

# Management of Microbial Contamination in Storm Runoff from California Coastal Dairy Pastures

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A survey of storm runoff fecal coliform bacteria (FCB) from working farm and ranch pastures is presented in conjunction with a survey of FCB in manure management systems (MMS). The cross-sectional survey of pasture runoff was conducted on 34 pastures on five different dairies over 2 yr under varying conditions of precipitation, slope, manure management, and use of conservation practices such as vegetative filter strips. The MMS cross-sectional survey consisted of samples collected during 1 yr on nine different dairies from six loafing barns, nine primary lagoons, 12 secondary lagoons, and six irrigation sample points. Pasture runoff samples were additionally analyzed for *Cryptosporidium* sp. and *Giardia duodenalis*, whereby detectable concentrations occurred sporadically at higher FCB concentrations resulting in poor correlations with FCB. Prevalence of both parasites was lower relative to high-use areas studied simultaneously on these same farms. Application of manure to pastures more than 2 wk in advance of storm-associated runoff was related to a  $\geq 80\%$  reduction in FCB concentration and load compared to applications within 2 wk before a runoff event. For every 10 m of buffer length, a 24% reduction in FCB concentration was documented. A one-half (75%), one (90%), and two (99%) log<sub>10</sub> reduction in manure FCB concentration was observed for manure holding times in MMS of approximately 20, 66, and 133 d, respectively. These results suggest that there are several management and conservation practices for working farms that may result in reduced FCB fluxes from agricultural operations.

REGULATIONS AND POLICIES have been enacted worldwide to reduce the impacts microbial pollution has on coastal watersheds. Programs such as the European Union's Water Framework Directive (CEC, 2000; CEC, 2006) and Australia's National Water Quality management Strategy, including fresh and marine water quality guidelines (ANZECC, 2000), are setting water quality criteria and directing water body assessment and mitigation. In the United States, similar action is being taken through total maximum daily loads (TMDL). For example, the San Francisco Region of the California Regional Water Quality Control Board (CRWQCB) implemented a pathogen TMDL for Tomales Bay in 2005 (CRWQCB, 2005) and is now implementing a conditional waiver program for grazing lands on livestock agricultural operations within the same watershed (CRWQCB, 2008). These policies establish ambient water quality standards for FCB to protect beneficial uses of shellfish harvesting and contact and noncontact recreation in the watershed. Standards are geometric means of 75 and 14 most probable number/100 mL in tributary streams and shellfish leases in the Bay, respectively. The TMDL and conditional waiver also direct managers of FCB sources, including livestock agriculture operations, to implement management measures that will reduce FCB concentrations in stormwater runoff.

Dairy farms and grazing livestock ranches in the watershed use pastures for on-farm feed production and grazing. These are critical management units because they reduce the need for and cost of imported feed. They range in size from tens to hundreds of hectares, in which calves and adult animals graze. In some cases, manure from loafing barns is spread and irrigated on these pastures for irrigation and fertilization of grasses and feed crops. Relative to more extensively grazed farm management units and

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**Abbreviations:** CFU, colony forming units; CRWQCB, California Regional Water Quality Control Board; FCB, fecal coliform bacteria; MMS, manure management system; TMDL, total maximum daily load; VTS, vegetative treatment system.

lands without grazing, these manure-applied pastures can generate runoff with FCB concentrations and loads that are one to two orders of magnitude higher, or  $10^2$  to  $10^3$  compared with  $10^4$  to  $10^6$  cfu/100 mL (Lewis et al., 2005). This is consistent with observations of elevated nutrient values in surface water from watersheds with pastures receiving manure applications (Rothenberger et al., 2009)

The resulting management challenge is how to maintain the vital production role these pastures provide while reducing FCB in storm runoff that can impact the Bay's aquatic resources. Improvements to water quality that can be realized by the containment of dairy waste and converting its use to land irrigation, similar to those documented in New Zealand by Wilcock et al. (2009), were realized in the Tomales Bay in the 1970s. Current steps taken on the studied farms to do this include application of manure from August through November, in advance of the winter storm season and any resulting surface runoff. It is anticipated that this advance application of manure affords time for the manure to desiccate and be incorporated into pasture soils, reducing the level of FCB available for transport and delivery to surface waters. Typically, the manure that farmers apply has been stored for a period of time (weeks to months) as liquid in lagoons and in some cases as solids in stockpiles. Reduction of microbial pollution in this stored manure is anticipated as a result of numerous conditions that take place in manure storage systems including temperature changes, aerobic conditions, and predation (Hill, 2005). Additionally, some producers have fenced streams and riparian areas to prevent livestock grazing and resulting manure deposition in or near surface waters, a common water quality management practice for grazing livestock operations.

Investigation of microbial pollution and management from livestock pasture systems has been conducted in other climates (Kay et al., 2005; Shanks et al., 2007), in groundwater (Close et al., 2010), and through the use of controlled runoff plots and rainfall simulation (Lim et al., 1997; Collins et al., 2004; Collins et al., 2005; Meals and Braun, 2006; Ferguson et al., 2007; Sullivan et al., 2007). Results from these and other investigations offer confirmation that vegetative treatment systems (VTS), as described by Koelsch et al. (2006), generate varying reductions of microbial concentrations and loads in pasture runoff. These reductions are functions of VTS design and maintenance, as well as the physical and chemical properties of the indicator bacteria or microbial water-borne pathogen studied (Ferguson et al., 2007). The capacity of VTS to reduce microbial pollution can be surpassed by extreme precipitation and runoff events and resulting channel flow and erosion (Collins et al., 2004; Collins et al., 2005).

There are also comprehensive reviews and additional studies on the role that manure storage and handling technology (Hill, 2005; Atwill et al., 2009) and field application approaches (Li et al., 2005; Meals and Braun, 2006; Sistani et al., 2010), including pasture rest (Knox et al., 2008), have in reducing microbial pollutant loading and availability for transport to surface and subsurface waters. In these cases, microbial pollutant levels in manure are reduced before storm runoff events as a result of environmental conditions that decrease the survival and persistence of indicator bacteria and microbial water-borne pathogens.

To better understand the risk and management of microbial pollution from pastures in Tomales Bay, we conducted a two-component study. First, we sampled and analyzed runoff from dairies and ranches in the watershed. We hypothesized that the currently used management practices, specifically duration in time since manure application, combined with VTS would reduce concentrations and loads for FCB, *Cryptosporidium* sp., and *G. duodenalis*. In conjunction, we documented additional farm factors, such as animal age group and numbers, site characteristics, and climatic factors that influence microbial pollution fate and transport from these pastures. Second, we conducted a sampling survey within cooperating farm manure management systems (MMS) to enumerate FCB concentration. In this survey, we investigated the impacts existing MMS have on FCB levels. In sampling manure from point-of-origin loafing barns to irrigation on the studied pastures, we hypothesized that FCB concentration is indirectly related to manure holding time.

The results presented in this paper complement our earlier findings for the management of microbial pollution in runoff from lots and corrals on these same dairies and ranches (Miller et al., 2007; Miller et al., 2008; Lewis et al., 2009) by providing the results from the pasture management units on the cooperating farms and ranches. This management-scale study also parallels soil box and runoff plot scale, as well as rainfall simulation research of microbial pollution impacts, by investigating the effectiveness of recommended conservation practices to water quality on working dairies and ranches. Accordingly, the presented research is unique in its practical significance for documenting on-farm results and providing direction on compliance with indicator bacteria water quality regulations and criteria.

## Materials and Methods

### Study Area

A detailed description of the study area, including climate and agricultural production history in the Tomales Bay watershed, is available in Lewis et al. (2009). Briefly, the watershed is characterized by the prevailing Mediterranean climate and livestock agriculture, including dairy production that began in approximately 1850. Additionally, there are records as far back as 1890 of a native oyster fishery. Current commercial oyster production occurs on approximately 280 ha of leased Bay tidal lands.

Five dairy farms and grazing ranches were selected for the pasture component. Nine dairies were selected for the MMS survey based on voluntary participation and their location within the Tomales Bay watershed. Thirty-four pastures were enrolled as specific study sites in the pasture component. Nine MMS were studied, composed of 33 different MMS units ranging from loafing barn effluent to irrigation sprinkler effluent.

### Study Design

This was an observational longitudinal study of a large cross section of pastures ( $n = 34$ ) experiencing management scale implementation of measures designed to reduce FCB levels in stormwater discharge. Studied pastures varied in size and slope (Table 1). Each studied pasture had one or more of the following management measures implemented during the study period: no management measures (control), duration of

time since manure application, and channeling pasture runoff through vegetative filter strip or grassed waterway (0–61 m in length). FCB concentration, stormwater runoff rate, and FCB instantaneous load, as well as *Cryptosporidium* sp. and *G. duodenalis* concentrations, were determined for samples from the 34 study sites over 2 yr (2002–2003 and 2003–2004). Multivariate analysis was used to determine associations between FCB levels discharged from pastures, site factors, and management practices. The size, slope, stocking rate, animal age, curve number and hydrologic group used to model and describe rainfall and runoff relationships (SCS, 1985), and precipitation variables (24-h and cumulative-to-date) were treated as covariates to account for site-specific differences between pastures and inherent variation among storms.

We conducted the cross-sectional survey of MMS from June 2000 to June 2001. The nine dairies cooperating in this survey ranged from approximately 100 to 450 milking cows. MMS material sampled included nascent loafing barn manure and manure in successive storage lagoons within the respective farm MMS. Studied lagoons consisted of open-air, earthen-berm structures engineered with clay liners to minimize percolation into the ground. Typically, barn effluent was scraped and flushed daily into a primary lagoon. Primary lagoon material was pumped to secondary lagoons for storage on an infrequent basis. Irrigation with stored manure occurred in late spring and early summer, and again in fall. Samples were collected from six loafing barns, nine primary lagoons, 12 secondary lagoons, and six irrigation sample points. Sampling was conducted at the effluent point for each respective MMS unit studied. For example, primary lagoon samples were collected from the effluent pipe taking manure from the primary lagoon to a secondary lagoon. For each MMS sample collected, we documented from which unit the sample was collected, if a solid separator was part of the MMS, and estimated the holding time or age of the manure sampled. Manure holding time was estimated with the following equation:

$$\text{Manure holding time (d)} = \frac{(\text{MMS volume in m}^3)(168 \text{ h/wk})(0.0000115 \text{ d/s})}{(\text{discharge in m}^3/\text{s})(\text{system operation in h/wk})}$$

where MMS volume (m<sup>3</sup>) was based on MMS construction records, discharge (m<sup>3</sup>/s) was measured as described in the sample collection section, and system operation (hr/wk) was estimated by dairy producer responses during seasonal interviews.

## Pasture Management Measure Implementation

### Manure Application Duration

Solid manure distribution is performed by spreader trucks. In some cases, irrigation systems are used to distribute liquid material before the predominantly solid material is loaded onto trucks. The timing of application is dependent on the availability of equipment and labor to distribute manure across each

**Table 1. Rainfall, discharge, and management conditions of 34 dairy and ranch pastures from which runoff samples were collected and analyzed during the 2002–2003 and 2003–2004 winter storm seasons.**

Pasture characteristic	Mean	Median	Min.	Max.
Precipitation and discharge				
24-h cumulative precip. (mm)	18.7	15.2	1.0	75.2
Annual cumulative precip. (mm)	434	510	78	665
Slope (°)	15	13	2	30
Discharge (m <sup>3</sup> /s)	0.017	0.004	0.0002	0.19
Storm runoff (m <sup>3</sup> )	260	70	0.007	2421
Management				
Size (ha)	7.2	6.1	0.6	32
Stock number	39	18	0	100
Animal concentration (no./ha)	12	5	0	62
Pasture ground cover (%)	88	92	2	99
Buffer length (m)†	5.6	0	0	61.0

† Statistics describe conditions for the water quality improving management practices below 11 of the 34 studied pastures.

pasture. In this applied study, manure application date was documented for each studied pasture, occurring from late August to early November. The time duration since manure application was calculated for each respective sample event, dictated by precipitation and storm events that generated runoff from each respective pasture. Duration times were lumped into four broad duration groups: <2.0 wk, 2 to 4 wk, 1 to 12 mo, and greater than 12 mo before sample collection.

### Vegetative Treatment System

We used existing conservation practices (vegetative buffers, grassed waterways) maintained for 11 of the studied pastures to improve the quality of storm runoff after exiting the pasture. In these instances, runoff was directed from the pasture through one of three best management practices: vegetative buffer strips (five pastures), grassed waterways (four pastures), or impoundments (two pastures). These studied conservation practices were “as built” and managed on these working farms in accordance with the NRCS Technical Field Guide (NRCS, 2004). Vegetation consisted primarily of annual grasses, with some associated forbs and perennial grasses that comprise California’s coastal rangelands. For the vegetative buffer strips and grassed waterways, the plant height and cover on these sites were dictated by the life cycle of these primarily annual plants, rainfall patterns, and sample collection timing with these life cycles. Germination took place with the first rains in October and November of each year, followed by moderate growth through the colder temperatures of December, January, and February, ending with increased growth to senescence in April, May, and June. As a result, cover was dominated by thatch and residual dry matter during early season storms, grass seedlings up to approximately 15 cm during the middle of winter, and fully grown grasses approximately 45 cm or taller in the spring. The two impoundments enlisted in this study were cement basins approximately 3 m wide, 10 m long, and sloped to 1 m of maximum depth. They were designed to facilitate solids settling.

### Sample Collection

It has been established that excessive FCB loading to Tomales Bay is rainfall dependent (O’Connell et al., 2000; Lewis et al.,

2005). This is consistent with findings from other systems along the Pacific Coast of North America (Shanks et al., 2006) and elsewhere (Kay et al., 2005), in which precipitation and storm runoff drive increased indicator bacteria values in tributary rivers or streams, and receiving bays. The California Department of Public Health uses 24-h cumulative precipitation from a local precipitation station, Tomasini Point, to regulate harvest closures of winter shellfish growing leases in the Bay. Accordingly, we conducted storm-based water sampling and analysis of storm runoff for FCB below each studied pasture.

Sample collection sites were identified that functioned as the downstream point of the microcatchment for each studied pasture. These downstream microcatchment sampling locations only have surface runoff during and immediately following storm events. Water samples for FCB concentration determination in pasture runoff and MMS were collected via grab sampling. Pasture samples were collected from each dairy or ranch, and respective sample locations during storms (two to six), when runoff was generated, across the entire season. In a few instances we collected a series of samples during each storm to directly characterize hydrograph position for collected samples.

MMS samples were collected from each studied MMS and respective barn, manure storage lagoon, or irrigation stage via grab sampling. Sample collection sites included respective effluent plumbing that provided access to manure moving through studied systems.

Instantaneous runoff was measured for each water sample collected using either the area-velocity method (velocity  $\times$  channel width  $\times$  channel depth  $\times$  0.85 to account for surface flow) (Mosley and McKercher, 1993) with a Global Waters flow meter (Global Waters Inc., Gold River, California, USA) or the time to fill a container of known volume. The method used was dependent on having a cross-sectional area of the running water sufficient to accommodate the flow meter.

## Microbial Enumeration

Enumeration of FCB concentrations was performed as described in Lewis et al. (2009), including the use of three to five 10- to 100-fold serial dilutions and adjustments for variable sample holding times and log<sub>10</sub> FCB concentration decay that resulted from the storm-based sampling.

The log<sub>10</sub> concentration of FCB followed a first-order decay process, such that  $\beta(t) = -0.0022$  with units of time set in hours (95% CI,  $-0.003$ ,  $-0.0014$ ). This decay coefficient did not vary significantly across the different sources of water ( $p$  value  $> 0.05$  for an interaction term between time and water source), indicating that a single decay coefficient can be used for adjusting FCB concentrations at  $t = x$  to a 24-h standard ( $t = 24$ ).

Enumeration method for *G. duodenalis* was performed as described in Miller et al. (2007), which used quantitative immunofluorescent microscopy with a percent recovery of 27.6%. Enumeration method for *Cryptosporidium* sp. was performed as described in Miller et al. (2008), which used quantitative immunofluorescent microscopy with a 21% recovery for water samples that had residual pellets of 50  $\mu$ L or less volume. For water samples that had residual pellets in excess of 50  $\mu$ L, quantitative immunofluorescent microscopy was preceded by

immunomagnetic separation that resulted in 47% recovery (Miller et al., 2008).

## Instantaneous Load Calculation

Using FCB concentration and instantaneous runoff rate for each sample event, we calculated instantaneous load of FCB for each sample event at each pasture, defined as:

$$\text{instantaneous load (cfu / s / ha)} = \frac{(\text{cfu / 100 mL})(10^6 \text{ mL / m}^3)(\text{m}^3 / \text{s})}{(\text{total surface area of loading unit in ha})}$$

where (cfu/100 mL) is the FCB concentration in the water sample and (m<sup>3</sup>/s) is the instantaneous runoff rate associated with that water sample. This calculation is necessary to compare between study areas on a standardized basis of per unit time and per unit area.

## Statistical Analysis

Linear mixed effects regression was used to test for differences in log<sub>10</sub> transformed FCB concentration and instantaneous load in pasture runoff as functions of site characteristics and management measure combinations (Pinheiro and Bates, 2000). Unique models were developed for FCB concentration and instantaneous FCB load. FCB concentration and instantaneous load were set as the outcome variables, with each pasture and MMS sample site set as a group effect to adjust the  $p$  values for repeated sampling at the same sites. A forward stepping approach was used to develop the multivariate regression models, with  $P \leq 0.1$  set as the criterion for inclusion of the variable in the final model. Our ability to develop multivariate regression models for *Cryptosporidium* sp. and *G. duodenalis*, as was done for FCB, was not possible given the small number of water samples that contained detectable levels of these protozoal parasites.

## Results and Discussion

### Pasture Storm Runoff

A total of 211 storm runoff samples were collected from the 34 studied pastures, including 86 in 2002–2003 and 125 in 2003–2004. Geometric mean FCB concentration for the entire dataset was 10,045 cfu/100 mL, ranging from 1 to  $7.6 \times 10^7$  cfu/100 mL. Geometric mean FCB instantaneous load for the entire dataset was  $9.1 \times 10^4$  cfu/ha/s, ranging from 1 to  $2.04 \times 10^9$  cfu/ha/s. Analogous geometric mean FCB concentration and load values in runoff from high-use areas on the same dairies and ranches were two to four orders of magnitude greater, or FCB concentration of  $10^4$  to  $10^5$  cfu compared with  $10^6$  cfu and more (Lewis et al., 2005; Lewis et al., 2009). The lower intensity use pastures studied in this investigation, however, represent one to two orders of magnitude more surface area than do the high-use areas. The result is that 1 ha of high-use area and 100 ha of pasture could present the same level of FCB loading to the watershed.

Of the 211 samples analyzed for protozoa, 8% ( $n = 17$ ) had detectable levels of *Cryptosporidium* sp. oocysts and 4% ( $n = 8$ ) had detectable levels for *G. duodenalis* cysts (Fig. 1). Mean *Cryptosporidium* sp. concentration for the 17 positive samples

was 77 oocysts/L, ranging from 2 to 716 oocysts/L. Mean *G. duodenalis* concentration for the eight positive samples was 10 cysts/L, ranging from 3 to 29 cysts/L. By comparison, the prevalence and concentration of these two protozoa were substantially higher in runoff from high-use areas with calf use on these same dairies and ranches. Specifically, *Cryptosporidium* sp. oocysts were found in 21% of the high-use area runoff samples, with a mean concentration of 642 oocysts/L and range of 2 to 1,818 oocysts/L (Miller et al., 2008). *G. duodenalis* was detected in 16% of the high-use area runoff samples, with a mean of 821 and range of 1 to 13,928 cysts/L (Miller et al., 2007). This difference in the prevalence and concentration of protozoa parasites in runoff from pastures compared to high animal use areas is likely the result of reduced animal density, manure deposition, and therein pathogen loading. It could also be explained by the observation that young calves (0 to 3 mo) are not allowed access to pastures, yet this younger population is the high-risk group for infection with *Cryptosporidium* sp. and *G. duodenalis*, resulting in higher levels of protozoa in runoff from calf high-use areas compared to locations dominated by older animals (e.g., pastures). Results presented for pastures in this paper (Fig. 1) and for high-use areas in Miller et al. (2007 and 2008) indicate that detection of *Cryptosporidium* sp. and *G. duodenalis* in dairy runoff only occurred for runoff samples with FCB concentrations in excess of  $1 \times 10^3$  cfu/100 mL.

With regard to precipitation, FCB concentration (Table 2) and instantaneous load (Table 3) in pasture runoff are significantly related to 24-h and cumulative precipitation at the time of sampling. Similar to results for high cattle use areas (Lewis et al., 2009), there are flushing dynamics of FCB concentration and load from pastures in relation to 24-h cumulative precipitation. This flushing is additionally related to cumulative precipitation at the date and time of sampling. There is an increase in FCB concentration up to approximately 40 mm of rainfall during a 24-h period (Fig. 2). Using the coefficients in Table 3, a threshold for maximum FCB instantaneous load is reached at approximately 500 mm of cumulative precipitation on the sampling date. The implication from these model coefficients is that FCB on these pastures is supply limited and subject to flushing and dilution during individual storms and as the storm season progresses.

Statistical model results indicate that directing pasture runoff through a vegetative treatment system and increasing the duration of time between manure application and storm-related runoff are both associated with reductions in FCB concentrations (Table 2) and instantaneous load (Table 3). With respect to FCB concentration and vegetative treatment systems, the model coefficient is negative, which indicates that as the length of the vegetative treatment increases, there is an associated decrease in the FCB concentration in pasture runoff (Table 2 and Fig. 3). Specifically, for every meter of VTS there is approximately a 2.7% reduction in FCB con-

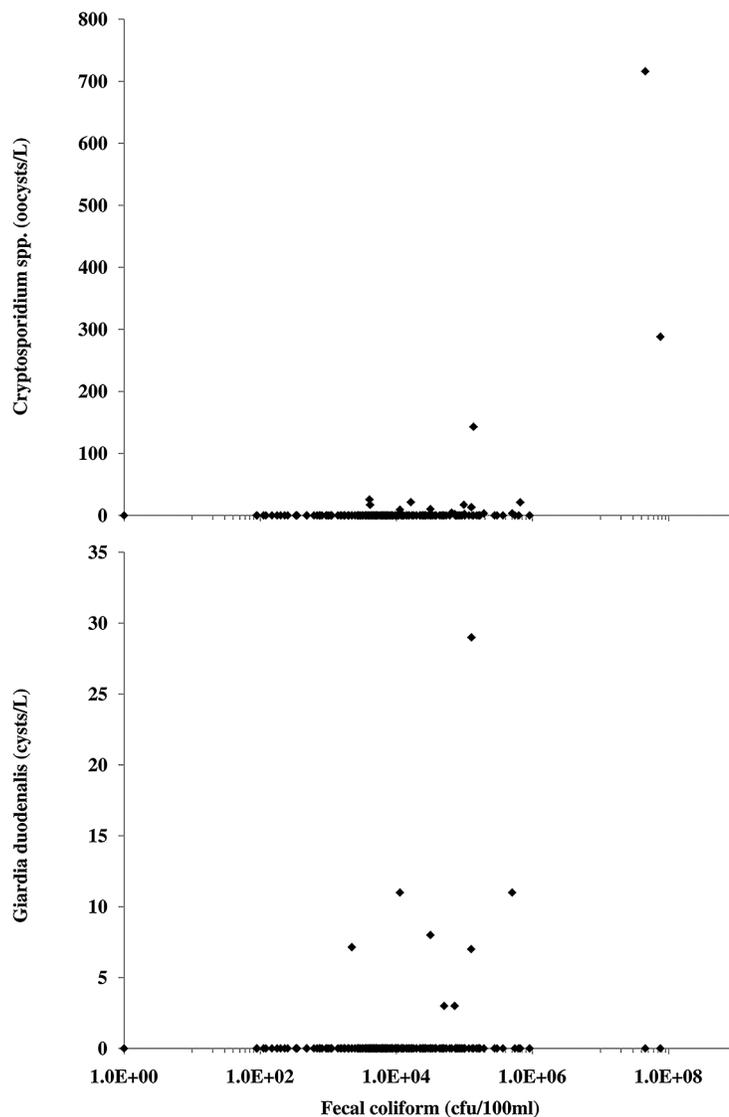


Fig. 1. Prevalence of *Cryptosporidium* spp. (top) and *Giardia duodenalis* (bottom) as a function of fecal coliform concentration in pasture storm runoff samples.

centration or cumulative reduction of 24% for every 10 m of VTS. This is consistent with previous work in which reductions in microbial pollution levels were documented as a function of VTS length in rainfall simulation, soil box (Davies et al., 2004;

Table 2. Linear mixed effects model for the associations of management practices and rainfall with fecal coliform concentration (log<sub>10</sub> value) in surface runoff from dairy and ranch pastures during storm conditions, 2002–2004, Tomales Bay, California.

Factor	Coefficient	95% CI†	P-value†
Constant or intercept term for the model	4.11	(3.67, 4.55)	<0.0001
24-h precipitation (mm)	0.07	(0.05, 0.09)	<0.0001
24-h precipitation <sup>2</sup> (mm)	-0.0009	(-0.001, -0.0006)	<0.0001
Manure application duration			
≤2 wk‡	0.0	-	-
2 to 4 wk	-0.80	(-1.20, -0.49)	0.0002
1 to 12 mo	-1.02	(-1.30, -0.69)	<0.0001
>12 mo	-1.46	(-1.91, -1.02)	<0.0001
Length of vegetated buffer (m)	-0.012	(-0.026, -0.001)	0.0738

† Adjusted for potential lack of independence due to repeated sampling of pastures across storms.

‡ Referent condition is manure application 2 wk or less before storm runoff generation and sampling.

**Table 3. Linear mixed effects model for the associations of management practices and rainfall with fecal coliform load (log10 value) in surface runoff from dairy and ranch pastures during storm conditions, 2002–2004, Tomales Bay, California.**

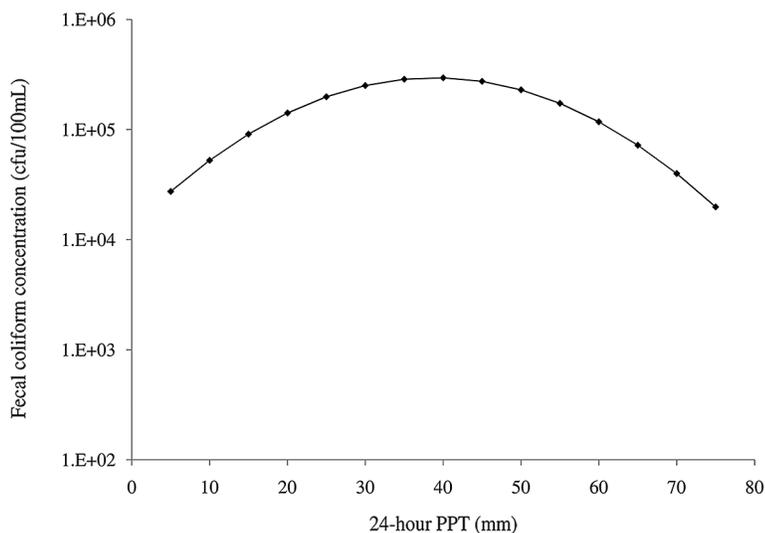
Factor	Coefficient	95% CI†	P-value‡
Constant or intercept term for the model	2.21	(1.26, 3.17)	<0.0001
24-h precipitation (mm)	0.14	(0.11, 0.16)	<0.0001
24-h precipitation <sup>2</sup> (mm)	-0.0017	(-0.0021, -0.0012)	<0.0001
Cumulative precipitation (mm)	0.01	(0.01, 0.02)	<0.0001
Cumulative precipitation <sup>2</sup> (mm)	-0.00001	(-0.00002, 0.000006)	0.0001
Manure application duration			
≤2 wk‡	0.0	-	-
2 to 4 wk	-0.84	(-1.41, -0.27)	<0.0002
1 to 12 mo	-0.99	(-1.54, -0.44)	<0.0001
>12 mo	-1.27	(-1.89, -0.64)	<0.0001

† Adjusted for potential lack of independence due to repeated sampling of pastures across storms.

‡ Referent condition is manure application 2 wk or less before storm runoff generation and sampling.

Ferguson et al., 2007), or runoff plot studies under natural rainfall (Tate et al., 2006; Sullivan et al., 2007). The magnitude of reduction resulting from VTS use on these hillside pastures is significantly lower, only 0.01 log<sub>10</sub> reduction per meter of VTS compared to one or more log<sub>10</sub> reduction indicated in controlled studies. Statistically significant differences in FCB concentrations and loads among the three different studied practices were not detected. Our study unit size (five vegetative buffer strips, four grassed waterways, and two impoundments) may have limited the ability to make this comparison, pointing to the need for a modified study design.

The association between FCB instantaneous load in pasture runoff and VTS length was not statistically significant ( $p > 0.1$ ). The relatively larger scale of these pastures is an environment in which load is dictated by precipitation and resulting runoff, which can overburden the ability of VTS to increase infiltration and therein reduce discharge and the resulting FCB load. These dairies and ranches, and the respective studied pastures, are on hillsides with relatively greater slopes (Table 1) than studied elsewhere (Kay et al., 2005; Shanks et al., 2006). These steeper slopes increase the amount of runoff gener-



**Fig. 2. Results from data-driven linear mixed effects model of fecal coliform concentration as influenced by 24-h cumulative precipitation. Manure application duration was <2 wk, or the model referent. The vegetated buffer length was set to zero.**

ated from rainfall relative to less-steep regions, contributing to the decreased ability of studied VTS to reduce FCB loads through infiltration and decreased discharge. Additionally, these conditions add to the difficulty of designing, implementing, and maintaining conservation practices that consistently approximate sheet flow as originally intended. Similar VTS limitations have been documented for *Escherichia coli* (Tate et al., 2006), *Cryptosporidium* sp. (Davies et al., 2004), and are explained by Atwill et al. (2009).

Both slope and length are driving factors for buffer efficacy and design (Koelsch et al., 2006), and the complex slopes in the study area restrict total VTS length and reduce the number of loca-

tions where VTS can be installed. The vegetation management required to maintain VTS effectiveness over an annual growth cycle, and therein its ability to improve runoff through sedimentation and infiltration (Bedard-Haughn et al., 2005; Koelsch et al., 2006), may not always be the highest priority for the farmer. It is because of these considerations and realities that many of the VTS we studied were vegetated channels or ditches lacking the sheet flow most desired for maximum reduction of pollutants in runoff (Koelsch et al., 2006; Knox et al., 2008).

Increasing the duration of time between manure application and storm-related runoff was negatively associated with both FCB concentration (Table 2) and instantaneous load (Table 3). This is evident by the increasingly negative coefficients as the duration is increased from less than 2 wk, 2 to 4 wk, up to 52 wk, and greater than 1 yr (Tables 2 and 3). Because we log<sub>10</sub> transformed the data before statistical modeling, the numerical values for these coefficients for duration are direct estimates of the mean log<sub>10</sub> reduction associated with these time durations. The longer the duration, the more negative the coefficient and

the greater the decrease in FCB concentration and load in the pasture runoff. Figure 3 demonstrates how each respective application duration group is associated with a subsequent reduction in FCB concentration. Relative to applying manure to a pasture less than 2 wk in advance of a runoff-generating storm event, waiting just a week or two more (i.e., 2 to 4 wk in advance of a runoff-generating storm event) was associated with a nearly 1-log reduction (~90% reduction) of FCB concentration and load. Further FCB concentration and load reductions were associated with even longer duration time between manure application and storm runoff, but at a diminishing rate of return. The reduction observed is similar to those documented by Meals and Braun (2006). In that study, *E. coli* levels in runoff from plots with 3-d old manure was 50% lower than for plots with fresh manure. Similarly, Li et al. (2005) documented how exposing fecal material to several days of ambient temperature typical for spring through fall conditions on California rangeland significantly reduced levels of *Cryptosporidium parvum*

oocysts in the manure. By taking advantage of a “treatment period” following manure application in advance of runoff-generating storms, producers are leveraging a combination of processes (e.g., desiccation, thermal stress, and predation) that reduce microbial pollutants (Atwill et al., 2009).

### Manure Management System Survey

A total of 115 samples were collected from cooperating farms with MMS. The geometric mean for the entire data set was  $4.6 \times 10^5$  cfu/100 mL and ranged from 70 to  $4.5 \times 10^8$  cfu/100 mL. The different MMS units, as well as the presence or absence of a solids separator, did not have significant associations ( $p > 0.1$ ) with FCB concentrations. However, the geometric mean and ranges for these units were not similar to each other. Barn effluent geometric mean FCB concentration was  $6.4 \times 10^6$  cfu/100 mL, ranging from  $4.1 \times 10^5$  to  $4.4 \times 10^8$  cfu/100 mL. Primary manure storage lagoon geometric mean was  $2.5 \times 10^6$  cfu/100 mL, ranging from 224 to  $4.5 \times 10^8$  cfu/100 mL. Secondary manure storage lagoon geometric mean was  $4.9 \times 10^4$  cfu/100 mL, ranging from 70 to  $2.6 \times 10^7$  cfu/100 mL. Irrigated manure geometric mean was  $2.6 \times 10^4$  cfu/100 mL, ranging from  $1.4 \times 10^3$  to  $1.7 \times 10^6$  cfu/100 mL.

Model results indicated that manure holding time or age of manure and FCB concentration are directly related in the studied MMS (Fig. 4). The material with the shortest holding time was barn effluent, with holding time increasing as manure effluent is moved from primary to secondary storage lagoons. The model’s log10 constant value was 6.51 cfu ( $p < 0.001$ ; 95% CI 6.16, 6.86), meaning that at a manure holding time of zero days the concentration of FCB in manure effluent was  $10^{6.51}$ , or about  $3.23 \times 10^6$  cfu/100 mL. Based on log10 transformed FCB concentration data, the first-order coefficients for manure holding time in days was  $-0.015$  cfu/100 mL/d ( $p < 0.0001$ ; 95% CI  $-0.019, -0.011$ ). This first-order die-off relationship is similar to previously documented modeling results for indicator bacteria (Crane and Moore, 1985; Moore et al., 1989). It is also interesting to note that Smith et al. (2009) found “longer storage times” of manure were associated with decreases of the pathogen *E. coli* 0157 on cattle farms.

These results predict FCB concentration reductions of one-half log 10 (75% reduction) for 20 d, one log10 (90%) for 66 d, two log10 reductions (99%) for 133 d, and three log10 reductions (99.9%) for 199 d of holding time. This negative association between holding time and FCB concentration was also evident in effluent samples being held for irrigation, indicating the important role that management of liquid manure can have on reducing FCB levels deposited on pastures (Fig. 4). Manure holding time for the collected samples ranged from <1 to 290 d. The studied farms have variable MMS infrastructure, with some lacking the resources to expand the infrastructure needed for increasing manure holding times. For these reasons, it is not typically feasible for producers to hold liquid manure

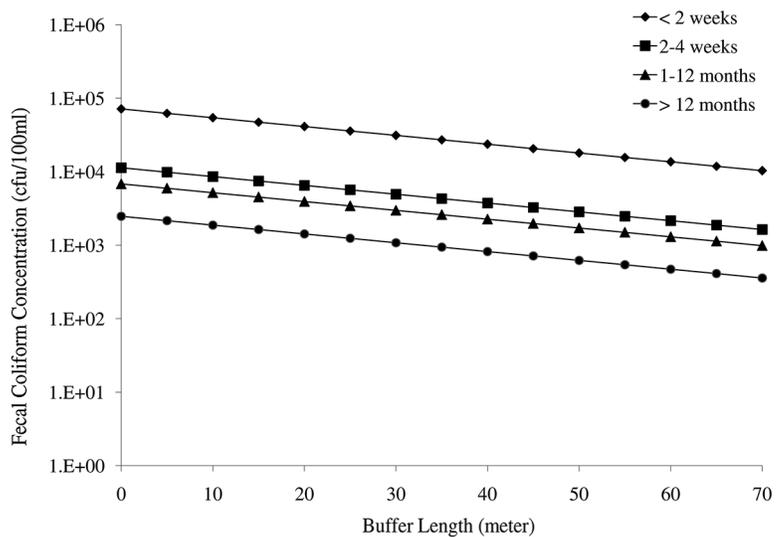


Fig. 3. Results from data-driven model of fecal coliform concentration as influenced by manure application duration and buffer strip length. The 24-h cumulative precipitation was held constant at 12.7 mm.

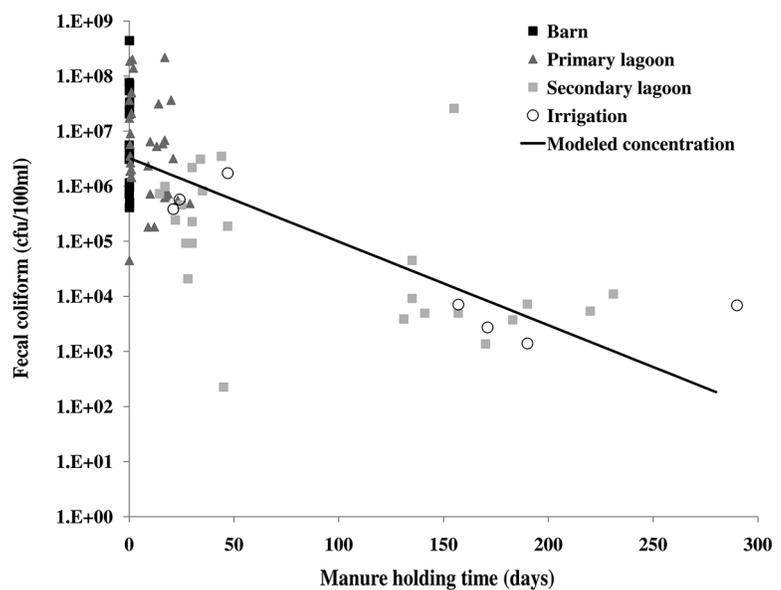


Fig. 4. Measured fecal coliform concentration values in manure management system effluent samples and data-driven model concentration as functions of manure holding time.

for periods approaching 200 or more days. However, facilitating manure holding times to between 60 d or 2 mo to 120 d or 4 mo before irrigation will significantly reduce the FCB concentration in the applied manure and therein reduce the FCB load on recipient pastures. It is important to point out that holding time, as a management practice to reduce FCB values before pasture application, can be compromised by the reinoculation of aged manure with fresh manure.

### Conclusions

Understanding and enhancing the ability of currently implemented conservation practices to reduce the risk of microbial pollution in runoff from agricultural sources to coastal waters is critical to meeting the dual objectives of viable agricultural production systems and water quality that maintains beneficial

uses downstream. Our management-scale study of grazed pastures receiving manure applications documents that reductions in FCB concentration and load are being realized from a combination of practices.

In the case of the studied pastures receiving applied manure, practices that manage the microbial levels in the applied material should be the first point of intervention. This includes practices that increase manure holding times to 1 mo or more and achieve a minimum 2- to 4-wk delay between manure application and potential microbial transportation during storm runoff events. With reductions in FCB and water-borne pathogens realized from those practices, use of VTS should be considered to facilitate additional improvements to water quality.

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