff characteristics vary with season, so does bacteria concentration (Christensen, 2002). The observed difference in bacteria concentration during the dry and rainy season could be due to prolonged dry days that allowed accumulation and build-up of microbial contaminants in the watershed before being washed off during a rainfall event or stormwater runoff which demonstrated the connection between rainfall intensity or runoff duration and fecal pollution (Paule et al., 2014).

The effect of water surface temperature was significant at the 0.0655 level. There was a moderate positive association between water temperature and fecal streptococci concentration. The result was expected since bacteria grow faster at higher temperatures and that the growth rate slows down at very low temperatures

(Water Research Watershed Center 2014). This finding was consistent with the result of previous researches (Clark and Norris, 2000; Christensen, 2001; David and Haggard, 2011; Ibekwe et al., 2011; Mwakalobo et al., 2013) and provided further evidence for the connection between temperature and bacteria growth.

The effect of pH was significant at the 0.0828 level. There was a moderate positive association between pH and fecal streptococci concentration. This finding was consistent with the result of previous research (David and Haggard, 2011) that showed a positive association between fecal streptococci concentration and pH in 1 of 11 sites for all flow conditions. This finding further supports that models incorporating various physicochemical properties used to predict bacteria concentration are site and bacteria group specific (David and Haggard, 2011).

The effects of dissolved oxygen and salinity on fecal streptococci concentration were not significant. Ferguson et al. (1996) indicated that dissolved oxygen was not a significant predictor of fecal streptococci concentration. David and Haggard (2011) also found that dissolved oxygen was not a significant predictor of fecal streptococci in 4 of 11 sites for all flow conditions. Ibekwe et al. (2011) observed that salinity levels were never significantly related to bacteria count levels in any of the bacteria groups (total coliform, fecal coliform, E. coli, and enterococci).

The results of the effects of the different physicochemical parameters on fecal streptococci concentration demonstrated the varying effects of environmental conditions on the survival rate of bacteria once they leave the digestive tract of warm-blooded animals (Clark and Norris, 2000).

The usefulness of the obtained model cannot be undermined despite the low coefficient of determination. Previous studies (Christensen et al., 2000; Christensen, 2001) argued that bacteria measurements are affected by the precision in the analytical method used (American Public Health Association, American Water Works Association, and Water Environment Federation 1992; Christensen, 2001). In addition, previous statistical models showed a range of explained variance in fecal bacteria relationships that were highly dependent upon included variables, flow, and site characteristics (David and Haggard, 2011; Ibekwe et al., 2011).

The statistical model established in the study is useful in developing scientific understanding of the Iloilo River bacteriological system. It is also useful in discovering possible dynamics on how this system is influenced by the different geographical, meteorological, and physicochemical parameters which can be used as a tool for river bacteriological monitoring and water quality assessment that will eventually lead to an improved understanding of Iloilo River water quality.

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