Using wildlife activity and antibiotic resistance analysis to model bacterial water quality in coastal ponds

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Using wildlife activity and antibiotic resistance analysis to model bacterial water quality in coastal ponds.

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Abstract

Models that help predict fecal coliform bacteria (FCB) levels in environmental waters can be important tools for resource managers. In this study, we used animal activity along with antibiotic resistance analysis (ARA), land cover, and other variables to build models that predict bacteria levels in coastal ponds that discharge into an estuary. Photographic wildlife monitoring was used to estimate terrestrial and aquatic wildlife activity prior to sampling. Increased duck activity was an important predictor of increased FCB in coastal ponds. Terrestrial animals like deer and raccoon, although abundant, were not significant in our model. Various land cover types, rainfall, tide, solar irradiation, air temperature, and season parameters, in combination with duck activity, were significant predictors of increased FCB. It appears that tidal ponds allow for settling of bacteria under most conditions. We propose that these models can be used to test different development styles and wildlife management techniques to reduce bacterial loading into downstream shellfish harvesting and contact recreation areas.

Keywords: Fecal coliform bacteria; antibiotic resistance; land cover; regression modeling

1. Introduction

The coast of the southeastern USA is changing. Large tracts of land once used for silviculture are being converted to residential, resort communities. In many cases this has led to decreased water quality even though best management practices (BMPs) are employed. Development in the coastal southeast typically includes residential homes, golf courses, parks, and light commercial establishments. In addition to increased impervious surface, these land uses all include maintained lawns, deciduous trees, and ornamental plants that are attractive to wildlife. In many cases, overall wildlife carrying capacity increases compared to climax forest or monoculture of conifer trees, and wildlife is often pushed to the wetland margins and marsh hammocks adjacent to estuarine waterways to avoid human activity (SCDNR, 2004). Consequently, bacterial water quality has been impaired even though human and domestic animal sources were well managed.

Increased impervious surface and channelization that accompanies development increases non-point source pollution of coastal waters (Corbett et al., 1997; Holland et al., 2004), and coastal retention ponds may play a role in the transport of contaminants to estuaries. In coastal South Carolina, there are over 8000 retention ponds and the number increased by approximately 13 percent per year from 1994 to 1999 (Siewicki et al., 2007). These ponds are designed to retain rainfall up to a certain point, above which they overflow into estuaries and coastal waters. Some BMP stormwater ponds are designed to exchange water with the estuary at high tide, leading to twice daily pond discharge in the absence of rainfall. In these tidal retention ponds, rainfall that coincides with ebbing tides can cause increased loading of non-point source runoff constituents such as fecal coliform bacteria (FCB).

Fecal coliform bacteria are an important non-point source runoff contaminant as they are the microbiological indicator organism used to classify areas used for shellfish harvesting and contact recreation in many states. The contamination of natural waters with untreated fecal material may indicate the presence of pathogenic bacteria and viruses such as *Salmonella spp.* and the Hepatitis A virus, both of which can cause severe gastroenteritis (USEPA, 1986). Exceedences of microbial water quality guidelines are relatively common even when the best available controls are employed. Common sources of fecal pollution include faulty septic systems, livestock operations, and pets. Local wildlife is also an important source of FCB. For example, a single deer, raccoon, duck and goose have been shown to deposit up to $5x10^8$, $1x10^8$, $1x10^{10}$, and $5x10^{10}$ colony forming units (cfu) per day, respectively (Hanson, 1997; Mara, 1974; USEPA, 2001; Yagow, 2000). Models that provide information about sources of FCB in coastal waters could be important tools for water quality management.

Improved understanding of sources of FCB will lead to more effective management strategies. There is substantial microbial source tracking (MST) literature describing attempts to determine the relative contributions of anthropogenic and non-anthropogenic sources of fecal pollution in environmental waters (Choi et al., 2003; Griffith et al., 2003; Parveen et al., 2001; Parveen et al., 1997; Wiggins, 1996). An increasing number of studies suggest wildlife is an important source of FCB. For example, Somarelli et al. (2007) showed wildlife were the primary source of FCB in lakes surrounded by agriculture, including dairy farms. They used genetic fingerprinting techniques to determine that geese and deer contributed about 70 percent of the *E. coli* in these lakes. Whitlock et al. (2002) used antibiotic resistance analysis (ARA) to identify sources of FCB in an urban watershed. They showed that wildlife were the primary source of FCB when concentrations were elevated above background levels. Using ARA of enterococci in Huntington Beach, CA, bird feces was found to be the source of surf-zone contamination under some conditions (Choi et al., 2003).

The study presented here builds upon our previously published work (Siewicki et al., 2007) by including wildlife activity prior to sampling to better predict FCB levels in coastal retention ponds. We provided models that predicted total as well as presumed wildlife FCB levels in coastal ponds using a combination of ARA, physical-chemical, environmental, and land cover variables. The objective of this study was to use similar variables along with photographic wildlife monitoring to model non-anthropogenic sources of FCB in coastal retention ponds. To date, we know of no other studies that have combined wildlife monitoring, land cover data, and antibiotic resistance analysis in an effort to model FCB concentrations in environmental waters. We propose that these refined models can be used to test both development and wildlife management strategies to protect the quality of water in ponds discharging into estuaries where both shellfish harvesting and contact recreation are allowed.

2. Methods

2.1 Study sites

Nine study sites on Kiawah Island, South Carolina (Fig. 1) represented the primary style of development expected to increase along the southeast coast. Specifically, all were located in a coastal golf resort community that utilizes retention ponds as Best Management Practices (BMPs) for stormwater management. Sites were selected to include a range of predominant land cover types. Pond 61 drained into Pond 56 which then drained into a tidal creek. All other sites drained directly into tidal creeks. Pond 35 was completely surrounded by golf course with elevations ranging from 1 to 4 m above mean sea level. Ponds 1, 74, 75, 93 and 94 were surrounded by a combination of golf course and residential areas. Ponds 56 and 61 were in a residential area with elevations of 1 to 2 m above mean sea level. Pond 96 was surrounded almost exclusively by wooded land cover. Finally, additional sample sites at the Kiawah Sewage Treatment Plant (KSTP) included untreated, pre-chlorinated sewage (KSTPPreChlor), as well as a retention pond (KSTPHoldPond) containing a mixture of secondary treated waste, well, and potable water used for irrigation of Pond 1 and Pond 75 catchment areas.

Catchments were manually delimited *in situ* using differential GPS after mapping all drainage pipes and grates. Sewage treatment plant sites were not affected by adjacent land cover, therefore catchments were not delimited for KSTP sites. Catchment size ranged from 2.4-45.2 hectares (Table 1). Land classes for all catchments were defined from combinations of aerial photography and *in situ* (differential GPS) measurements. All spatial data layers were processed in a GIS (ArcView 3.x or ArcMap 8.x or 9.x, ESRI, Redlands, CA, USA) and the amounts and locations of specific land classes or other aspects of topology were associated to contaminant concentrations. Fifteen land use and land cover types were classified and ground-truthed within the study areas. Building footprints were obtained from scanned engineering drawings, edited, and registered to parcel maps to create a detailed impervious surface data layer. To assess the accuracy of our land cover classification method, 30 points were randomly selected for each land cover type in each catchment and verified *in-situ*. Accuracies for pond sites ranged from 82.9-94.8% (mean = 90.8%).

2.2 Sample collection

Samples were collected and measured for FCB at Ponds 56 and 61 beginning in June, 2002. Samples were collected and measured for FCB at all other sites beginning in February, 2005. Samples were collected at four to six week intervals through January, 2006 in sterile Nalgene™ bottles three to four hours after high tide and kept on ice while transported to the laboratory where they were immediately analyzed (<six h). Salinity, water temperature, pH and specific conductance were measured near the surface with either an YSI Environmental model 63 or YSI 556 handheld meter (Yellow Springs, Ohio, USA) at the time of sampling.

2.3 Water Quality Analyses

Fecal coliform bacteria concentrations were measured using the membrane filtration method (APHA, 1989). All plates were incubated in a water bath for 24-h (± 4 h) at 44.5 °C. After the incubation period, each plate was inspected for dark blue colonies. Each dark blue colony was counted as one colony forming unit (cfu). Data are expressed as cfu per 100 ml unless otherwise specified. Antibiotic resistance analysis was used to measure the resistance of *E. coli*, which were identified using the hydrolysis of methylumbelliferyl-β-D glucoronide (MUG, Difco Laboratories, Detroit, MI, USA) assay. Up to ten *E. coli* isolates from each sample were measured for antibiotic resistance. *Staphylococcus aureus* ATCC 25923 and *Psuedomonas aeruginosa* ATCC 27853 were used as control isolates.

Two methods were used to measure antibiotic resistance during this study. From June, 2002 through June, 2003, a previously published technique was used which measured resistance to single concentrations of 10 antibiotics (Parveen et al., 1997). Beginning in June, 2003, an improved ARA method was used that measured resistance to multiple concentrations of 26 antibiotics on a 96-well plate (Table 2). Plates were pre-made with dehydrated antimicrobics in each well. We used MicroScan® Research Use Only panels (Dade Behring Inc., W. Sacramento, CA). Bacteria were transferred from an 18-24 hour nutrient agar plate to 3 mls of autoclaved deionized water to a turbidity equivalent to a 0.5 McFarland Barium Sulfate turbidity standard. Then 0.1 ml of the sterile water/bacteria suspension was transferred into 25 mls of cation adjusted Mueller Hinton broth and inoculated the 96-well panels. Panels were incubated for16-20 hours at 37° C. An isolate was considered resistant if it grew at the highest concentration of a drug.

2.4 Estimating Wildlife Activity

To quantify shorebird and waterfowl activity within the watersheds, digital cameras were deployed for four days prior to sampling. Nikon Coolpix 8700 cameras (Tokyo, Japan) coupled to a Harbortronics Digisnap 2000 electronic shutter release (Gig Harbor, Washington, USA) were deployed on the edge of the ponds to photograph a series of five pictures (spaced 15 minutes apart) at dawn, midday and dusk. To estimate terrestrial wildlife, we adapted a previously used technique (Jacobson et al., 1997) utilizing motion sensitive, infrared illuminated trail cameras (Highlander Sports, Inc., Huntsville, Alabama, USA) deployed over shelled corn bait and a single furbearer animal attractant scent wafer (Pocatello Supply Depot, Pocatello, Idaho, USA). All digital photographs were examined and the highest number of each species in each picture each day was recorded. For deer, individual types (collared, antlered, non-antlered, or fawn) were recorded and summed. Image resolution was occasionally insufficient to identify animals to species, and the best possible animal identification was performed based on image quality. For wildlife activity, the average daily maximum for each type of animal per deployment was used.

2.5 Weather and tide measurements

A Li-Cor Biosciences (Lincoln, NE, USA) LI-1400 weather station was maintained approximately midway between the sites on Kiawah Island. Rainfall, temperature, wind speed, wind direction, relative humidity, solar irradiation, and soil temperature were continuously recorded. Tide was monitored at two locations at Kiawah using Global model WL15X water level loggers (Global Water, Inc., Gold River, CA, USA).

2.6 Data analysis

Bacterial isolates showing no drug resistance (MARnegFCB) were assumed to be primarily from wildlife sources for this study, based on the assumption that the proportion of antibiotic resistant FCB is equal to the proportion of tested *E. coli* from the same sample. For example, if the total cfu concentration at a site was 1000 per 100 ml and 20% of the isolates tested were antibiotic resistant then it was assumed that the

MARnegFCB concentration for this sample was 80% or 800 cfu. This was the response variable in our MARnegFCB models. Total cfu concentration was the response variable in the TotalCfu model.

Linear regression modeling was used to develop compact predictive models for microbial contaminants using combinations of land cover, antibiotic resistance, meteorological data, tide, and wildlife activity prior to sampling. Bootstrapping was used to further evaluate the regression models and provide confidence intervals of regression coefficients. Data were re-sampled by bootstrapping 1000 to 100,000 times. Correlation analysis (Pearson) was done to further characterize relationships among variables. All statistical tests were done in SAS 8.2 or 9.1 (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1 Fecal Coliforms in Coastal Ponds

A total of 202 water samples were analyzed throughout this study. Fecal coliform bacteria concentrations ranged from 0-710 CFU/100 ml in ponds and 2-46, 000 CFU/100 ml in KSTPPreChlor and KTSPHoldPond. Average FCB levels (± SE) were 66.2 ± 10.9 for ponds and 7, 345.3 ± 3, 420.6 for KSTPPreChlor and KTSPHoldPond. Among ponds, mean FCB concentrations were highest in Pond 1 and lowest in Pond 94 (Table 3).

3.2 Antibiotic resistance analysis

During the period of June 2002 through June 2003, wildlife activity was monitored within the watersheds of Ponds 61 and 56 while water samples were measured for antibiotic resistance using the 10-antibiotic method of Parveen et al. (1997). Beginning in June, 2003 we began using the 26-drug method described above. The MARnegFCB and total FCB concentrations from both time periods were not significantly different from one another ($p<0.05$; t-test), therefore data from both time periods were combined and analyzed.

A total of 1387 *E. coli* isolates were tested for antibiotic resistance throughout this study. Multiple antibiotic resistant bacteria were found at all sites on multiple occasions. Resistance was greatest at the KSTPPreChlor and KSTPHoldPond sites, followed by Pond 74 and Pond 1, and lowest at Pond 93 and TCPE (Table 3). Among retention pond sites, overall percentage of *E. coli* resistant to one or more drugs ranged from 5-24%, compared to 42-45% for the sewage treatment plant (Table 3). Among drugs, the most frequently observed resistance was to the Beta-Lactam drugs penicillin and ampicillin, while we seldom observed resistance to aminoglycosides amikacin and apramycin (Table 2).

3.3 Wildlife Activity

A total of 11, 080 trail camera images and 7, 818 pond camera images were analyzed during this study. The most commonly observed terrestrial wildlife included white tailed deer (*Odocoileus virginianus*) and raccoon (*Procyon lotor*). Less frequently observed species included gray squirrel (*Sciurus carolinensis*), bobcat (*Lynx rufus*), and grackle (*Quiscalus spp.*). The most commonly observed waterfowl and shorebird species included coots (*Fulica americana*), hooded mergansers (*Lophodytes cucullatus*), mallards (*Anas platyrhynchos*), great blue herons (*Ardea herodius*), and egrets (*Egretta alba*, *Egretta thula*).

3.4 FCB pond models and correlations

Three data points were found to be outliers, and one data point was treated as a leverage point. These four data points were excluded from analysis. Abbreviations and descriptions of model terms are in Table 4. The models were:

$$FCB = 3.9 * \left(e^{(0.0588\alpha + 2.82\beta - 4.12\delta + 22.3\varepsilon + 0.0000119\varphi - 0.816\gamma)}\right) - 1 \quad (1)$$

$$MARnegFCB = 4.8 * \left(e^{(0.0493\alpha + 3.81\beta + 4.21\chi + 19.8\varepsilon + 0.0000148\varphi - 0.0486\eta)}\right) - 1 \quad (2)$$

Where:

$$\alpha = \log(RainThreeDaysBeforeSampling)$$
$$\beta = \%WoodedLandCover$$
$$\chi = \%imperviousCover$$
$$\delta = (Building + Cartpath) \div PondVol$$
$$\varepsilon = Duck2/(water * ViewPer) * \%TreedPondPerimeter$$
$$\phi = HighTide - Avg.14dHighTide$$
$$\varphi = Solar * Solar$$
$$\gamma = WarmSeason(May - September)$$
$$\eta = Avg2hAirTemp$$

For the Total FCB model wildlife activity, meteorological parameters, land cover, and season were all useful predictors of FCB concentrations, and our model explained 47% of the observed variation in total cfu concentrations. Increased duck activity on ponds significantly increased FCB concentrations ($p = 0.0005$). For weather variables, increased solar irradiation six hours before sampling ($p = 0.0041$) and high cumulative rainfall over three days prior to sampling ($p < 0.0001$) were significant predictors of increased FCB. In addition, the greater the percentage of wooded land cover ($p = 0.0119$) and ratio of building and cart path to pond volume ($p < 0.0001$) in a watershed, the higher the expected concentration of FCB. Lower FCB concentrations were associated with the warm months of May through September.

For the MARnegFCB model, wildlife, land cover, weather, and tide variables were useful predictors of cfu levels, and our model explained 48% of the observed variation in presumed wildlife (no antibiotic resistance) FCB. This model included four of the same variables as the TotalCfu model, as well as two additional terms. Average air temperature two hours prior to sampling was associated with decreased FCB ($p = 0.008$).

Increased percent impervious surface was also a significant predictor of increased FCB concentrations ($p<0.001$).

3.5 Bootstrapping and model confidence limits

Bootstrapping provided 95% confidence intervals for coefficients of each parameter associated with FCB measurements while also providing an estimated sample distribution. Model coefficients were generated by re-sampling the pond FCB data 100, 000 times (Table 5). The coefficients are similar to the linear regression coefficients for most terms. Very little difference in variability among coefficients was observed regardless of data sampling frequency. The 95% CI for the solar irradiation term coefficient in the total cfu model and the average air temperature term in the MARneg model included zero (Table 5), suggesting that a few influential data points caused these terms to be significant in the regression model.

3.6 Correlations

Forty nine variables and combinations of variables were tested during the model building process (Table 6). There were some significant correlations between bacteria levels and variables that were not significant in multiple regression models. Specifically, for MARNegFCB, the irrigation and percent treed pond perimeter terms were significant and positively correlated with increased FCB (Table 6). Activity of animals such as egrets ($p=0.16$), raccoons ($p=0.42$), and average daily maximum deer ($p=0.83$) was not significantly correlated with MARNegFCB.

4. Discussion

Duck activity was a useful predictor of total and MARNeg FCB in coastal ponds in combination with other variables. Duck activity varied greatly and was highest during the cool migratory season. All ducks were observed in autumn and winter, with 94% in December and January. Many species of ducks migrate south along the Atlantic flyway every year beginning in late fall through early winter. It was not uncommon to see large groups of multiple species of ducks on our study ponds throughout the winter months. Ponds with high percentages of treed perimeter were the preferred habitat for waterfowl and shore birds, and percent treed perimeter was used to normalize duck activity although duck activity was significant without inclusion of treed pond perimeter in the term. Repelling waterfowl and shorebirds in areas of marginal water quality might be effective during the limited time periods they normally occur.

Our results agree with previous studies that suggested birds were important sources of FCB in environmental waters (Choi et al., 2003; Grant et al., 2006; Somarelli et al., 2007), however none of these studies used photographic monitoring to quantitatively link bacterial water quality to avian wildlife. Photographic monitoring appeared to measure shorebirds and waterfowl well. There may be ways to improve upon this method. For example, using more cameras on large ponds, as well as taking more pictures at different times throughout the day could be helpful. In this study, camera battery life was the limiting factor and determined the number of pictures that could be taken prior to sampling. Other birds such as cormorants and anhinga were frequently observed in and around several ponds, but they were not significant in our models. Both birds are common throughout the spring and summer months and tend to feed throughout mid day.

However they were not often observed in photographs during early morning or at dusk when cameras were programmed to take pictures and as such, their effects on FCB levels in coastal ponds may have been underestimated.

The contribution of FCB from terrestrial animals like deer and raccoon, although known to be abundant on Kiawah Island, was less evident using our approach. Models might have been improved by using more cameras per sample site. Alternatively, it is possible that under most conditions deer and raccoon have less of an impact on bacterial water quality in this system. The transport of FCB from terrestrial wildlife to ponds is strongly dependent upon recent rainfall and the flat terrain at our sites may inhibit transport of fecal material off land.

Two other techniques were used to determine if the influence of additional wildlife species could be included in the models. Total daily bacterial loading (cfu/day) was calculated by multiplying average daily maximum by loading factors (Table 7) for terrestrial (deer and raccoon) and aquatic (egrets, herons, and ducks) wildlife, but these terms were not significant in the model. Daily total numbers of each species summed over the camera deployment were also tested but were less significant in the models compared to average daily maximum observed in a single picture. We suggest that average daily maximum is a better estimate of total activity since many of the species we tested travel in groups and tend to feed or raft often throughout the day (e.g., ducks, deer, and raccoons).

Our results agree with similar studies (Kelsey, 2006) and suggest the relationship among wildlife, tidal ponds, and bacterial water quality is multifaceted. After recent heavy rainfall on an ebbing tide, ponds may be less effective at slowing the transport of runoff contaminants from upland areas into downstream tidal creeks and estuaries. Raccoon, deer, bobcat, otter, and mink were commonly found on most marsh islands, likely because islands provide a degree of security lacking on the mainland (SCDNR, 2004). Heavy rainfall before a large incoming tide likely leads to wash-off and transport of bacteria from hummocks and marsh islands into retention ponds. The inclusion of the unusually high recent tide term in the model supports this conclusion.

The most important predictor variables in both the Total FCB and MARnegFCB models, based upon type II sums of squares, were impervious cover and rainfall. Specifically, total FCB levels were higher in watersheds where either percentage of total impervious surface or the ratio of buildings and golf cart paths to pond volume was high. This is consistent with what has been found in previous studies (Schoonover and Lockaby, 2006; Tong and Chen, 2002), that suggested increased impervious cover leads to increased overland flow and transport of non-point source contaminants to adjacent water bodies. Both total and MAR negative FCB increased with cumulative three day rainfall prior to sampling. This agrees with results from rain event studies we have conducted in this area (results not published) which showed that increases in pond FCB levels from recent rainfall were observable for up to 72 h.

The ratio of buildings plus golf cart paths to pond volume was a useful predictor of total FCB. The majority of buildings at our sites were residential, either single family homes or multi-family condominiums, and it is likely that pet waste in yards near residential buildings contributed to elevated FCB levels in adjacent ponds, especially smaller ponds. This finding has management implications, suggesting that larger, deeper ponds in areas of high density housing could contribute to improved bacterial water quality. Wooded land cover was also associated with increased FCB in coastal ponds. Many wildlife species prefer wooded habitat. Wooded habitats along with measures of impervious surfaces that are unattractive to wildlife are better predictors of bacteria concentrations then terrestrial wildlife activity.

The warm season (May through September) was a significant, negative predictor in the total FCB model. Similarly, both average air and soil temperature were highly correlated to season. Several of our study ponds were shallow and occasionally exceeded 30°C when sampled. The warm season also coincides with typical stratification of the ponds, which limits mixing. Warm weather correlated with reduced FCB concentrations, suggesting higher die off.

Bootstrapping analysis showed that some predictor variables were highly variable and insignificant (Table 5). Specifically, the 95% CI for coefficients for the solar irradiation term in the Total FCB bootstrap model and the air temperature term in the MARnegFCB model included zero. This suggests that a few influential data points caused these variables to be significant in the multiple regression models.

The MARnegFCB model was based on the assumption that antibiotic susceptible bacteria were more likely to come from wildlife. Although this might not always be the case, clearly there is a trend towards more resistance in anthropogenic bacteria. For example, in this study resistance was much higher in sewage treatment samples compared to environmental samples. We assert that the MARnegFCB more accurately models wildlife sources of bacteria and should be useful when trying to manage these bacteria sources.

5. Conclusion

Regression models for predicting FCB levels in coastal retention BMP's were improved by the addition of wildlife activity monitoring prior to sampling. Specifically, ducks were important predictors of pond FCB levels, especially in the winter months when they were present in high numbers. The areas in and around our study areas are known to support large populations of migratory birds, especially during the winter months. Management strategies aimed at reducing FCB from avian wildlife may be useful and targeted at systems where water quality is impaired during the brief migratory season.

Land use variables were important predictors of both total and MARNeg FCB. Increased percent impervious cover and woods were associated with increased FCB in coastal ponds. In addition, small ponds that were surrounded by a large number of houses or condominiums had higher total FCB concentrations. Resource managers should consider increasing the ratio of pond volume to housing density as one way to reduce bacteria levels in waterways contiguous with shellfish harvesting and contact recreation areas. The usefulness of reducing wooded pond perimeters should be investigated. Tidal retention ponds appear to work well under most conditions, as FCB levels are frequently lower in ponds than in tidal creeks just downstream of ponds (data not shown), suggesting that settling of contaminants occurs. Minimizing tidal flushing of ponds can reduce estuarine impacts.

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Figure 1. Ponds studied on Kiawah Island, SC, land uses within the pond watershed are shown.

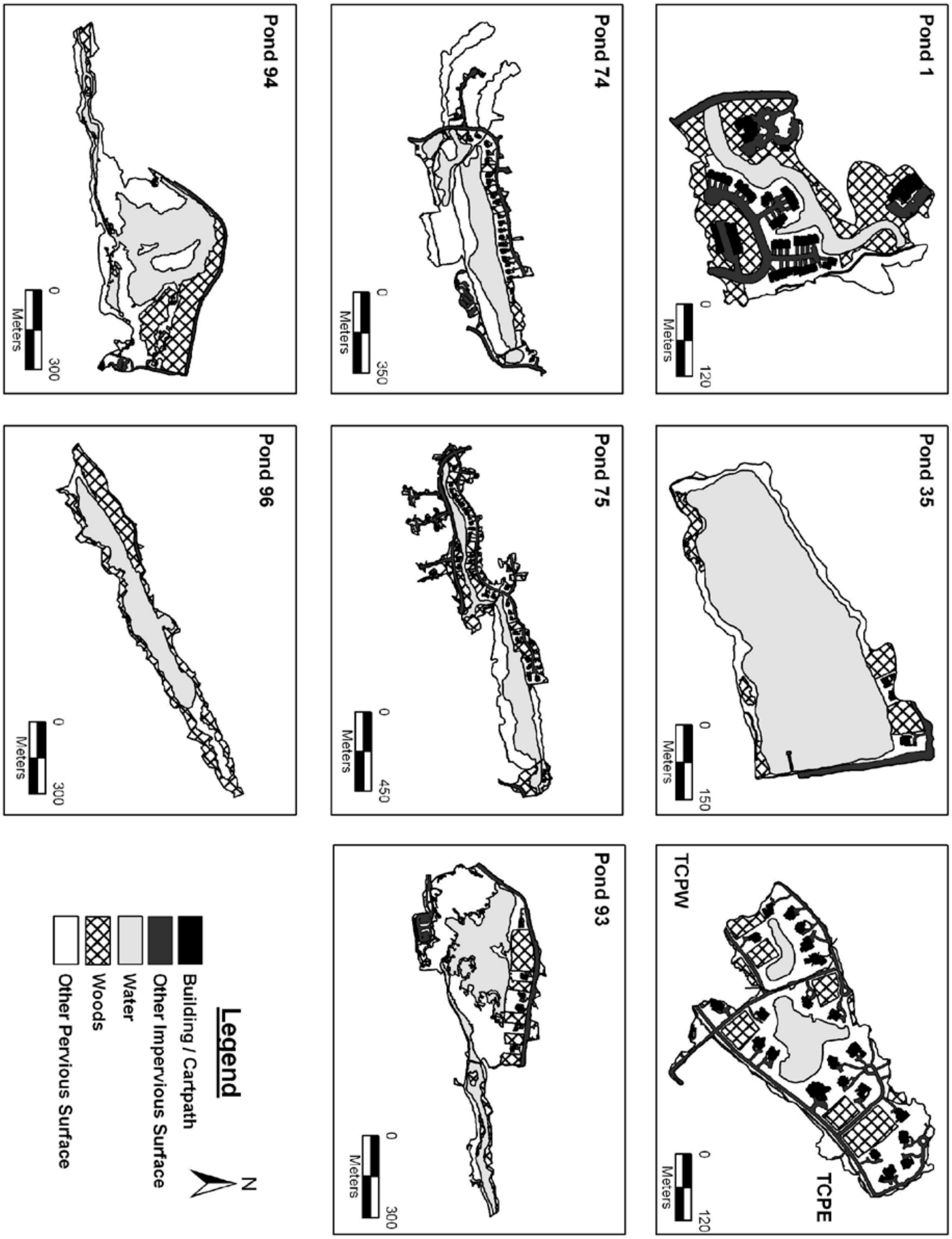

Table 1. Catchment size with percentage of impervious surface and woods at pond sites. Antibiotic resistance was greatest at Pond 74, which had the highest ratio of percent impervious surface to percent woods.

Site	Catchment size (ha)	% IS	% Woods	%IS / %Woods
Ponds				
P1	9.1	30.0%	35.0%	0.86
P35	17.1	4.3%	6.8%	0.63
P74	35.3	16.0%	5.7%	2.81
P75	45.2	23.0%	16.8%	1.37
P93	27.2	9.9%	13.7%	0.72
P94	32.1	4.7%	21.2%	0.22
P96	20.5	1.6%	44.6%	0.04
TCPE	8.5	24.0%	14.4%	1.67
TCPW	2.4	23.0%	16.5%	1.39

Table 2. Antibiotics used in resistance testing. A total of 1 387[*] isolates were analyzed throughout the study. Superscripts in drug class column denote generation. The concentration ranges describe those drugs used in the Dade-26 method. For example, 4-32 means the concentrations tested were 4, 8, 16, and 32. Values in parentheses reflect changes in concentrations.

Class	Drug Name	Abbr.	Conc. (ug/ml)	Resistant Isolates
aminoglycoside	Amikacin	Ak	8-64	0
beta-lactam	Amoxicillin	Amx	4-32	130
beta-lactam	Ampicillin*	Am	10*; 4-32	133
aminoglycoside	Apramycin	Apr	8-32	4
macrolide	Azithromycin	Azi	1-4 (2-8)	43
ephalosporin[2]	Cefoxitin	Cfx	8-32	43
cephalosporin[3]	Ceftriaxone	Cax	8-64	1
cephalosporin[1]	Cephalexin	Cex	16-128	62
cephalosporin[1]	Cephalothin	Cf	3-32 (16-128)	119
bacteriostatic	Chloramphenicol	C	8-32	15
quinolone[2]	Ciprofloxacin	Cp	1-4	13
macrolide	Erythromycin	E	4-32 (16-128)	82
aminoglycoside	Gentamicin	Gm	2-16	12
carbapenem	Imipenem	Imp	2-16	12
aminoglycoside	Kanamycin*	Kan	25*	1
carbapenem	Meropenem	Mer	2-16	12
quinolone	Moxifloxacin	Mox	1-8 (0.25-4)	10
quinolone	Naladixic Acid*	NA	25*; 4-32	43
aminoglycoside	Neomycin*	Neo	50	1
	Nitrofurantoin	Fd	16-128	96
quinolone	Ofloxacin	Ofl	1-8	9
tetracycline	Oxytetracycline*	Otet	50*; 4-32	104
beta-lactam	Penicillin*	P	75*; 8-64 (16-128)	153
aminoglycoside	Streptomycin*	St	12.5*; 16-128	27
sulfonamide	Sulfathiazole*	Sz	500*; 250-500	62
tetracycline	Tetracycline*	Te	4-32	32
bacteriostatic	Trimethroprim	T	2-16	50
	Trimethroprim/ Sulfamethoxazole	TS	2/38-4/76	36

* denotes drugs and concentration used in the MAR-10 method.

Table 3. Pond and sewage treatment plant FCB concentrations and antibiotic resistance.

Site	Avg cfu	(median)	SE	Max cfu	Antibiotic Res
Ponds					
P 1	162.2	(166)	40.0	360	19.2%
P 35	24.3	(3)	11.1	104	11.4%
P 74	33.8	(12)	22.0	228	23.1%
P 75	56.8	(30)	16.8	182	14.9%
P 93	51.3	(36)	16.9	138	7.0%
P 94	21.6	(16)	6.3	58	13.8%
P 96	58.3	(15)	25.0	256	9.5%
TCPE	44.5	(16)	12.2	340	5.6%
TCPW	73.7	(18)	26.8	710	9.5%
Sewage Treatment					
KSTP PreChlor	7598.2	(1939)	3933.1	46000	41.7%
KSTP HoldPond	833.2	(17)	773.8	31000	44.7%

Table 4. Abbreviations and explanation of terms tested in models. Rain is in mm, distances are m, areas are m^2, temperatures are °C, turbidity is NTU's, volume is m^3, tide height is m above mean sea level, and salinity is ppt.

Term	Description	N	Min	Max	Mean	StdDev
LCfu	Log (cfu+1)	121	0.00	6.567	3.090	1.486
LMarneg	Log (cfu * MarNegPer+1)	93	0.92	6.567	3.299	1.258
Deer	Avg. daily max. number * distance to pond edge	102	0.00	2.10E+03	311.393	407.152
Duck	Avg. daily max. number	102	0.00	28.400	2.049	5.308
Duck_tree	Avg. daily max. ducks / proportion of pond * (percent treed pond edge)	102	0.00	0.140	0.005	0.018
Egret	Avg. daily max. number	102	0.00	13.250	0.726	1.914
Heron	Avg. daily max. number	102	0.00	9.250	0.320	0.970
Raccoon	Avg. daily max. number * distance to pond edge	102	0.00	768.450	76.911	114.955
%Fairway	Percent fairway	121	0.00	0.137	0.014	0.036
%ImpSurf	Percent impervious surface	121	0.02	0.301	0.180	0.085
%Road	Percent road	121	0.02	0.191	0.116	0.052
%Rough	Percent golf course rough	121	0.00	0.161	0.024	0.043
%UpLand	Percent upland	121	0.23	0.879	0.765	0.162
%VegLawn	Percent VegLawn	121	0.00	0.502	0.292	0.208
%Water	Percent water	121	0.12	0.768	0.235	0.162
%Woods	Percent wooded land cover	121	0.06	0.446	1.84E-01	9.86E-02
BCVolume	Bldg + cart path areas / pond vol.	121	0.00	0.466	0.178	0.120
Fairway	Area (m2)	121	0.00	4.83E+04	5.04E+03	1.30E+04
ImpWood	ImpSurf area / woods area (m)	121	0.04	2.727	1.205	0.656
NU1	Summed area of lawn, woods, water, and golf course	121	0.00	1.74E+05	4.15E+04	5.01E+04
RoadImpSurfRatio	Road area / impervious surface area	121	0.56	1.000	0.697	0.132
TreePer	Percent treed pond perimeter	121	0.52	1.000	0.874	0.177
Upland	Upland area	121	2.11E+04	3.28E+05	1.11E+05	9.35E+04
VegGolf	Vegatated or landscaped golf course area	121	0.00	1.80E+04	2.05E+03	4.76E+03
VegLawn	Vegatated or landscaped lawn course area	121	0.00	7.65E+04	2.59E+04	2.06E+04
Water	Water area	121	2.91E+03	1.32E+05	4.86E+04	5.01E+04
AirTemp	Avg. air temp 2 h before sampling	121	1.38	30.699	18.196	7.124
LRain2	Log(2 day rain +1)	121	0.00	3.497	1.095	1.147
LRain7	Log(7 day rain +1)	121	0.00	4.868	2.286	1.366
Rain2	rain during 2 d prior to sampling	121	0.00	32.000	4.562	6.147
Rain3	rain during 3 d prior to sampling	121	0.00	37.000	7.595	9.138
Rain3_7	rain between 3 and 7 d before sampling	121	0.00	97.000	13.479	17.282
Rain7	rain during 7 d prior to sampling	121	0.00	129.000	18.041	20.380
SO	Solar irradiation2	119	2.27	2.29E+05	1.95E+04	3.78E+04
WindSpeed	Avg. wind speed six hours prior to sampling	121	0.00	4.830	0.677	0.524
LandLoading	Sum of all avg daily max land animal * loading factor	102	0.00	2.44E+10	6.29E+09	5.28E+09

Table 4 Continued. Abbreviations and explanation of terms tested in models. Rain is in mm, distances are m, areas are m^2, temperatures are °C, turbidity is NTU, volume is m^3, tide height is m above mean sea level, and salinity is ppt.

Term	Description	N	Min	Max	Mean	StdDev
PondLoading	sum of all avg daily max pond animal * loading factor	102	0.00	8.82E+12	4.03E+11	1.09E+12
TotalLoading	sum of all avg daily max land and pond animal * loading factor	121	1.28E+09	8.82E+12	3.49E+11	1.01E+12
CurrentTide	Current tide height (m)	121	0.06	1.564	0.712	0.299
LTideTime	log(TideTime)	121	1.07	1.803	1.411	0.149
pH	pH	121	6.81	9.150	8.017	0.453
Salinity	Salinity	121	5.05	32.000	17.062	7.374
Tide	Previous high tide - avg. 14 d high tide	121	-0.51	0.638	-0.026	0.219
TideStage	Current tide - previous high tide	121	-1.86	-0.498	-1.182	0.309
Turb	Turbidity	121	2.11	128.400	9.675	13.916
$Turb^2$	$Turbidity^2$	121	4.45	1.65E+04	285.659	1553.263
Volume	Pond vol.	121	1.02E+04	1.65E+05	5.11E+04	4.95E+04
WaterTemp	Water temp.	121	8.50	32.000	21.339	6.866
Aerated	Indicates ponds with aeration	121	0.00	1.000	0.562	0.498
Irrigation	Indicates ponds adjacent to irrigation	121	0.00	1.000	0.149	0.357
Mon	Indicates May-Sept or Oct-Apr	121	0.00	1.000	0.455	0.500

Table 5. Variable coefficients from re-sampling data 100,000 times.

(a) TotalFCB boostrapping model

Confidence limits	Constant	$Solar^2$	Rain3	%Woods	Duck3	BCVolume	Mon
Lower	0.95	$-1.5x10^{-6}$	0.03	0.02	6.7	2.165	-1.21
Median	1.54	$1.0x10^{-5}$	0.06	2.43	21.5	3.916	-0.74
Upper	2.18	$1.7x10^{-5}$	0.08	5.01	38.6	5.591	-0.26

(b) MARneg bootstrapping model

Confidence limits	Constant	$Solar^2$	Rain3	%Woods	Duck3	ImpSurfPer	AirTemp
Lower	1.08	$4.2\ x10^{-6}$	0.01	0.33	1.65	0.599	-0.0704
Median	1.96	$1.3\ x10^{-5}$	0.04	3.24	19.2	3.52	-0.0334
Upper	2.92	$2.1\ x10^{-5}$	0.06	5.76	39.0	6.31	0.0045

Table 6. Pearson's correlation coefficients of variables used in regression models. Both cfu and MarnegCfu concentrations were log (x + 1) transformed. Bold indicates significant p<0.05. Explanations of other terms are included in Table 4.

Variable	cfu	Non-Res cfu	% Res
Deer	-0.115	0.024	0.159
Duck	**0.227**	**0.231**	-0.128
Duck_tree	**0.313**	**0.364**	-0.044
Egret	-0.065	-0.161	-0.155
Heron	0.028	0.078	-0.138
Raccoon	-0.026	-0.093	**0.254**
%Fairway	-0.130	-0.105	0.177
%ImpSurf	**0.215**	**0.205**	0.131
%Road	**0.217**	**0.219**	0.114
%Rough	-0.120	-0.112	0.142
%UpLand	0.169	0.175	0.099
%VegLawn	0.031	0.016	-0.003
%Water	-0.169	-0.175	-0.099
%Woods	**0.202**	**0.259**	0.118
BCVolume	**0.316**	**0.287**	0.149
Fairway	-0.143	-0.134	0.158
ImpWood	-0.121	-0.073	0.083
NU1	-0.091	-0.081	0.110
RoadImpSurfRatio	-0.081	-0.023	-0.096
TreePer	**0.178**	**0.217**	0.058
Upland	-0.027	-0.037	0.030
VegGolf	-0.152	-0.145	0.152
VegLawn	0.008	0.018	0.023
Water	-0.132	-0.158	-0.054
AirTemp	-0.096	-0.129	0.199
LRain2	**0.228**	0.102	**0.266**
LRain7	**0.193**	0.016	0.178
Rain2	**0.206**	0.132	**0.221**
Rain3	**0.316**	**0.218**	**0.321**
Rain3_7	0.072	-0.039	0.074
Rain7	0.123	0.010	0.151
SO	0.110	0.058	-0.059
WindSpeed	0.069	0.194	0.139
LandLoading	-0.051	-0.050	0.054
PondLoading	-0.074	-0.087	-0.117
TotalLoading	-0.073	-0.086	-0.104
CurrentTide	0.011	0.119	**-0.206**
LTideTime	-0.051	-0.191	**0.234**
pH	0.040	-0.075	-0.067
Salinity	-0.101	-0.002	-0.095
Tide	**0.270**	**0.242**	-0.097
TideStage	-0.170	-0.162	-0.111
Turb	-0.099	-0.041	-0.119
Turb 2	-0.076	0.005	-0.065
Volume	-0.176	-0.183	0.028
WaterTemp	-0.127	-0.133	**0.238**
Aerated	-0.068	-0.089	-0.090
Irrigation	**0.306**	**0.236**	0.174
Mon	**-0.212**	-0.199	0.172

Table 7. Daily fecal coliform bacteria loading rates used to calculated individual species contributions and tested as model terms.

Animal	**Loading (cfu/d/animal)**	**Explanation**	**References**
Deer	$4.24x10^8$	Average of two literature values	Hanson, 1997; Yagow, 2001
Raccoon	$1.13x10^8$	Literature	Yagow, 2001
Duck	$4.54x10^9$	Average of three literature values	Mara, 1974; Roll & Fujioka, 1997; EPA, 2001
Egret	$3.17x10^9$	Average wt. times average cfu/g of ducks and geese	USEPA, 2001; USGS, 2002
Heron	$3.45x10^9$	Average wt. times average cfu/g of ducks and geese	USEPA, 2001; USGS, 2002

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