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Fecal indicator bacteria and *Salmonella* in ponds managed as bird habitat, San Francisco Bay, California, USA

Gregory G. Shellenbarger[a,*], *Nicole D. Athearn*[b], *John Y. Takekawa*[b], *Alexandria B. Boehm*[c]

[a]*California Water Science Center, US Geological Survey, Placer Hall, 6000 J Street, Sacramento, CA 95819, USA*
[b]*Western Ecological Research Center, US Geological Survey, San Francisco Bay Estuary Field Station, 505 Azuar Drive, Vallejo, CA 94592, USA*
[c]*Department of Civil and Environmental Engineering, Environmental Water Studies, Stanford University, Stanford, CA 94305, USA*

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ABSTRACT

Throughout the world, coastal resource managers are encouraging the restoration of previously modified coastal habitats back into wetlands and managed ponds for their ecosystem value. Because many coastal wetlands are adjacent to urban centers and waters used for human recreation, it is important to understand how wildlife can affect water quality. We measured fecal indicator bacteria (FIB) concentrations, presence/absence of *Salmonella*, bird abundance, and physico-chemical parameters in two coastal, managed ponds and adjacent sloughs for 4 weeks during the summer and winter in 2006. We characterized the microbial water quality in these waters relative to state water-quality standards and examined the relationship between FIB, bird abundance, and physico-chemical parameters. A box model approach was utilized to determine the net source or sink of FIB in the ponds during the study periods. FIB concentrations often exceeded state standards, particularly in the summer, and microbial water quality in the sloughs was generally lower than in ponds during both seasons. Specifically, the inflow of water from the sloughs to the ponds during the summer, more so than waterfowl use, appeared to increase the FIB concentrations in the ponds. The box model results suggested that the ponds served as net wetland sources and sinks for FIB, and high bird abundances in the winter likely contributed to net winter source terms for two of the three FIB in both ponds. Eight serovars of the human pathogen *Salmonella* were isolated from slough and pond waters, although the source of the pathogen to these wetlands was not identified. Thus, it appeared that factors other than bird abundance were most important in modulating FIB concentrations in these ponds.

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1. Introduction

Saltwater and freshwater wetlands and ponds serve as important habitats for juvenile fish and shellfish, and migratory birds, some of which are threatened or endangered (Shenker and Dean, 1979; Kwak and Zedler, 1997; Beck et al., 2001). In San Francisco Estuary (SFE), nearly 95% of the saltwater wetlands and ponds present in the 1800s have been diked for agriculture, flood control, urban development, and salt production (Nichols et al., 1986) and have lost most of their original ecosystem functions. Collapse of the wetlands and ponds has been implicated as a threat to bird populations and a cause for commercial fishery decline, degradation of water quality, and changes in productivity within the bay

*Corresponding author. Tel.: +1 916 278 3191; fax: +1 916 278 3013.
E-mail addresses: gshellen@usgs.gov (G.G. Shellenbarger), nathearn@usgs.gov (N.D. Athearn), john_takekawa@usgs.gov (J.Y. Takekawa), aboehm@stanford.edu (A.B. Boehm).
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(Nichols et al., 1986). Similar degrees of wetland and pond degradation and associated effects have been reported around the world (e.g., Findlay and Houlahan, 1997; Davis and Froend, 1999).

Researchers and managers have now begun to understand fully the range and values of ecosystem services provided by wetlands, marshes, and managed ponds. Thus, efforts are being made around the world to restore wetlands to their original ecosystem functioning. However, there is increasing concern about the potential for waters from such wetlands to degrade receiving water quality and potentially pose a health risk, particularly due to their use by large numbers of birds (Grant et al., 2001).

Recreational waters in the United States and around the world are subject to water-quality criteria based on concentrations of fecal indicator bacteria (FIB), including total coliform (TC), *Escherichia coli* (EC), and enterococci (ENT). Bird feces are known to contain FIB, which may degrade water quality relative to national criteria (e.g., Grant et al., 2001; Harwood et al., 2000; Alderisio and DeLuca, 1999). In fact, some recreational water advisories and closures in southern California are thought to be caused by FIB emanating from bird feces in a coastal marsh (Grant et al., 2001). However, the health risk associated with exposure to FIB from bird feces remains unknown (Boehm et al., in press). For example, bird feces can contain zoonotic organisms such as *Salmonella* (Hubalek, 2004; Roy et al., 2002). Efforts to document pathogens associated with waters impacted by birds are needed to characterize potential health risks associated with exposure to waters from wetlands, managed ponds, and marshes along the world's coastlines.

The impact of bird use of a wetland on environmental water quality was investigated by Grant et al. (2001) in southern California. These researchers did not identify a correlative link between bird densities and water quality, but they identified bird feces as a probable source of ENT in a tidal wetland. *Salmonella* have been detected in surface waters in Canada (Gannon et al., 2004; Johnson et al., 2003), along the Mediterranean Coast (Baudart et al., 2000; Martinez-Urtaza et al., 2004; Touron et al., 2005), and the US Gulf coast (Goyal et al., 1977; Haley et al., 2006). However, no work has previously determined the prevalence of these organisms in California surface waters.

There are several objectives of the work presented here. First, the microbial water quality was characterized within and around several ponds that are being restored and managed as bird habitat within the southern SFE, and the measured water quality was compared to state recreational standards. Even though SFE is one of the largest estuaries in the United States, no studies have documented the concentrations of FIB in its adjacent wetlands. Second, concentrations of FIB and the presence/absence of *Salmonella* in the ponds were examined to understand how they varied with bird use and other environmental conditions to gain insight into occurrence, sources, fate, and transport of these organisms. Third, a number-balance box model was applied to the ponds to determine if they act as sources or sinks of FIB. Although this work was carried out in one set of wetlands, it allows insight into the extent to which wildlife can affect water quality in coastal wetlands and managed ponds, and how these habitats can potentially affect coastal microbial water quality along coastlines.

2. Material and methods

2.1. Study area

The study was conducted on two former evaporative salt production ponds, A9 and A10, in the Don Edwards San Francisco Bay National Wildlife Refuge north of Alviso, CA (Fig. 1). The ponds are part of the South Bay Salt Pond Restoration Project (www.southbayrestoration.com) in SFE, which is an effort to restore about 65 km^2 of wetlands that were diked and used as evaporation ponds for commercial salt production. The restoration project has several aims; one is to restore and maintain habitats to support residential and migratory birds, and a second is to provide for human recreational activities such as bird watching and boating. Ponds A9 and A10 are currently owned by the US Fish and Wildlife Service and managed as part of a flow-through pond system that predominantly serves as bird habitat. Pond A9 has an area of 1.42 km^2 and an average depth of about 0.5 m. Pond A10 has an area of 0.957 km^2 and an average depth of about 1.1 m. These two ponds were selected for this study because they experience very high bird abundance, are the subject of numerous other studies, and the salinities in these ponds did not exceed ocean salinities (as is the case in some of the former evaporative salt ponds). The ponds are impounded by levees that are adjacent to the far southern end of SFE and bordered by tidal reaches of Coyote Creek to the north and Alviso Slough to the west and south. The watershed for the ponds includes only the pond surface and the adjacent levees. Coyote Creek (which will be referred to as a slough for simplicity) and Alviso Slough drain mixed-use watersheds containing urban and agricultural areas. Because Central California has a Mediterranean climate, dry summers and rainy winters, the sloughs primarily contain SFE water and a small amount of nuisance runoff in the summer, and SFE water and stormwater runoff in the winter. During the winter, the ponds are closed off from the sloughs by gates, while, during the summer, the gates of pond A9 are open so that it receives water from Alviso Slough during high tides. Gravity-driven flow though culverts between ponds A9, A10, and A11 was low during the winter but higher during the summer. In addition to other differences in these habitats between winter and summer (e.g., water temperature, salinity, bird use), there was an extensive mat of filamentous green algae in pond A9 during the summer study period.

2.2. Water sampling and analysis

A total of eight sampling events (weekly events for 4 weeks during each winter and summer) took place during 2006 (Table 1). During most sampling events, water samples were collected from 10 locations around the perimeter of each pond (from the levees) and from five locations in the adjacent sloughs (one sample from Coyote Creek and four samples from Alviso Slough). Sampling during the last winter trip was abbreviated because of weather conditions, and only five

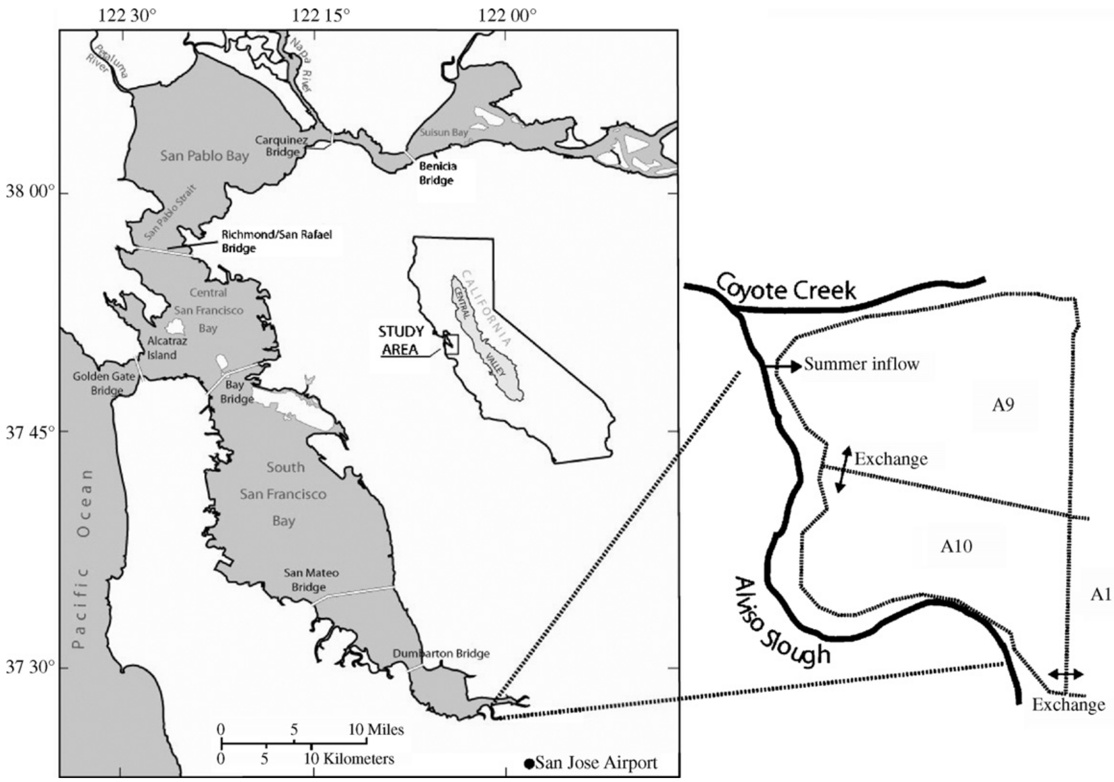

Fig. 1 – Study area detailed in southern San Francisco Bay. Levees surround the ponds (A9 and A10) and isolate them from the local sloughs (Coyote Creek and Alviso Slough). There is inflow to pond A9 only during the summer at the labeled arrow. Year around, there is exchange flow between the ponds (double-headed arrows), although the gravity-driven flow tends to be toward ponds of higher numbers (i.e., from A9 into A10 into A11).

Table 1 – Bird count dates, FIB/*Salmonella* sampling dates, and number of fecal indicator bacteria [*Salmonella*] water samples collected for the 2006 study

Bird count date	FIB sampling date	Pond A9	Pond A10	Sloughs
6 February	7 February	10 [–]	10 [–]	5 [–]
13 February	14 February	10 [2]	10 [2]	5 [1]
20 February	21 February	10 [2]	10 [2]	5 [1]
15 March	14 March	5 [2]	5 [2]	4 [1]
10 July	10 July	10 [2]	10 [2]	5 [1]
17 July	18 July	10 [2]	10 [2]	5 [1]
24 July	24 July	10 [2]	10 [2]	5 [1]
31 July	1 August	10 [2]	10 [2]	5 [1]

The long interval between the third and fourth sampling periods existed because weather conditions prohibited access to the ponds.

samples were collected from each pond and four samples from the sloughs. In addition, during all but the first week of sampling, five water samples were collected to analyze for the presence of *Salmonella*; two samples were collected from each pond, and one sample was collected from Alviso Slough. Sampling was conducted during a single nighttime or early morning high tide for each sampling event to minimize the potential for sunlight to reduce the bacterial concentrations (Boehm et al., 2002).

Physical (temperature and turbidity) and chemical (dissolved oxygen (DO), pH, and salinity) parameters were measured with a Hydrolab MiniSonde (Hach, Loveland, CO) that was calibrated following the methods of Wagner et al. (2006) prior to each sampling period. Salinities are reported using the unitless Practical Salinity Scale. Water levels in the ponds were read from gauged staff plates that are permanently installed near the culverts that connect two adjacent ponds (in pond A9 the gauge was located next to the culvert connection to pond A10, and in pond A10 there were two gauges—one at the culvert connection to pond A9 and one at the culvert connection to pond A11).

Water samples were analyzed for FIB using Colilert (TC and EC) and Enterolert (ENT) defined—substrate assays implemented in a 97-well format (IDEXX, Westbrook, ME) following manufacturer's directions. These results are compared to California state marine recreation contact (REC-1) single sample standards (www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/Regulations-OceanBeaches.pdf; accessed 14 April 2008): TC = 10,000 most probable number (MPN)/100 mL, EC = 400 MPN/100 mL, and ENT = 104 MPN/100 mL. These ponds are not necessarily subject to the REC-1 standards, because they are not available for in- or on-water activity (part of the perimeter of each pond has public recreation on the levees). However, other ponds in the Alviso complex are available for on-water activities, particularly for seasonal waterfowl hunting, and recreation is allowed in the sloughs. Discharge from the pond system and sloughs is transported ultimately to SFE, parts of which are subject to REC-1 standards. Importantly, the ponds that were the subject of our study are similar to wetlands that are present along the world's coastlines, often adjacent to high recreational-use beaches. An understanding of water quality in these ponds can yield information that will be useful in understanding the influence of coastal wetlands, in general, on coastal water quality.

The presence/absence of *Salmonella* was determined in water samples using a modified EPA method 1682 for *Salmonella* in biosolids. Up to 500 mL of water were membrane filtered for each sample. Filters were incubated in tryptic soy broth (TSB) overnight. A matrix control was prepared by adding a loopful of stationary phase *Salmonella enterica* subsp. *enterica* serovar Typhimurium LT2 to a water sample and filtering as described above. Positive and negative controls were prepared with *Salmonella* ser. Typhimurium LT2 and *Enterococcus faecium*, respectively. Six 30 μl aliquots of each TSB enrichment were dropped onto modified semi-solid Rappaport-Vassiliadis (MSRV) media. After incubation, motile organisms were picked, streaked on xylose lysine deoxycholate (XLD) agar, and incubated at 37 °C for 24 h. Several colonies displaying typical *Salmonella* morphology on each plate were picked and subjected to biochemical assays: lysine iron agar, triple sugar iron agar, and urea broth. Selecting only a few colonies could underestimate the total number of different serovars present. Presumptive positives were confirmed using a polymerase chain reaction (PCR) assay that targets the salmonellae-specific *invA* gene. Primers and conditions for the PCR reactions are given in Malorny et al. (2003). PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. Positive and negative PCR and extraction controls were run in conjunction with unknowns. An estimate of the theoretical lower limit of detection was two cells per 1 L, based on the fact that at least one cell must have been captured in each 500 mL sample that was positive for *Salmonella*. One to two colonies from each positive XLD plate were preserved and serovars were identified by the Pennsylvania Animal Diagnostic Laboratory System-New Bolton Center, University of Pennsylvania. The *Salmonella* nomenclature that we report conforms to nomenclature as reported by Brenner et al. (2000).

2.3. FIB box model

We used a simple box model of FIB for each pond to determine if the presence of a source or sink term S^* is needed to explain changes in FIB concentrations between sampling events:

$$S^* = \Delta(V_{pond}C_{pond}) - V_{in}C_{in} + V_{out}C_{out} \quad (1)$$

Here S^*, the source or sink term in units of MPN per interval between sampling events, represents the number of FIB entering or exiting the system in that period. S^* values were normalized by the number of days between sampling events to obtain S with units of MPN per day. Sources could include direct deposition in the ponds, growth, or local runoff (from the levees). Sinks include bacterial death, settling, inactivation, and predation by zooplankton. V_{in} and V_{out} are the volumes of water transported into and out of the pond between sampling events; C_{in} and C_{out} are concentrations of FIB in water transported into and out of the ponds in V_{in} and V_{out}, respectively; and $\Delta(V_{pond}C_{pond})$ is the change in the average number of FIB in the pond between sampling events. This number balance assumes (1) C_{in} and C_{out} can be adequately estimated by averaging the concentrations measured closest to the inlet and outlet of each pond measured during one sampling event and the subsequent one ($n = 2$ for C_{in} and C_{out} for each calculation), and (2) V_{pond}, V_{in}, and V_{out} can be adequately estimated from the Salt Pond Box Model (or SPOOM, Lionberger et al., 2008). SPOOM is a box model that simulates salinity, temperature, and volume for multiple ponds connected in a series. Required inputs to SPOOM include rainfall and water level. Water level was measured during each sampling event as described earlier, and rainfall data from the San Jose International Airport were gathered from the National Climatic Data Center at (www.ncdc.noaa.gov). During the winter for pond A9, V_{in} is zero (because the tide gate to the slough was closed during the winter), and V_{out} is estimated from the SPOOM. During the summer sampling period, both terms are estimated from the SPOOM.

2.4. Bird abundance

Using binoculars and spotting scopes, weekly bird abundances at ponds A9 and A10 were obtained. No birds were counted in the sloughs because of the difficulty with accessing these habitats. Counting was usually conducted on the day preceding the nighttime water sampling (Table 1). Birds were identified to species and their location recorded within 250 by 250 m (6.25 ha) UTM grid cells superimposed on a graphical schematic of the pond. Surveys were conducted during daylight within 3 h of the highest high tide, when the largest number of waterbirds roosted in the salt ponds. Error estimates from simultaneous counts of ponds by separate observers suggests that assessment of total bird numbers varies by less than 20% among observers.

2.5. Statistical analysis

Statistical analyses were conducted using S-PLUS version 7.0 for all statistics, except Kendall's tau correlations, which used Matlab version 7.1 and the Statistics Toolbox. Non-parametric statistics were used for comparative analyses, in part, because of some small sample sizes (specifically for bird abundance) and a violation of the normality requirement for testing variables using parametric statistics. Kendall's tau correlations were selected for comparisons of the combined seasons between the FIB and physio-chemical variables, because tau is not affected by right-censored data (i.e., FIB). Kendall's tau correlations are no less sensitive than Pearson's correlation coefficients, but are generally smaller than the Pearson's coefficients, because they are on a different scale (Helsel and Hirsch, 2002). Correlations for pH and turbidity were weak and, for the most part, were not significant; hence their results are not reported here.

3. Results

3.1. Bird abundance results

The bird abundance on each pond was about ten times higher in the winter than in the summer (Fig. 2). During the winter, ducks represented about 92% of the birds on both ponds, with pond A9 (mean = 7470, 95% confidence interval = 1070) having greater than 300% more birds than pond A10 (mean = 2430, 95% confidence interval = 323). During the summer, birds were dominated by cormorants and California gulls (about 67% of the birds), and during this period, there were about 40% fewer birds using pond A9 (mean = 221, 95% confidence interval = 44) than pond A10 (mean = 380, 95% confidence interval = 19).

3.2. FIB results and environmental conditions

3.2.1. *Winter*

During the winter, the ponds were closed off from the sloughs by gates, so there was no exchange of material between sloughs and ponds. Water temperatures were typical for this time of year, approximately 11–12 °C (Table 2). Salinity in the sloughs was significantly lower than salinities in the ponds ($p<0.001$, Kruskal–Wallis test), reflecting the relatively large percentage of freshwater runoff in the sloughs during the rainy season. DO and pH levels suggested oxygenated conditions in the sloughs and ponds. FIB concentrations in the sloughs were significantly higher than in the ponds ($p<0.001$, Kruskal–Wallis test). FIB concentrations in the sloughs usually exceeded California recreational water-quality standards (Table 3). This suggests that the sloughs probably contained significant amounts of stormwater runoff from the watershed, which is typically rich in FIB (Ahn et al., 2005; Olivieri et al., 2007). TC and ENT were significantly higher in A9 compared to A10 ($p<0.001$ and $p=0.025$, respectively, Wilcoxon rank-sum test), but EC did not differ significantly ($p=0.41$, Wilcoxon rank-sum test)

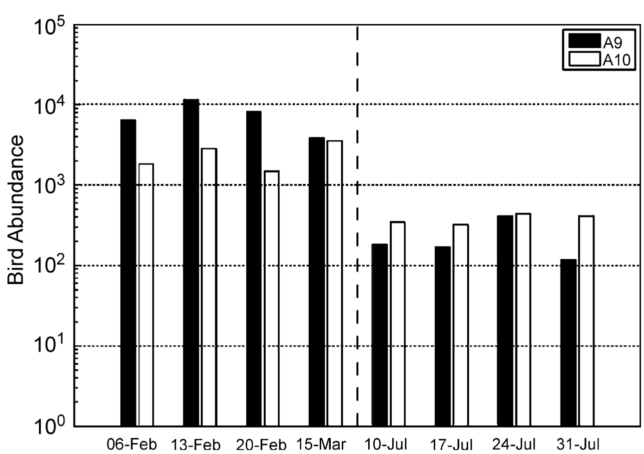

Fig. 2 – Bird abundance for ponds A9 and A10 during each 2006 sampling event.

Table 2 – Mean and 95% confidence interval in parentheses for temperature, salinity, turbidity, pH, dissolved oxygen (DO), TC, EC, ENT, and bird abundance by season and location

	Temperature (°C)	Salinity (–)	Turbidity (NTU)	pH	DO (mg/L)	TC (MPN/100 mL)	EC (MPN/100 mL)	ENT (MPN/100 mL)	*Salmonella* number positive	Bird abundance (count)
Winter										
A9	11.4 (0.8)	22.0 (0.2)	137 (34)	7.8 (0.2)	7.6 (0.5)	5452 (962)	247 (77)	67 (17)	0 [6]	7472 (1071)
A10	11.5 (0.7)	25.6 (0.3)	85 (34)	8.0 (0.1)	6.3 (0.3)	1115 (308)	308 (93)	47 (17)	1 [6]	2432 (323)
Sloughs	12.1 (0.9)	6.6 (1.7)	136 (80)	8.0 (0.3)	7.7 (0.6)	20,310 (2570)	1218 (371)	423 (132)	2 [3]	N/A
Summer										
A9	22.8 (1.1)	21.0 (0.9)	91 (49)	8.2 (0.2)	2.7 (0.5)	15,720 (2708)	3114 (1537)	6836 (2937)	2 [8]	221 (44)
A10	23.8 (0.9)	22.1 (0.4)	79 (27)	8.3 (0.1)	5.1 (0.6)	17,820 (2664)	1044 (540)	429 (285)	0 [8]	380 (19)
Sloughs	23.0 (1.2)	11.8 (2.6)	144 (62)	7.8 (0.2)	4.1 (0.6)	23,290 (1057)	5281 (3470)	477 (229)	2 [4]	N/A

For *Salmonella*, the number of positive samples is shown, while the number of collected samples is given in brackets. N/A refers to no bird abundance measured in the sloughs.

between the two ponds (Fig. 3). The rates of exceedance of the state standard were not substantially different between ponds, and the FIB concentrations were typically lower than California water-quality standards for contact recreation (Table 3).

Table 3 – Percent of the FIB samples from each location and season that were greater than the California state marine contact (REC-1) standard

	Winter			Summer		
	A9	A10	Sloughs	A9	A10	Sloughs
TC	6	0	95	70	78	100
EC	26	28	100	82	55	80
ENT	17	14	95	88	40	90

The single-sample standards are: TC = 10,000 MPN/100 mL, EC = 400 MPN/100 mL, and ENT = 104 MPN/100 mL.

3.2.2. *Summer*

In the summer, pond A9 was hydrologically connected via a tide gate to Alviso Slough (Fig. 3). Thus, there was potential for substantial amounts of material transport between ponds and sloughs. Slough water temperature was greatly increased in the summer (mean = 23.0 °C) relative to the winter (mean- 12.1 °C, Table 2), as would be expected from solar heating of surface waters. Salinity in the sloughs was higher in the summer (mean = 11.8) than it was in the winter (mean = 6.6), due to reduced runoff inputs, but it was still lower in salinity than the two ponds (21.0 in A9 and 22.1 in A10). Pond A10 had significantly lower salinity in the summer relative to the winter ($p < 0.001$, Wilcoxon rank-sum test), while the salinity in pond A9 did not differ between seasons. This suggests that the loss of water via evaporation from the ponds, which would tend to raise the salinity in the summer, was likely balanced by the input of lower salinity slough water. pH in the ponds was higher in the summer than winter ($p < 0.05$, Wilcoxon rank-sum test), but pH in the sloughs was lower in the summer than winter ($p < 0.01$, Wilcoxon rank-sum test).

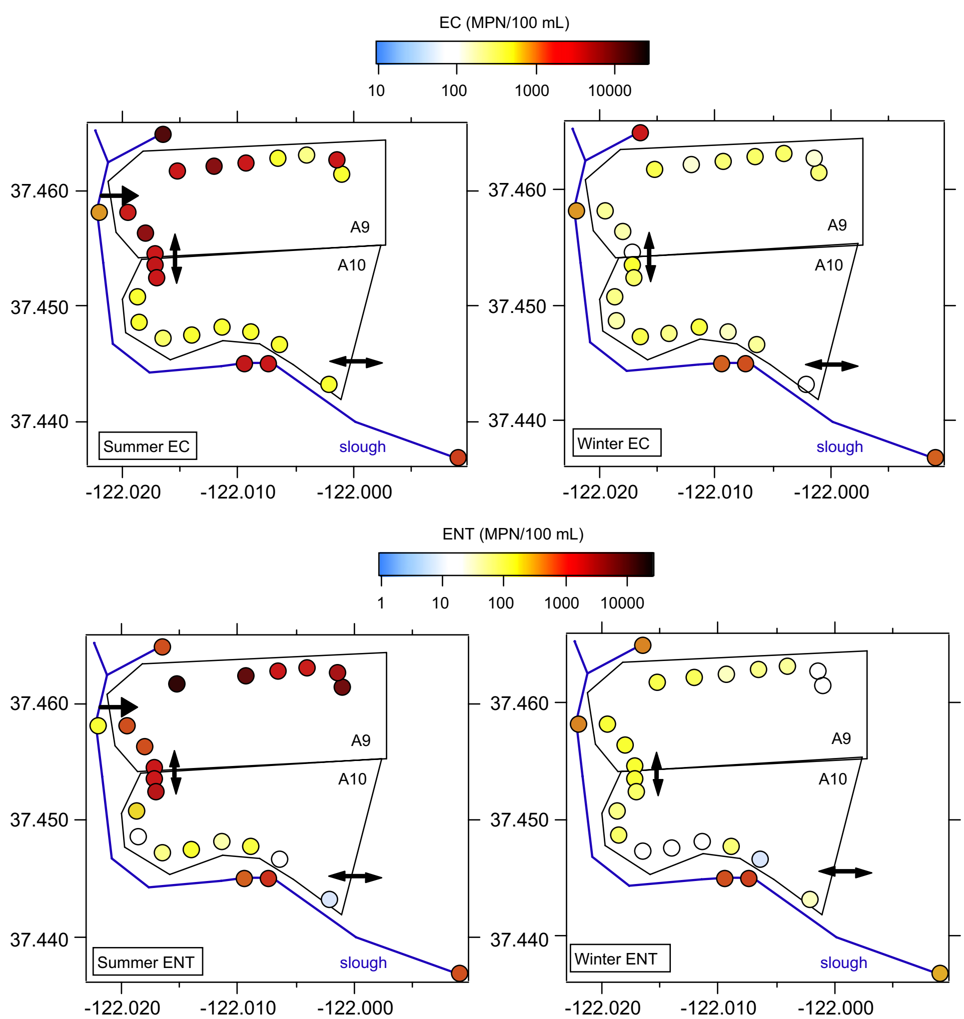

Fig. 3 – Geometric mean (GM) of EC (top) and ENT (bottom) at each sampling point during winter (right) and summer (left) sampling. The color of each marker is related to the GM of concentrations. Note different color scales for EC and ENT. Arrows show the direction and location of flow between water bodies during sampling.

The DO concentrations were significantly lower in the summer than in the winter for both ponds and sloughs ($p<0.005$, Wilcoxon rank-sum test, Table 2). However, the DO percent saturation in pond A10 was not significantly different between seasons ($p = 0.31$, Wilcoxon rank-sum test, data not shown). While only TC concentrations in the sloughs were significantly different between seasons (higher in summer, $p = 0.04$ for TC, $p = 0.20$ for EC, and $p = 0.86$ for ENT, Wilcoxon rank-sum test); TC, EC, and ENT concentrations in each pond during the summer were significantly higher than in the winter ($p<0.005$ for all FIB, Wilcoxon rank-sum test). In addition, during the summer, EC and ENT in pond A9 were significantly elevated relative to pond A10 ($p<0.001$ for each, Wilcoxon rank-sum test). The rates of FIB concentration exceedance of the state standard in the summer were highest in the sloughs, then followed by pond A9 and pond A10 (Table 3).

3.3. Correlations between FIB and environmental variables

The relationship between FIB concentrations in the ponds and sloughs, and environmental variables was investigated by considering the data in aggregate (Table 4). The correlations between pond FIB concentrations and measured environmental variables tended to be stronger and more significant than correlations obtained using slough FIB concentrations. Correlations between pond FIB and temperature were significantly positive, indicating warmer temperatures were coincident with higher FIB concentrations. Pond FIB and salinity, DO, and bird abundance were negatively correlated ($p<0.01$), indicating that lower salinities, DO, and bird abundance were coincident with high concentrations of FIB. It should be noted that pond TC and bird abundance were not correlated. The only significant correlations observed for the slough FIB concentrations were between TC and DO (negative) and EC and salinity (positive). It must be noted that some of the FIB sample concentrations in both seasons (particularly TC in the sloughs) were right censored; that is the measured concentrations exceeded the upper detection limit for the test (24,196 MPN/100 mL). The sloughs had about 50% of the winter samples and 75% of the summer TC samples over the detection limit. The ponds had 32% (A9) and 42% (A10) of the summer TC samples, and no winter samples, over the detection limit. Therefore, the actual FIB concentrations could have been higher. This limitation likely reduces the strengths and the significance of the correlations that we present, particularly for the sloughs.

Table 4 – Kendall's tau correlation coefficients and levels of significance between indicator bacteria and the physico-chemical variables for both seasons combined

	Temperature	Salinity	DO	Bird abundance
	$n = 149$	$n = 150$	$n = 130$	$n = 16$
Pond TC	0.46***	−0.51***	−0.32***	−0.33
Pond EC	0.28***	−0.16**	−0.46***	−0.56**
Pond ENT	0.20**	−0.31***	−0.37***	−0.54**
	$n = 39$	$n = 39$	$n = 34$	
Slough TC	0.21	0.18	−0.40**	N/A
Slough EC	0.09	0.37**	−0.24	N/A
Slough ENT	−0.04	0.05	−0.01	N/A

Bird abundance data were only available in the ponds (N/A = not applicable), and n refers to the number of data pairs used to calculate tau. The pond data have been combined, and the seasons have been combined for both the ponds and sloughs. The levels of significance are as follows: *** $p<0.0001$; ** $p<0.01$; * $p<0.05$.

3.4. FIB box model results

S values were computed for TC, EC, and ENT for ponds A9 and A10 in both summer and winter (Table 5). The three S values reported each season represent the change in MPN per day for a given FIB in the pond over the interval between consecutive sampling events (four sampling events per season allow for calculation of three S values). Given that there was a high degree of variability in FIB concentrations between sampling events, the computed values of S also exhibit a high degree of variability (Table 5). This is not surprising, because the processes (physical, chemical, and biological) that affect FIB concentrations are variable on many time scales (Boehm, 2007). The seasonally averaged values for S better capture the general tendency of the highly variable processes in each pond that can affect FIB concentrations. During the winter, the box model results suggest that both ponds, on average, acted as sinks for TC (S is negative) and sources for EC and ENT (S is positive). The summer data suggest more varied results. On average, both ponds served as sinks for EC; however, the ponds showed terms opposite to each other for TC and ENT. Pond A9 acted as a sink for TC and a source for ENT, whereas pond A10 acted as a source for TC and a sink for ENT.

3.5. *Salmonella* results

Eight serovars of *Salmonella* in total were isolated and identified during this study. During the winter, *Salmonella* were isolated from zero of six samples collected in pond A9, and one of six samples in pond A10, while two of three samples from the slough were positive. Three serovars were detected in winter: *Salmonella* serovars Typhimurium, Javiana, and Heidelberg. Serovar Typhimurium was isolated from pond A10, while Javiana and Heidelberg were isolated from the slough. In the summer, *Salmonella* were isolated from two of eight samples in pond A9 and two of four samples in the slough; no *Salmonella* were isolated from pond A10 samples. Five different serovars were isolated in the summer: *Salmonella* serovars Kentucky, Glostrup, and Infantis were isolated from the slough, while serovars Bovismorbificans and Give were isolated from pond A9. Note that two serovars were isolated in a single sample from the slough (serovars Glostrup and Infantis were present together). Taken in aggregate, the frequency of detection of *Salmonella* was more likely in the slough (where 57% of water samples contained culturable

Table 5 – Values for S (MPN/day) calculated for each FIB and pond over the interval between sampling events (three intervals for each season: weeks 1–2, weeks 2–3, and weeks 3–4 presented, respectively), and the seasonally averaged S value (in italics)

	TC	EC	ENT
Winter			
A9	-3.8×10^{11}, -1.2×10^{12}, -1.3×10^{11}	2.4×10^{11}, 2.1×10^{11}, -1.3×10^{11}	-2.2×10^{10}, 2.0×10^{9}, 4.2×10^{10}
A9 average	$\mathbf{-5.7\times10^{11}}$	$\mathbf{1.1\times10^{11}}$	$\mathbf{7.3\times10^{9}}$
A10	-1.3×10^{11}, -3.0×10^{11}, -3.9×10^{11}	7.5×10^{11}, -2.5×10^{10}, -1.3×10^{11}	-1.5×10^{10}, -1.2×10^{10}, 4.0×10^{10}
A10 average	$\mathbf{-2.7\times10^{11}}$	$\mathbf{2.0\times10^{11}}$	$\mathbf{4.3\times10^{9}}$
Summer			
A9	1.2×10^{13}, -9.9×10^{12}, -1.6×10^{13}	-2.4×10^{11}, 2.6×10^{11}, -9.6×10^{11}	8.5×10^{11}, 9.8×10^{12}, -3.3×10^{12}
A9 average	$\mathbf{-4.9\times10^{12}}$	$\mathbf{-3.1\times10^{11}}$	$\mathbf{2.4\times10^{12}}$
A10	7.1×10^{12}, 1.8×10^{12}, -2.5×10^{12}	-6.1×10^{11}, 1.2×10^{12}, -1.6×10^{12}	-7.1×10^{11}, -4.7×10^{10}, -2.9×10^{10}
A10 average	$\mathbf{2.1\times10^{12}}$	$\mathbf{-3.5\times10^{11}}$	$\mathbf{-2.6\times10^{11}}$

A positive value for S implies pond sources, while a negative value for S implies pond sinks.

Salmonella, $n = 7$) compared to the ponds (where only 11% of water samples contained culturable *Salmonella*, $n = 28$).

4. Discussion

We hypothesized that birds would be the major source of FIB to the ponds and, thus, that concentrations of FIB would mirror bird abundance. We found that FIB concentrations were much lower in the ponds during the winter compared to the summer, even though bird abundance was much greater in the winter. During the summer, water in the ponds was warmer, fresher, and lower in DO than the winter; hence temperature, salinity, and DO were well correlated with FIB concentrations. One factor that appeared to cause higher concentrations of FIB in the ponds during the summer compared to winter was inflow of slough water. During the summer, a tide gate operated between pond A9 and the slough, allowing FIB-rich slough water to enter the pond system. When taking this inflow into account, as well as outflow from the ponds, a number-balance box model indicates that, on average during the summer, pond A9 acted as a sink for TC and EC (loss of 4.9×10^{12} and 3.1×10^{11} MPN/day, respectively) and had a net source of ENT (input of 2.4×10^{12} MPN/day). Pond A10 acted as a net source of TC (2.1×10^{12} MPN/day) and a net sink for EC and ENT (3.5×10^{11} and 2.6×10^{11} MPN/day, respectively). Possible sources in the ponds included bird feces and bacterial re-growth within the ponds, potentially on dense mats of filamentous green algae (predominantly *Enteromorpha* and *Cladophora*; Schraga, 2007). Whitman et al. (2003) have shown that *Cladophora* in freshwater systems can increase the persistence or instigate growth of FIB. Sinks for FIB include die-off, inactivation (Burkhardt et al., 2000), predation (Boehm et al., 2005), or deposition (Fries et al., 2006).

FIB (with the exception of TC) were negatively correlated with bird abundance when data were taken in aggregate, indicating lower FIB were coincident with higher bird abundances that occur in the winter. However, the box model indicates that both ponds acted as net sources of EC and ENT during the winter. This source could be a result of bird feces input. The box model estimated that the net source of EC and ENT to the pond was on the order of 10^{11} MPN/day EC and 10^{9} MPN/day ENT. This source term can be compared to the expected inputs from the birds using the ponds to put the source terms in context. Bird abundance on the ponds during the winter was dominated (92%) by dabbling and diving ducks. If we assume that all the birds using each pond were ducks, an estimate of the potential loading of fecal coliform (FC) by birds to each pond can be calculated. The average bird abundances per day on ponds A9 and A10 during the winter were 7472 and 2432, respectively. Valiela et al. (1991) estimated that ducks in Buttermilk Bay, MA delivered about 10^{9} FC/bird/day to the bay. Assuming that all FC in the ponds were EC (EC is a subset FC), this suggests that birds may be responsible for approximately 10^{12} MPN EC/day for each pond. The calculated net EC source terms S are about one order of magnitude less than the direct estimate of bird loading. Thus, we conclude that it is conceivable that that bird feces could have been a source of EC in the ponds during the winter, especially in light of potential EC sinks in the ponds that would reduce concentrations below those from direct input. Additional sources of FIB within the ponds could include bacterial re-growth, feces from other wildlife, and FIB-contaminated runoff from the levees entering the pond during rain events.

In the winter, pond A9 had higher concentrations of TC and ENT than pond A10. Because pond A9 had 300% more birds than pond A10 during the winter, this finding potentially could be explained by the increased input of bird feces into pond A9 relative to A10. In the summer, pond A9 had higher EC and ENT than pond A10, even though pond A10 had higher bird abundance. As illustrated in Figs. 1 and 3, pond A9 directly received FIB-contaminated slough water through the tidal gates in the summer. Thus, it appeared that pond A9 was more adversely impacted by slough water than pond A10.

Of the 35 samples assayed for *Salmonella*, seven were positive. The frequency of detection was higher in the sloughs than the ponds. It is interesting to note that the sloughs also had higher concentrations of FIB than the ponds. A total of eight serovars was detected in the seven positive *Salmonella* samples, with one sample yielding two serovars. Serovars isolated during the summer were entirely different from those isolated in the winter. During the summer, *Salmonella* serovars Typhimurium, Javiana, and Heidelberg were found, while in the winter, serovars Kentucky, Glostrup, Bovismorbificans, Give, and Infantis were detected. We should note that all of the serovars isolated in this study, with the exception of Glostrup, have been isolated from humans in California suffering from salmonellosis (CDC, 2005). Baudart et al. (2000) found seasonal differences between *Salmonella* serovars isolated in river waters in the south of France, but the serovars biased for winter-time detection were not those identified in our study. Refsum et al. (2001) found seasonality in the occurrence of *Salmonella* in Norwegian passerines, although the isolated serovars were almost always Typhimurium (which was isolated from pond A10 in the winter). The difference in serovar prevalence between summer and winter may be a result of different sources of *Salmonella* during these seasons. It is well established that birds can be carriers of *Salmonella*. However, *Salmonella* are not exclusively found in bird feces; they also emanate from reptiles (Corrente et al., 2004) and mammals, including cattle (Van Donkersgoed et al., 1999) and can be found in sewage and stormwater runoff (Baudart et al., 2000). The difference in serovars between seasons and sampling events could also result from our screening method, where only one to three colonies were selected from each XLD plate. This likely underestimated the total number of serovars collected.

5. Conclusions

1. FIB concentrations in sloughs and managed ponds of southern SFE can exceed the California REC-1 water-quality standards, and surprisingly, the sloughs had the highest frequency of exceedance in both seasons.
2. FIB concentrations in the ponds were higher during the summer when the ponds received FIB-rich waters from the adjacent sloughs, and, contrary to expectation, lower in the winter when the ponds were isolated from the sloughs and bird abundance was highest. Management of the ponds allows inflow from the sloughs during the summer to the detriment of pond microbial water quality.
3. Bird feces deposited in the ponds in the winter likely contributed large quantities of FIB to the ponds, but this did not lead to high rates of exceedance of water-quality standards.
4. The ponds can act as net wetland sinks for FIB from the sloughs, particularly during the summer. These ponds can serve as net sources of FIB (particularly in the winter), but since these ponds do not discharge directly to SFE, the effect of FIB-contaminated discharge from these ponds on SFE is assumed to be low. However, other similar managed ponds in the restoration project area discharge water to SFE and its tributaries.
5. *Salmonella*, including serovars that are human pathogens, were present in these wetlands. The higher frequency of detection in the sloughs than the ponds corresponded with the higher FIB concentrations in the sloughs than ponds.

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