Seasonal influence of environmental variables and artificial aeration on *Escherichia coli* in small urban lakes

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ABSTRACT

This study investigated patterns of *Escherichia coli* in urban lakes in Lubbock, Texas. Specific objectives were to (1) document seasonal patterns in abundance of *E. coli* over a 3-year period, (2) identify environmental factors, including effects of migratory geese and artificial aeration devices that may influence *E. coli* abundance, and (3) determine if *E. coli* abundance over time was similar for individual lakes. Water samples were collected monthly for 36 months from six lakes, three of which contained artificial aeration devices (fountains). Regression models were constructed to determine which environmental variables most influence *E. coli* abundance in summer and winter seasons. *Escherichia coli* is present in the lakes of Lubbock, Texas year-round and typically exceeds established bacterial thresholds for recreational waters. Models most frequently contained pH and dissolved oxygen as predictor variables and explained from 17.4% to 92.4% of total variation in *E. coli.* Lakes with fountains had a higher oxygen concentration during summer and contained consistently less *E. coli*. We conclude that solar irradiation in synergy with pH and dissolved oxygen is the primary control mechanism for *E. coli* in study lakes, and that fountains help control abundance of fecal bacteria within these systems.

Key words | Canada geese, *Escherichia coli*, fecal contamination, urban lakes

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INTRODUCTION

Water contaminated with feces has long been recognized as posing potential health risks to its users. Concern especially arises when fecal contamination occurs in drinking water, irrigation water, or water near populated areas. Although fecal deposits contain many different types of bacteria, screening for the presence of *Escherichia coli* has become a standard method for identifying waters contaminated with feces because identification of this species is relatively rapid and inexpensive (American Public Health Association 2005). *Escherichia coli* are facultative, anaerobic Gram-negative rods belonging to the large family of enteric bacteria. They are classified as mesophiles, which are associated with the intestinal tracts of warm-blooded animals and temperate and tropical ecosystems. The temperature growth range of *E. coli* is approximately 8 ° to 48 °C, with optimum growth ranging from 37 ° to 39 °C (Madigan 2002). Most strains of *E. coli* are harmless, but some types can cause illness. Furthermore, their presence is associated with an increased likelihood of other disease-causing bacterial pathogens including *Campylobacter* and *Salmonella* (Savill *et al.* 2001; American Public Health Association 2005; Vereen *et al.* 2007; Wu *et al.* 2011).

Despite our knowledge that *E. coli* can survive outside the digestive tract of warm-blooded organisms, the vast majority of research on *E. coli* and other fecal bacterial constituents has emphasized understanding their biochemistry, genomics, and host specificity in a clinical or controlled laboratory setting (Whitman *et al.* 2004; van Elsas *et al.* 2011). Much less is known about factors that influence the presence, abundance, persistence, and survival of *E. coli* and other fecal bacteria in non-host associated environments such as aquatic ecosystems (van Elsas *et al.* 2011). The

doi: 10.2166/wh.2016.020

need to better understand the fate and persistence of harmful bacteria in the environment and the physical and chemical factors that influence their life-history is highlighted by an increasing number of human infectious outbreaks that have arisen through environmental pathways (e.g., Soloman *et al.* 2002a, 2002b; Wachtel *et al.* 2002). Studies that have investigated *E. coli* and other fecal contaminants in open aquatic environments have typically been limited in their spatial and temporal scale. These studies are characterized by small sample sizes collected over brief time periods (1 year or less) and only during summer months (e.g., Lipp *et al.* 2001; Abulreesh *et al.* 2004; Warren *et al.* 2004; Davis *et al.* 2009). Furthermore, entities that are required by law to monitor fecal contamination, such as water treatment facilities, do not typically conduct ecological data analyses of the factors that influence survival and growth of the bacterial communities in those water sources. Routine site monitoring does provide an accurate snapshot in time of the bacteriological communities and potential contamination; however, it provides little in the way of long-term assessment of microbial community patterns or insight into the ecology of these communities.

Situated at the southern extent of the Great Plains Escarpment is a semi-arid region dotted with numerous small ephemeral wetlands known as playa lakes. Playa lakes are typically less than one acre in surface area, and it is estimated that there are more than 25,000 of these small wetlands in the region (Smith 2003). Lubbock, Texas, a city of approximately 250,000 people, is located within an area of particularly high playa lake density. Playa lakes that naturally occur within the landscape that became the city of Lubbock have been modified for storm-water drainage and now contain water year-round. These urban lakes attract large numbers of migratory Canada geese, *Branta canadensis*, which overwinter in this region from approximately October through March. Geese use the lakes during afternoon rest periods and for roost sites at night. During their 5-month stay, large quantities of fecal material are continually deposited in and around the lakes. These urban lakes, often located in city parks, are frequently used by humans and other domestic and wild animals; thus, contact with contaminated water can pose potential health risks for these organisms.

The purpose of this study was to investigate seasonal patterns in presence and abundance of *E. coli*, in six urban lakes in and around the city of Lubbock, Texas USA. Specific objectives of the study were to (1) document long-term seasonal patterns in abundance of *E. coli* in small urban lentic ecosystems over a continuous 3-year period, (2) identify environmental factors, including effects of migratory Canada geese and artificial aeration devices that might influence *E. coli* abundance, and (3) determine if patterns in *E. coli* abundance through time were similar for individual lakes. The results of our study provide needed information about environmental and ecological factors that influence seasonal abundance of fecal bacteria found in open environments, which could be used to help minimize the health risks of contaminated water.

MATERIALS AND METHODS

Six urban lakes in and around the city of Lubbock, Texas were chosen for sampling based on size, location, and the presence of artificial aeration devices. Study lakes ranged in size from approximately 1 ha to 9 ha in surface area (Shavlik 2000), and three of the lakes contained city-installed and operated fountains. Within each lake, three sampling locations were chosen across the longest axis of the lake, and the global positioning system coordinates of each of these locations were recorded for consistency of sampling over time. Each location was coded as either L (left), C (center), or R (right) from a pre-determined fixed point on the shore. At each location, instantaneous measurements of depth, temperature, dissolved oxygen, conductivity, salinity, pH, and turbidity were recorded. Two 1-L water samples were aseptically collected from a boat at a depth of between 12 and 18 inches beneath the surface at each location. Samples were collected from each lake and location once per month for 36 consecutive months. One litre of sampled water from each location was treated with 2 ml/L of concentrated sulfuric acid and transported to Advanced Analysis (Lubbock, Texas), a commercial analytical chemistry laboratory certified by the United States Environmental Protection Agency, for determination of ammonia, nitrate, and phosphate concentrations. The remaining litre of sampled water from each location was

transported to the Lubbock Christian University Microbiology Laboratory for microbial analysis.

The number of Canada geese was counted once per month at each lake on the day of water sampling during summer months. During winter months, when geese migrate to the area, counts were taken at each lake three times per month at approximately 10-day intervals. On occasions, when the number of geese at a lake were sufficiently large to preclude an accurate count while in the field, multiple photographs of the lake were taken from elevated positions and geese were counted in the laboratory from the best available digital image. The three winter counts from each month were averaged for statistical analyses. For all lakes and seasons, goose counts were taken between 11:00 am and 3:00 pm to coincide with their rest period between morning and evening feeding excursions.

Microbiological methods employed standard laboratory materials and procedures for isolating, culturing, and quantitating bacteria and measuring total solids (American Public Health Association 2005). Duplicate ten-fold dilution plates were prepared for each lake location sample using the standard plate count method to enumerate colony forming units (CFU) and thus determine bacterial loads. Peptone broth (1%) was used for dilutions, and Himedia™ Hi-Veg Plate Count Agar was used for plating. Plates were incubated at 25 °C for 10 days and then CFUs counted. Dilution blanks used for the standard plate counts were also used to inoculate duplicate chrome agar plates, which were incubated for 48 hours at 35 °C and counted. We used Coliscan Easygel® Chromagenic Agar, available from Micrology Labs, Goshen, Indiana, to determine coliform numbers indicative of fecal contamination and specifically *E. coli.* On chromagenic agar plates, *E. coli* forms blue colonies while other coliforms form red colonies. Blue colonies, indicative of *E. coli*, were transferred from the chrome agar plates to eosin methylene agar, a selective/differential agar medium suitable for culture and visual recognition of *E. coli*. After transfer to a second eosin methylene agar plate, isolates were then grown on tryptic soy agar plates and subjected to a battery of biochemical tests (indole, citrate, motility, H_2S production) to support their identity as *E. coli*. If all tests indicated the isolate was *E. coli*, the bacteria were stored on tryptic soy agar slants as reference material. Total dissolved solids were determined in the laboratory by evaporating and weighing 50-ml duplicate samples in aluminum pans until dry in a 95 °C oven for 48 hours.

Statistical analyses

All statistical analyses were conducted using SPSS version 22 (IBM Corp.). A one-way analysis of variance (ANOVA) was conducted for all variables collected from each lake to determine if any spatial gradient patterns existed between the left, center, and right sampling locations in each lake. For all other analyses, data were separated into a 'summer' season and a 'winter' season to control for effects of temperature on growth of bacteria. The summer season included samples taken during the months of May-October and the winter season included samples taken during the months of November-April. Samples taken in August 2011 were omitted from statistical analyses because they were taken just after a heavy rainfall and were not representative of the other 35 months of sampling.

For each lake and season, a total of nine (summer) and ten (winter) environmental variables were entered into a stepwise multiple linear regression model with a backward elimination search procedure to determine the most influential environmental variables predicting *E. coli* abundance. Variables for summer models were: pH, salinity, dissolved oxygen, water temperature, phosphate, ammonia, total solids, precipitation, and nitrate. Variables for winter models were the same as the summer season with the addition of goose count. A backwards elimination search procedure begins with all independent variables in the model, and a final model is determined by an iterative fitting process in which the least significant variable at each iteration is removed from the model. A final 'best' model is determined when no additional variables can be removed based on the *a priori* determined significance criteria. A significance level of 0.10 was used because of the exploratory nature of this study. The backwards elimination search procedure is preferred over forward or other search procedures when there is a small to moderate number of independent variables because the most influential variables begin in the model. In a forward stepwise selection procedure, the most influential variables are often left out of initial steps, leading to inflated mean square error values. The

backwards stepwise procedure also allows for an evaluation of each independent variable adjusted for all other variables before elimination of any variable (Mantel 1970; Kutner *et al.* 2004).

Assumptions of the multiple regression model were evaluated using standard procedures. Visual inspection of scatterplots indicated that the data for the dependent variable, *E. coli*, was not reasonably normally distributed. A log-transformation was conducted on this variable to normalize the data. Visual inspection of residual scatterplots indicated that all independent variables that were in each final model were reasonably normally distributed. Heteroscedasticity was assessed with the Brown-Forsythe test. All final models were non-significant ($P > 0.05$). Finally, multicollinearity that existed between predictor variables was ignored because studying specific relationships between predictor variables was not an objective of the present study and multicollinearity does not affect statistical significance or final model fit (Graham 2003). Plots were then created for the most frequently appearing variables in the final model to further investigate their effects on patterns of *E. coli* abundance.

To determine the similarities or differences among individual lakes and seasons, principal component analysis (PCA) was conducted using individual lakes as variables along with the *E. coli* data for each lake. Scree plot inspection revealed no more than two important components for each of the two season analyses. Therefore, component derivation was manually set at two in order to produce two-component plots for both seasons. For each run of the model, a Varimax rotation was used.

RESULTS

Escherichia coli was present in 75.6% of all individual samples over the 3-year study period. Presence of *E. coli* was only slightly higher in summer samples (78.4%) than in winter samples (73.15%). Across all lakes and sample dates, average *E. coli* was 482 ± 915 CFU/100 ml (Table 1). Mean abundance of *E. coli* in individual lakes was highly variable and ranged from 200 CFU/100 ml at Huneke in the summer season to 1,063 CFU/100 ml in Ribble during the summer season (Table 1). With only one exception (Maxey Lake during winter season), Huneke Lake contained the lowest abundance of *E. coli* while Ribble contained the highest abundance of *E. coli* across all 36 months of the study and for each season (Table 1).

Abundance of *E. coli*, although variable across lakes, was relatively stable within individual lakes for the duration of the study and only two clear patterns of abundance across lakes were discernable (Figure 1). First, there appeared to be a modest increase in *E. coli* in most lakes during the beginning of the winter seasons, particularly in October through January. The second across-lake pattern observed was two major spikes in *E. coli* abundance that occurred in the August 2011 and the June 2013 samples, which were collected just after major precipitation events (Figure 1).

Both visual inspection of graphs and results of one-way ANOVAs revealed that for all lakes and variables, there were no systematic differences between samples taken from the left, center, and right locations of each lake. Test *P*-values were all non-significant and ranged from 0.50 to 0.99. Therefore, in subsequent analyses, we averaged the

Table 1 | Mean abundance of *E. coli* (CFU/100 ml) for all lakes combined and individual lakes across all 36-months of sampling, and separated by summer and winter seasons

Lake	36-month mean	Summer mean	Winter mean
All lakes	482 ± 915 (0.0–6,667)	527 ± 1,011 (0.0–6,000)	440 ± 823 (0.0–6,667)
Elmore[a]	362 ± 544 (0.0–25)	361 ± 576 (0.0–2,500)	360 ± 529 (0.0–2,233)
Huneke[a]	257 ± 376 (0.0–1,933)	200 ± 237 (0.0–10.0)	311 ± 473 (0.0–1,933)
Patterson[a]	435 ± 787 (0.0–4,167)	494 ± 975 (0.0–8,333)	378 ± 580 (0.0–2,500)
Jennings	395 ± 708 (0.0–3,833)	444 ± 924 (0.0–3,833)	349 ± 440 (0.0–1,533)
Maxey	423 ± 922 (0.0–5,333)	598 ± 1,268 (0.0–5,333)	257 ± 355 (0.0–1,333)
Ribble	1,023 ± 1,547 (0.0–6,667)	1,063 ± 1,480 (300–6,000)	984 ± 1,649 (0.0–6,667)

[a]Lakes with fountains.

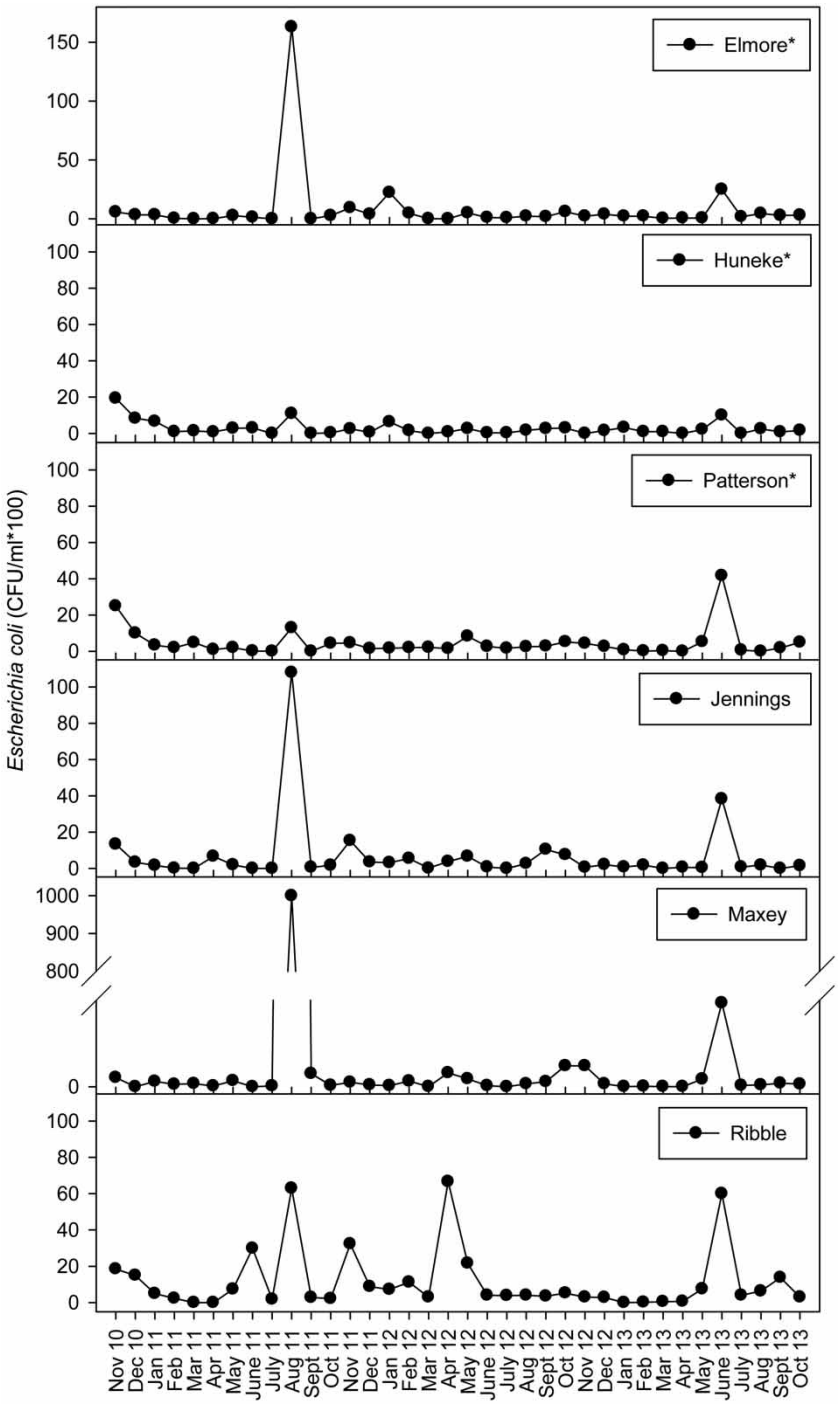

Figure 1 | Mean abundance (CFU/100 ml) of *Escherichia coli* for individual lakes. Asterisks indicate lakes with fountains.

three samples taken from each lake on each date. Thirty-six-month and seasonal means with ranges of selected study variables are presented in Table 2.

Final regression models for the summer season explained between 39.6% (Maxey) and 80.6% (Ribble) of the total variation in *E. coli* abundance. Of the nine

Table 2 | Means for selected variables across all six lakes and separated by seasons

Variable	36-month mean	Summer mean	Winter mean
pH	7.73 ± 0.76 (6.1–9.92)	7.61 ± 0.83 (6.10–9.92)	7.86 ± 0.69 (6.70–9.43)
Dissolved oxygen, mg/L	10.18 ± 3.51 (0.52–19.14)	7.78 ± 2.25 (0.52–13.95)	12.44 ± 2.95 (5.19–19.14)
Total solids, g/L dry weight	0.024 ± 0.012 (0.01–0.08)	0.023 ± 0.013 (0.009–0.08)	0.025 ± 0.01 (0.01–0.053)
Water temperature, °C	13.32 ± 6.69 (0.14–28.08)	18.42 ± 4.54 (6.3–28.1)	8.50 ± 4.50 (0.14–17.93)
Goose number	–	–	325.0 ± 484.6 (0.0–2,627)

predictor variables assessed, pH was a significant variable in four of the six final models (Table 3). Dissolved oxygen was also a significant predictor variable in four of the six final summer models. Salinity and water temperature were significant predictor variables in three of the six models for summer. All other variables were present in less than half of the models, although all nine variables assessed were represented in at least one of the final summer models.

Final regression models for the winter season explained between 17.4% (Jennings) and 92.4% (Ribble) of the total variation in *E. coli* abundance. Of the ten predictor variables assessed in winter, pH was a significant variable in five of the six final models (Table 4). Total solids was a significant variable in three of the six models. Of the other variables, all

Table 3 | Predictor variables in final regression models for each lake sampled during summer, coefficient value, significance of each variable, and proportion of variation in *Escherichia coli* explained by each final model

Lake	Variables in final model	Coefficient value	Significance (*t*)	Final model adjusted R^2
Elmore[a]	pH	0.469	0.001	0.675
	Dissolved oxygen	−0.159	0.03	
	Salinity	−7.205	0.009	
	Water temperature	−0.044	0.045	
Huneke[a]	Precipitation	0.137	0.009	0.424
	Dissolved oxygen	−0.159	0.014	
	Phosphate	3.04	0.031	
	pH	0.220	0.059	
	Water temperature	−0.057	0.059	
Patterson[a]	pH	−0.194	0.008	0.692
	Salinity	8.41	0.014	
	Dissolved oxygen	0.106	0.049	
	Phosphate	3.156	0.074	
	Total solids	−32.31	0.095	
Jennings	Precipitation	0.129	0.018	0.429
	Ammonia	1.166	0.045	
	Water temperature	−0.40	0.081	
	Total solids	0.129	0.090	
Maxey	Nitrate	7.088	0.003	0.396
Ribble	pH	0.252	0.002	0.806
	Salinity	−2.26	0.039	
	Dissolved oxygen	−0.107	0.064	

[a]Lakes with fountains.

Table 4 | Predictor variables in final regression models for each lake sampled during winter, coefficient value, significance of each variable, and proportion of variation in *Escherichia coli* explained by each final model

Lake	Variables in final model	Coefficient value	Significance (*t*)	Final model adjusted R^2
Elmore[a]	pH	0.143	0.004	0.424
	Water temperature	−0.091	0.006	
	Precipitation	0.122	0.048	
	Phosphate	−2.587	0.062	
Huneke[a]	pH	0.248	<0.001	0.650
	Total solids	−81.235	0.002	
	Water temperature	−0.033	0.067	
Patterson[a]	pH	0.179	0.001	0.560
	Dissolved oxygen	−0.072	0.021	
	Nitrate	−3.884	0.056	
Jennings	Total solids	23.812	0.043	0.174
Maxey	Dissolved oxygen	−0.101	0.002	0.470
	Goose count	0.001	0.004	
	pH	0.140	0.006	
Ribble	Goose count	0.003	<0.001	0.924
	Total solids	110.07	<0.001	
	Precipitation	0.485	<0.001	
	pH	−0.425	<0.001	
	Ammonia	−0.646	0.004	
	Nitrate	−6.541	0.048	

[a]Lakes with fountains.

were present in less than half of the models except for salinity, which was the only variable not represented in any of the winter models.

As temperatures declined during the winter months, the number of geese in and around the study lakes increased (Figure 2). Peak goose abundance occurred each year at the same time as the coldest water temperatures were observed. The overall mean winter season goose number across all six lakes was 325 geese per lake (Table 2). Although means are presented, it was not uncommon for

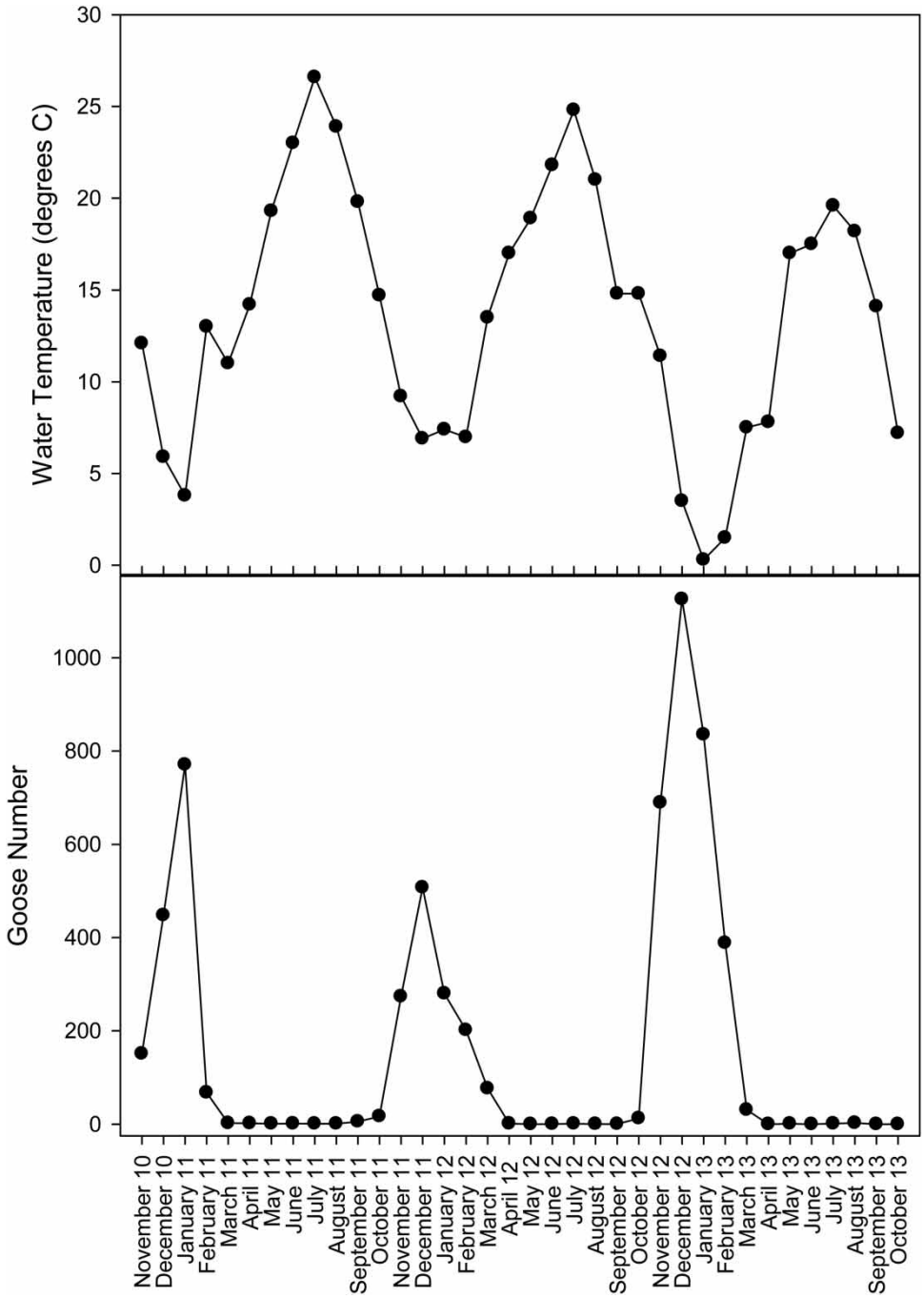

Figure 2 | Monthly mean water temperature and mean goose number across all six study lakes.

goose numbers on individual lakes to exceed several thousand birds. The highest count on an individual lake during our study was 6,372 geese, counted on Maxey Lake on November 29, 2012.

Differential seasonal abundance of *E. coli* in lakes with fountains versus those without was observed. In the summer season, *E. coli* abundance was greater in lakes without a fountain (Jennings, Maxey, Ribble) than in lakes with a fountain (Elmore, Huneke, Patterson) in 15 out of 17 months (Figure 3). However, in the winter season, greater abundance in *E. coli* occurred equally in lakes with fountains and without fountains (Figure 4).

Results of the principal components analyses for the summer season revealed one main component vector and one minor component vector. Loadings for Component 1 explained 72.6% of the variation in lakes based on *E. coli* abundance and Component 2 explained 12.1% of the variation for a total of 84.7% (Figure 5). Results of the principal components analyses for the winter season revealed two component vectors. Loadings for Component 1 explained 54.2% of the variation in lakes based on *E. coli* abundance and Component 2 explained 20.0% of the variation for a total of 74.2% (Figure 6). Spatial

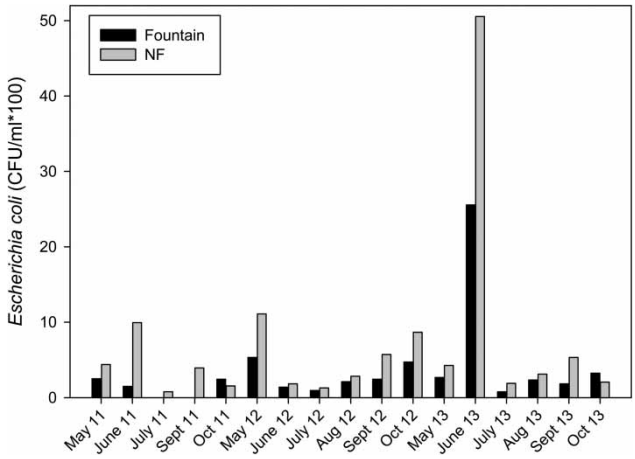

Figure 3 | Monthly mean *Escherichia coli* abundance (CFU/100 ml) in lakes with and without fountains in the summer season.

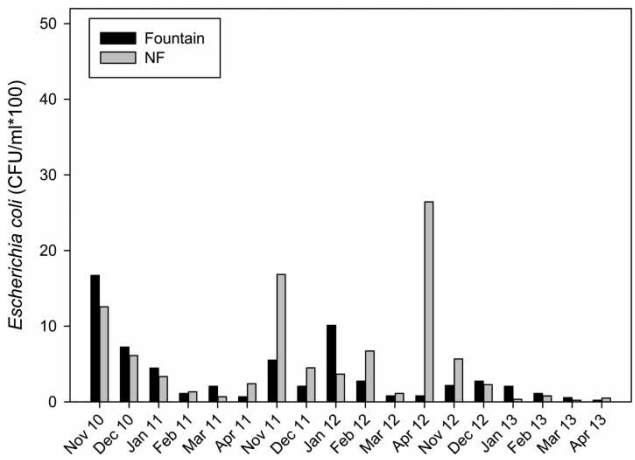

Figure 4 | Monthly mean *Escherichia coli* abundance (CFU/100 ml) in lakes with and without fountains in the winter season.

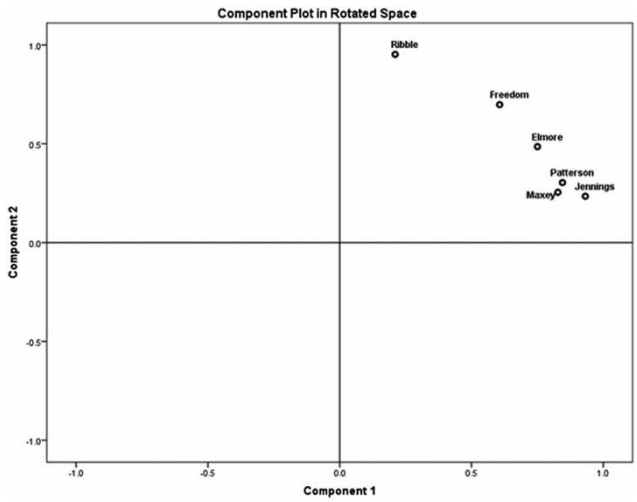

Figure 5 | Principal components analysis plot for study lakes in the summer season. Predictor variable is $\log_{10}$ of *Escherichia coli* abundance.

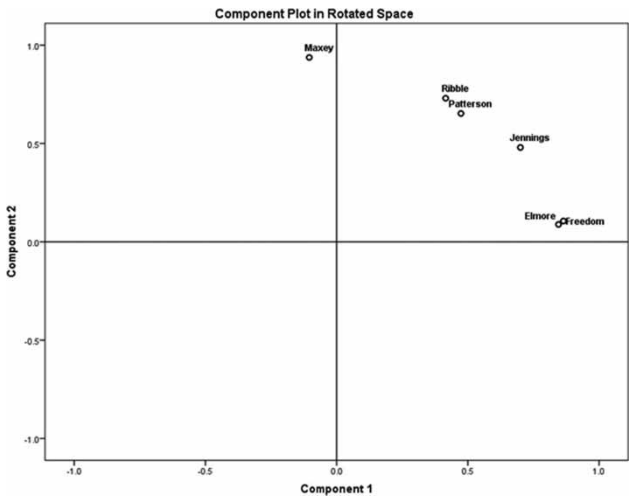

Figure 6 | Principal components analysis plot for study lakes in the winter season. Predictor variable is $\log_{10}$ of *Escherichia coli* abundance.

separation of lakes in both seasons indicated that patterns in *E. coli* are variable among individual lakes. Overall, lakes were clustered closer together during the summer season than during the winter season, and were arranged differently in space between summer and winter. With only one exception (Maxey), the spatial arrangement of lakes for the largest component in the winter season reflected the average number of Canada geese on each lake.

DISCUSSION

Escherichia coli is present in the urban lakes of Lubbock, Texas, year-round and typically exceeds the established bacterial loading threshold for recreational waters of 126 CFU/100 ml (United States Environmental Protection Agency 2003). Abundance of *E. coli* within individual lakes was relatively stable throughout the duration of the study and was unexpectedly similar between seasons. The modest increase in *E. coli* abundance, observed in most lakes during the beginning of the winter season, coincided with the arrival of migrating Canada geese to the area. Although water temperatures are declining during this time period, the region does not typically experience its coldest temperatures until January and February (Figure 2). Therefore, we suspect that the observed increase in *E. coli* abundance is due to the initial arrival of the geese and associated abrupt increase in bacterial deposits into the lakes prior to temperatures becoming too cold for the bacteria to readily reproduce.

The ability of regression models to explain the abundance of *E. coli* based on environmental factors was quite variable for individual lakes, ranging from less than 20% to over 90%. Models explained an average of 56.7% and 53.3% of variation in *E. coli* abundance for summer and winter seasons, respectively. The most frequent environmental variable that occurred in final regression models for both seasons in our study was pH. With only one exception, regression models indicated a positive correlation between *E. coli* and pH. The variable pH has been reported as a primary factor in determining *E. coli* abundance and survival in many studies. However, reports documenting the relative positive or negative effect of high or low pH on the abundance, growth, and survival of the bacterium have been inconsistent. Review of the literature suggests that the effect of pH on *E. coli* may depend on both the particular strain of bacterium as well as the type of environment and location in which it is found (Curtis *et al.* 1992; Lin *et al.* 1996; Davies-Colley *et al.* 1999; Sang *et al.* 2000; Bergholz & Whittam 2007; van Elsas *et al.* 2011; Wang *et al.* 2014; Ouali *et al.* 2015). These studies and others that have identified a specific directional relationship between indicator bacteria and pH have most often been conducted in non-natural and extreme environments, such as simulated gastric environments, wastewater ponds, or microcosms where observed pH was generally less than 3.0 or greater than 8.0.

Despite evidence that pH is of critical importance to the survival and abundance of *E. coli*, there has been extremely limited study of the effects of environmental variables in natural, non-host associated aquatic environments. One study (Beutel & Larson 2015) recently reported a decrease in fecal indicator species when high levels of pH (>8.5) and dissolved oxygen (>20 mg/L) were achieved in pond water passed through biofilters, but did not document long-term seasonal abundance in their associated open study system. The general positive relationship between *E. coli* and pH reported by the present 3-year study is among the first to document the long-term seasonal relationship between *E. coli*, pH, and other physical and chemical variables in an open aquatic environment where values of pH were not at extremes. Therefore, observed influences of pH in these systems may not be directly comparable to other systems, strains, and locations that have been studied. Additional study is needed to better understand these relationships in natural environments.

Dissolved oxygen was also an important environmental factor during the summer season, as indicated by regression models. As a facultative anaerobe, *E. coli* should not be precluded from growing in an environment solely due to the presence or absence of oxygen. However, our analyses revealed an inverse relationship between *E. coli* abundance and oxygen concentration during warmer months, when extremes in oxygen concentration due to algal photosynthesis during the day and consumption at night are most likely to be observed. The severe drought that occurred in Texas during 2011 created receding water conditions, and extremely low dissolved oxygen values were recorded in early morning samples on several sampling occasions.

Thus, it appears that oxygen is somehow involved as a key explanatory factor in *E. coli* abundance.

We were unable to directly compare relationships between dissolved oxygen and *E. coli* across lakes, because we sampled lakes in the same order on each sampling date and cannot account for the differential ability of water to hold oxygen at different temperatures. However, even without accounting for temperature, the lakes in our study that contained a fountain had substantially higher oxygen levels. The average oxygen concentration during summer for lakes with fountains was 8.35 mg/L compared to 6.92 mg/L for lakes without fountains. This, coupled with the clear result that lakes with fountains had a lower abundance of *E. coli* than those without fountains, provides strong evidence that oxygen concentration plays an important role in determining the abundance of *E. coli* in natural systems.

Methods for inducing inactivation of pathogenic bacteria in wastewater applications have been extensively studied. Many of these studies have focused on sunlight (solar ultraviolet radiation) as an effective and economical method of bacterial inactivation, and have frequently provided information regarding the synergistic effects of sunlight and other physical and chemical variables (Ouali *et al.* 2015). Among environmental variables, dissolved oxygen and pH have been most frequently implicated in bacterial inactivation. For example, Curtis *et al.* (1992) reported that pH was a major factor in achieving bacterial inactivation, and concluded that the ability of solar energy to damage fecal coliforms was completely dependent upon the presence of oxygen. Likewise, Davies-Colley *et al.* (1999) and Beutel and Larson (2015) reported that inactivation of *E. coli* increased strongly with increasing dissolved oxygen.

The results of our study combined with information gleaned from the literature regarding solar inactivation of bacteria and its relationship to dissolved oxygen and pH lead us to conclude that solar radiation is likely the primary control mechanism for *E. coli* in the urban lakes we studied, and that the extent to which solar activity decreases bacterial abundance is influenced by the relative presence of dissolved oxygen and pH. Four major lines of evidence support this conclusion. First, all study lakes are open bodies of water with little to no canopy cover to shade the surface of the water. Second, the city of Lubbock receives the equivalent of 263 days of sunshine per year (National Oceanic and Atmospheric Administration 2005). Third, lakes with fountains, and therefore higher dissolved oxygen concentrations, consistently showed lower summer bacterial levels. Indeed, Huneke Lake had the lowest *E. coli* levels of any lake and it is the only study lake to have multiple fountains (three in total). Finally, all of our samples were collected from between 12 and 18 inches below the surface of the open water, which would be within the photic zone subject to solar irradiation.

The many urban lakes and their associated parks in and around the city of Lubbock, Texas, provide residents with primary locations for frequent outdoor recreational activities such as walking/jogging, enjoying time with pets, and fishing. The mild climate also allows residents to use these areas on a nearly year-round basis. High levels of fecal indicator bacteria in these systems (Moorhead *et al.* 1998; this study) and recent identification of harmful (Westerfield 1996; Adams *et al.* 2015) and antibiotic resistant (Warren *et al.* 2004; Rutherford unpublished data) strains of bacteria do put people at risk when frequenting these recreational areas. A very practical application of our analyses would be to recommend that cities or appropriate managing entities install fountains in urban impoundments such as the ones studied herein. Not only do fountains serve as an aesthetic enhancement to the lake and the experience of those using it, they would act to lower fecal bacterial counts, and therefore mitigate the risk of exposure and sickness to the public.

All nine of the variables included in our regression analyses were significant in at least one final model for the summer season, and nine out of ten variables were significant in at least one final model during the winter season. Furthermore, two is the maximum number of significant variables shared by the same lake between seasons. This result suggests that the most influential factors affecting the abundance of *E. coli* vary in both time and space. Support for this conclusion is further provided from the results of our PCA analysis. Lakes were grouped more closely during the summer than during winter, indicating that lakes shared more similarity between factors influencing *E. coli* abundance in the summer season, though significant variation did exist for both seasons. Additionally, individual

lakes showed little similarity in their spatial positioning between seasons. For example, Maxey Lake occupies the position of highest loading of Component 2 in the winter PCA analysis, but in summer occupies a position much more strongly along the axis of Component 1. Many of the other lakes also occupy dissimilar positions between seasons. Such a result would suggest an extremely complex nature of the relationship between biotic and abiotic portions of the environment in individual lakes, and that the most influential factors for *E. coli* abundance often differ between individual lakes at any given time.

One of the major limitations of PCA is that it is not possible to identify the exact variable constituents of the component vectors after the variable reduction routine is completed. Any conclusion of causal patterns in the spatial arrangement of the results of PCA must be recognized as speculative. However, it is noteworthy that during the winter season, the spatial arrangement of lakes along Component 1 of the PCA, with only Maxey Lake as an exception, reflected the average number of geese using the lakes. We could identify no other clear potential causal patterns from the PCA analyses.

It is hypothesized that the large number of Canada geese that migrate to the area each fall is a primary source of *E. coli* in the lake water. Although other potential sources of fecal bacteria do exist in these environments, including resident birds, dogs, cats, and other wildlife, a look at the soles of your shoes after a winter's walk through one of the parks makes it understandable why the geese would be implicated. Indeed, Kullas *et al.* (2002), studying the quantity of feces deposited in an urban park, estimated that for a one-mile walk in a park heavily utilized by geese, a person would likely encounter 424 pieces of feces. However, the presence of pathogenic forms of *E. coli* and other bacteria in goose feces has been reported to be quite low (Hussong *et al.* 1979; Kullas *et al.* 2002), and it is not known to what extent feces deposited on the ground surrounding a lake affect fecal loading within the water itself, although one would expect it to be a potential factor. Several possibilities exist regarding the effect of the geese on the aquatic bacterial community. First, because they come to the study area during the coldest months, bacteria present in their fecal deposits may have no effect, being completely mitigated by temperature. Alternatively, fecal bacteria may remain present in low numbers due to the cold, but when temperatures warm in the spring, bacteria previously deposited by the geese become active. In this scenario, geese are a major source, but there is a lag time until temperatures warm to within the bacteria's optimal growth range. Molecular techniques will likely be required to definitively link migratory geese to *E. coli* present in the open water of the lakes.

The present study also raises additional questions and identifies information gaps regarding the nature of the presence, location and abundance of *E. coli* in urban lakes. Clearly, *E. coli* is present in or within close proximity to the lakes as indicated by the two instances in our study (August 2011 and June 2013 samples), where extremely high abundances were observed shortly after rainfall events large enough to create runoff. On these two dates, samples were taken just after single rainfall events of more than 6 cm (2.4 inches). Equivalent and even greater total monthly amounts of rainfall occurred during the study, but were spread over several small precipitation events. Thus, it appears that heavy rainfall somehow affects the amount of *E. coli* in the water column. One possible explanation is that bacteria could be in greater abundance in lake-bottom sediments that get stirred up during runoff events, or perhaps they are in greater abundance in soil surrounding the lakeshore than in open water. Such a conclusion seems reasonable, if solar inactivation indeed occurs more readily in the upper portions of the water column than in deeper portions of the lake as was observed by Maiga *et al.* (2009). Our own preliminary findings of spatial distribution of *E. coli* extending from the open water substrate onto the shore indicates a high presence within the soils (Hutton, unpublished data).

CONCLUSIONS

Escherichia coli is present in the urban lakes of Lubbock, Texas year-round and is in high enough concentrations to warrant concern. Although several environmental variables play a role, pH, and dissolved oxygen, in concert with solar irradiation, appear to be the most important variables determining the abundance of fecal bacteria in the lake ecosystems. There is a high likelihood that the primary source of fecal contamination in the lakes comes from the

Canada geese that migrate to the area each winter, although additional studies will need to verify this supposition. Increasing the dissolved oxygen concentration of the lake water, particularly in the summer months, through the use of fountains or other aeration devices, is a relatively simple method to reduce the abundance of *E. coli* in the lakes. The numerous and complex interactions that occur among environmental variables in dynamic open aquatic systems makes it challenging to fully understand the ecology of an organism such as *E. coli*. Information regarding the sources, growth, persistence, and control of urban bacterial communities will only continue to grow in importance. This exploratory study, having revealed some of the most likely important relationships, will provide guidance to future investigations as they seek answers to more specific questions about bacterial communities in urban aquatic systems.

ACKNOWLEDGEMENTS

Direct and in-kind funding for this project was graciously provided by M. Rodriguez of Advanced Analysis, Inc. Without this support, the project would not have been possible. We thank B. Baker and D. Neel for conducting goose counts for the project. We thank M. Valles, S. McBride, N. Rutherford, R. Roper, and N. Kemei for significant assistance in microbiological preparation and quantification of samples. We thank C. Tomlinson, M. Valles, B. Ho, C. Anderson, B. Marsing, B. Baker, and G. Howard for signification assistance in the collection of field samples and chemical analyses. We thank Dr P. Baumgardner for statistical advice. Dr D. Swartz provided a helpful editorial review of the manuscript.

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First received 14 January 2016; accepted in revised form 8 July 2016. Available online 4 August 2016