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Impact of irrigation water type and sampling frequency on Microbial Water Quality Profiles required for compliance with U.S. Food Safety Modernization Act Produce Safety Rule standards

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ABSTRACT

The U.S. Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) requires that farmers generate a Microbial Water Quality Profile (MWQP) from 20 samples per agricultural water source, taken over 2–4 years and five annual samples thereafter. Farmers must use the MWQP to ascertain a geometric mean (GM) of ≤126 CFU/100 mL and statistical threshold value (STV) of ≤410 CFU/100 mL of generic *Escherichia coli*. Farmers are responsible for collecting samples and paying for testing, incurring a financial and time burden. To determine if testing frequency can be reduced without compromising accuracy, water samples (n = 279) were collected from twelve sites in the U.S. Mid-Atlantic region from 2016 to 2018 comprising tidal brackish river, non-tidal fresh river, pond, vegetable processing, and reclaimed water. The GM and STV were calculated for all sites and water types using all samples, and for multiple sub-samples of <20 from each site and water type. A Monte Carlo simulation was used to determine the proportion of sub-sample sizes that yielded the same determination as the entire sample size of PSR standard compliance. Four sites, two pond and two reclaimed water sites, complied with PSR GM and STV requirements when using the entire sample set. When a water source's calculated GM and STV using the entire sample set hovered close to the PSR thresholds, sub-sample sizes approached the recommended 20 samples to reach a congruent compliance determination. However, 99% agreement was obtained with a sub-sample of five when the absolute difference between the GM and STV from total samples and the PSR thresholds was ≥2.6 and 4.5 log CFU/100 mL E. *coli*, respectively. These findings suggest that under certain conditions the MWQP may be generated with well below 20 samples, reducing the economic burden on farmers while still maintaining a representative MWQP.

1. Introduction

Foodborne diseases cause approximately 600 million illnesses per year leading to 420,000 deaths worldwide (World Health Organization, 2015; Scallan et al., 2011). Within the United States, foodborne diseases cause 48 million illnesses, 128,000 hospitalizations, and 3000 deaths annually (World Health Organization, 2015; Scallan et al., 2011). Produce (including seeded vegetables, herbs, vegetable row crops, and fruits) caused the most foodborne illnesses (28%), followed by chicken (12%) and pork (10%) in the U.S. from 2009 to 2015 (Dewey-Mattia

Abbreviations: Reclaimed water, (RW); Non-tidal fresh river water, (NF); Tidal brackish river water, (TB); On-farm pond water, (PW); Vegetable processing water, (PP); Geometric Mean from the entire sample set for each site, (GMTotal); Statistical Threshold Value from the entire sample set for each site, (STVTotal); Geometric Mean for each simulated combination of sub-samples, (GMSub); Statistical Threshold Value for each simulated combination of sub-samples, (STVSub).

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et al., 2018).

The Food Safety Modernization Act (FSMA) was passed by Congress in 2011 to improve food safety in the United States by identifying sources of possible contamination in the food chain, including those in the farm environment (Boys et al., 2015). One possible source of contamination on farms is water, which has multiple applications including irrigation, pesticide application, and cooling (Bowen et al., 2006; Castillo et al., 2004; Gagliardi et al., 2003; Geldreich and Bordner, 1971; Gelting et al., 2011; Riordan et al., 2001). FSMA has addressed the need to evaluate farm water sources through the Produce Safety Rule (PSR) agricultural water standards. Farmers falling under the PSR must generate a Microbial Water Quality Profile (MWQP) by calculating the Geometric Mean (GM) (a type of average) and Statistical Threshold Value (STV) (a measure of variation) of generic *Escherichia coli*, a widely used indicator of fecal contamination (Bihn et al., 2017). Any water source that will be used for irrigation and likely to contact fresh produce commonly consumed raw must meet the standards of GM $\leq$ 126 CFU/100 mL ($\leq$2.1 log CFU/100 mL) and STV $\leq$410 CFU/100 mL ($\leq$2.6 log CFU/100 mL) of *E. coli*.

The U.S. Food and Drug Administration (FDA) estimates the cost of water testing to be $87.30 per sample, although the United Fresh Produce Association calculated $120 per sample to be a more accurate estimate because of higher expected analysis costs and differences between farm sizes (United Fresh Produce Association, 2013; Wall et al., 2019). At a 2018 U.S. Water Summit that brought together farmers, academics, industry, and government to discuss needs and concerns related to the agricultural water section of the PSR, many farmers expressed concerns about the PSR's water testing requirements and the use of *E. coli* as an indicator organism (Wall et al., 2019). The frequency of testing was specifically cited as a concern. Many argued that more research is needed to justify the additional cost of more frequent testing than is recommended by Good Agricultural Practices (GAPs) standards that many farmers had previously followed and based on FDA's previous GAPs Guidance to Industry (FDA, 1998; Good Agricultural Practices/Good Handling Practices, n.d.; Wall et al., 2019). The FDA GAPs specify that more than one test is preferable, while the Maryland Department of Agricultural GAPs Certification program requires three surface water samples per season (FDA, 1998; Good Agricultural Practices/Good Handling Practices, n.d.). It is common for farmers to use multiple water sources, raising costs and logistical efforts exponentially (Wall et al., 2019). Many agricultural stakeholders also raised concerns that variations between different water types could influence the persistence and survival of pathogens, arguing that a one-size-fits-all approach may not be the best option (Haymaker et al., 2019; Wall et al., 2019). Additional research is needed on how farmers and regulators can feasibly implement the PSR requirements and how water type and sampling frequency can impact the MWQP (Gradl and Worosz, 2017; Rock et al., 2019).

Limited data exists on the impact of water type and sampling frequency on the GM and STV calculations required by FSMA's PSR. To our knowledge, only one study has examined the empirical and theoretical basis of the PSR's sampling requirements (Havelaar et al., 2017). No studies have compared multiple agricultural water types to systematically test how a range of sampling frequencies impact compliance with PSR standards. Therefore, using several agricultural water types, the objective of our study was to determine if reducing the number of samples required for a MWQP to less than 20 samples would yield GM and STV values similar to those calculated from a sample size of 20 or more.

2. Materials and methods

The current study is part of CONSERVE: A Center of Excellence at the Nexus of Sustainable Water Reuse, Food, & Health (conservewaterforfood.org). Through the sampling efforts of the CONSERVE team, we have access to water quality data spanning two years (2016–2018) and 12 sites in the Mid-Atlantic U.S. (Solaiman et al., 2020).

2.1. Sample sites

The sites selected for this study were either being currently used for irrigation of food crops or are potential future sources of irrigation water and have been previously described in detail (Panthi, 2019; Solaiman et al., 2020). Water types represented include tertiary treated municipal wastewater, also known as reclaimed water (RW), non-tidal fresh river water (NF), tidal brackish river water (TB), on-farm pond water (PW), and vegetable processing water (PP). Sample sites, water type, and number of samples are shown in Table 1. Samples were collected over two years, from September 2016 to October 2018, twice per month during the growing season (mid-March to mid-November). Sampling dates and the total number of samples for each site were variable depending on accessibility (Table 1).

2.2. Sample collection and processing

Sample collection and processing have been described previously (Haymaker et al., 2019; Sharma et al., 2020; Solaiman et al., 2020). Briefly, at each site, 1 L of water was collected into sterile 1 L polypropylene bottles (Thermo Fisher Scientific, Waltham, MA, USA). For surface water sites, bottles held with a sampling stick (Zenport Industries, Portland, OR, USA) were inverted, submerged until 15–30 cm below the water surface, and turned sideways until full. For reclaimed water sites, water was collected from spigots close to field release sites (e.g., sprinklers used for groundwater recharge or animal feed crop irrigation). Before sample collection from spigots, water was allowed to run for 1 min. For all water types, bottles were immediately transferred to coolers containing ice packs for transport to the laboratory. Samples were stored overnight at 4 °C.

Analysis for *E. coli* quantification is described in Solaiman et al. (2020). Briefly, within 12 h, standard membrane filtration was carried out according to EPA Method 1604 for enumeration of *E. coli* (EPA, 2002). Four different volumes (0.1 mL, 1 mL, 10 mL and 100 mL) of water, were filtered through 0.45 μm, 47 mm cellulose ester membrane filters (Pall Corporation, Ann Arbor, MI, USA). Smaller volumes (0.1 mL and 1 mL) of water were adjusted to 10 mL with sterile DI water before filtration. Membrane filters (Pall Corporation, Ann Arbor, MI, USA) were placed aseptically onto MI agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for quantification of *E. coli*. All MI plates were incubated for 24 h at 37 °C. Blue colonies on MI plates were recorded as *E. coli*. A fluorescent lamp was used for counting to give maximum visibility of colonies.

2.3. Statistical analysis

To construct the MWQP, we first calculated the GM by summing the log-transformed *E. coli* counts from each water source (measured in CFU/100 mL), then dividing by the number of samples for that site (Bihn et al., 2017). We then converted the log-transformed GM to the regular scale by taking the antilog to calculate the GM. We next calculated the STV using the formula:

$$\log(\text{STV}) = (\text{log-transformed GM}) + 1.282*\text{std}(\log \text{values})$$

We calculated the GM and STV for each site using all samples (n = 13–27 depending on site, Table 1). Since the PSR requires that a MWQP be constructed using at least 20 data points, we refer to the GM and STV calculated from the entire sample set for each site as GM_{Total} and STV_{Total}. The specific sample sizes used to calculate GM_{Total} and STV_{Total} for each site are provided in Table 1.

For analyses by water type, sites with the same water type were combined (Table 1). Tidal brackish and vegetable processing water were not included in the water type comparison analysis as there was only one

Table 1
Geometric mean (GM_{Total}) and statistical threshold values (STV_{Total}) calculated for agricultural water sources from each sampling site collected from October 2016–October 2018 using the entire data set.

Site(N)	Water Type	GM_{Total}[a] in CFU/100 mL E. *coli* (Compliant/Non-Compliant[b])	GM_{Sub}[c]% agreement(n)	STV_{Total} in CFU/100 mL E. *coli* (Compliance/Non-Compliant	STV_{Sub} % agreement(n)
MA01 (19)	Reclaimed Water	15.91 (C)	94% (3)	315.89 (C)	97% (17)
MA02 (17)	Reclaimed Water	4.59 (C)	99% (2)	56.32 (C)	92% (4)
MA03 (25)	Non-tidal Fresh River Water	126.98 (NC)	68% (24)	1058.20 (NC)	91% (11)
MA04 (26)	Non-tidal Fresh River Water	80.87 (C)	90% (14)	907.75 (NC)	90% (9)
MA05 (24)	Non-tidal Fresh River Water	273.11 (NC)	92% (4)	1416.86 (NC)	90% (4)
MA06 (24)	Reclaimed Water	15.03 (C)	90% (3)	711.03 (NC)	93% (21)
MA07 (25)	Non-tidal Fresh River Water	126.57 (NC)	56% (24)	2488.21 (NC)	93% (6)
MA08 (26)	Tidal Brackish River Water	538.92 (NC)	95% (2)	3157.44 (NC)	96% (3)
MA09 (26)	Non-tidal Fresh River Water	236.77 (NC)	91% (8)	1822.17 (NC)	91% (6)
MA10 (27)	Pond	12.16 (C)	91% (2)	208.48 (C)	96% (19)
MA11 (27)	Pond	13.50 (C)	91% (2)	249.44 (C)	90% (18)
MA12 (13)	Vegetable Processing Water	587.93 (NC)	91% (5)	29606.62 (NC)	91% (2)

[a] GM_{Total} and STV_{Total} were used to calculate the percentage of sub-samples that agreed with the PSR standard determination using Monte Carlo simulations is shown, with n representing the size of the sub-sample used to calculate agreement.
[b] Compliance to the PSR standard is given in parentheses, with C = compliant to PSR standard and NC = non-compliant PSR standard. The FSMA PSR standard is ≤ 126 CFU/100 mL generic *E. coli* for the geometric mean and ≤410 CFU/100 mL generic *E. coli* for statistical threshold value.
[c] GM_{Sub} and STV_{Sub} refer to the GM and STV obtained from sub-sets of the data for that site.

site each for those water types and therefore the combined data is no different than the site data. To evaluate differences in GM_{Total} by water type, the Bonferroni method was used to calculate a confidence interval for the GM_{Total} for each water type (Table 2). Statistical significance was determined by overlap of the confidence intervals for GM_{Total} between water types.

Table 2
Geometric mean (GM_{Total}) and statistical threshold values (STV_{Total}) calculated for agricultural water sources grouped by water type collected from October 2016–October 2018 using the entire data set.

Water Type (N)	GM_{Total}[a] in CFU/100 mL E. *coli* (Compliant/Non-Compliant[b])	GM 95% Confidence Interval[c]	STV_{Total} in CFU/100 mL E. *coli* (Compliant/Non-compliant)
Tidal Brackish River (26)	538.92[A] (NC)	(234.40, 1239.02)	3157.44 (NC)
Non-tidal Fresh River (126)	152.13 [A] (NC)	(95.89, 241.35)	1554.18 (NC)
Pond (54)	12.81[B] (C)	(5.28, 31.11)	222.13 (C)
Vegetable Processing (13)	587.93 [A] (NC)	(32.13, 10759.03)	29606.62 (NC)
Reclaimed (60)	10.93[B] (C)	(4.18, 28.61)	289.20 (C)

[AB]Indicate statistically significant differences in the geometric means between water type at a significance level of 0.05.
[a] GMTotal and STVTotal were used to calculate the percentage of sub-samples that agreed with the PSR standard determination using Monte Carlo simulations is shown, with n representing the size of the sub-sample used to calculate agreement.
[b] Compliance to the PSR standard is given in parentheses, with C = compliant to PSR standard and NC = non-compliant PSR standard. The FSMA PSR standard is ≤ 126 CFU/100 mL generic *E. coli* for the geometric mean and ≤410 CFU/100 mL generic *E. coli* for statistical threshold value.
[c] The Bonferroni method was used to calculate GM 95% confidence intervals.

We also calculated the GM and STV for each simulated combination of sub-samples, which we define as GM_{Sub} and STV_{Sub}, to determine if the values calculated from a sub-set of samples diverged from GM_{Total} and STV_{Total} for that water source. To evaluate this, we ran a Monte Carlo simulation. A Monte Carlo simulation is a probability simulation that informs users on how likely a particular outcome is by running a model hundreds or thousands of times, each time using different randomly-selected values. The first step in the data analysis was to generate all possible combinations of samples for sub-sample sizes of two through the total number of samples for each site (Table 1). The number of sample combinations was capped at 50,000 since increasing the number of combinations past this point did not significantly impact the results. We then calculated a binomial proportion for each sub-sample size at each site to determine the percentage of combinations where the PSR compliance determination (according to the GM_{Sub} and STV_{Sub}) was congruent with PSR compliance determination based on the GM_{Total} and STV_{Total}. For example, site MA01 had a GM_{Total} of 15.9 CFU/100 mL E. *coli* (n = 19) which is less than the PSR standard of 126 CFU/100 mL and therefore determined to be in compliance. Using a sub-sample size of 2, 88% of the sub-sample combinations also had a GM_{Sub} less than 126 CFU/100 mL and therefore in those cases using a smaller sample size to calculate the GM was congruent with GM_{Total}.

We also performed simple linear regressions to assess the relationship between the number of samples required to achieve a binomial proportion greater or equal to 99% congruency between GM_{Total} and GM_{Sub} or STV_{Total} and STV_{Sub}, and the absolute difference of GM_{Total} or STV_{Total} from the PSR standards for both individual sites and water type. These absolute differences were calculated in log CFU/100 mL and compared to GM and STV thresholds of 2.1 and 2.6 log CFU/100 mL, respectively.

In all cases, *p*-values of ≤0.05 were defined as statistically significant. All statistical analyses were performed using Statistical Analysis Software (SAS) Studio version 9.4 (Cary, NC) or Microsoft Excel (Redmond, WA).

3. Results

3.1. Microbial Water Quality Profiles

Using the entire sample set (n = 279 samples from 12 sites), 33% (4/12) of sites complied with the PSR requirements for both GM (≤126 CFU/100 mL E. *coli*) and STV (≤410 CFU/100 mL E. *coli*). Fifty percent (6/12) of the sites met the PSR GM standard but exceeded the STV standard (Table 1).

When samples were grouped by water type to analyze *E. coli* concentrations, pond water (n = 54 samples from two sites) and reclaimed water (n = 60 samples from three sites) met the PSR standards for both GM and STV. Tidal brackish river water (n = 26 samples from one site), non-tidal fresh river water (n = 126 samples from five sites) and vegetable processing water (n = 13 samples from one site) did not meet the PSR standards for GM or STV (Table 2). GMs for pond water and reclaimed water were significantly different ($p < 0.05$) from the GMs for tidal brackish water, non-tidal fresh river water, and vegetable processing water (Table 2).

3.2. Sampling frequency assessment

For nine out of 12 sites, a sub-sample size of eight or less was sufficient for at least 90% of sub-sample combinations to reach the same determination of compliance with the PSR GM requirement (≤126 CFU/100 mL E. *coli*) (Table 1, Fig. 1). Although sites MA01, MA02 and MA12 had sample sizes less than 20 (as required for the PSR MWQP), we observed similar results as those sites that had 20 or more total samples (Table 1).

Sites MA01, MA02, MA10 and MA11 complied with the PSR standards based on GM_{Total} and STV_{Total} (n = 17–27, Table 1). For these sites, GM_{Sub} yielded 91–99% agreement with the determination of compliance to the PSR standard based on GM_{Total} with sub-sample sizes of 2–3 (Table 1). Sites MA03 and MA07 (n = 25) had a GM_{Total} that hovered close to the PSR threshold of ≤126 CFU/100 mL E. *coli* and whose STV_{Total} exceeded the PSR threshold of ≤410 CFU/100 mL (Table 1,

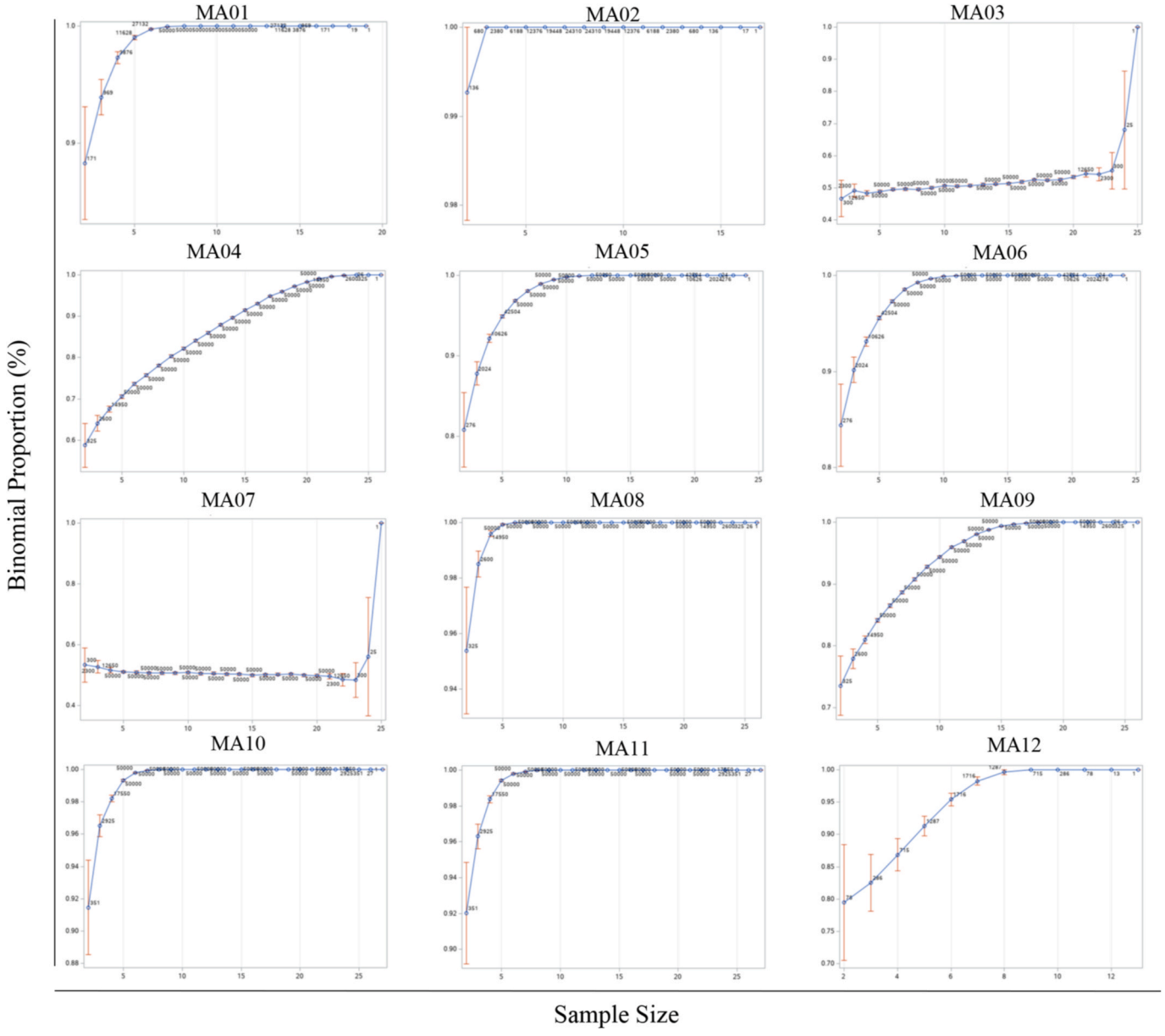

Fig. 1. Binomial proportions using different combinations of sub-samples of the geometric mean (GM_{sub}) of *E. coli* agreeing with the GM_{Total} Produce Safety Rule compliance determination (GM ≤ 126 CFU/mL) by sampling site.

Fig. 1). These two sites required sub-sample sizes of 24 to reach agreement with the determination based on GM_{Total} at least 56% of the time.

Nine samples or less were sufficient for 90–96% of STV_{Sub} combinations to agree with the PSR standard determination based on STV_{Total} for seven out of 12 sites (Table 1, Fig. S1). A sub-sample size of less than half of the total sample size was sufficient for sites MA02, MA03, MA04, MA05, MA07, MA08, MA09, and MA12 for STV_{Sub} agreement with compliance determination based on STV_{Total} at least 90% of the time (Table 1, Fig. S1).

When non-tidal fresh river water, reclaimed water, and pond water samples were combined by water type, a sub-sample size of five or less was sufficient for at least 90% of pond and reclaimed sub-sample combinations to agree with the PSR standard determination based on GM_{Total} (Fig. S2). However, pond water and reclaimed water required a higher proportion of the total number of samples to reach agreement with the PSR STV standard determination (Fig. S3). A sub-sample size of 25/54 for pond water and 47/60 for reclaimed water was required for agreement with the STV_{Total}-based PSR STV standard determination 90% of the time (Fig. S3). For non-tidal fresh river water, a sub-sample size of 70/126 was required for agreement with the GM_{Total}-based PSR standard determination 91% of the time (Fig. S2) and a sub-sample size of 8/126 was required for 92% agreement with the PSR STV_{Total} standard determination 91% of the time (Fig. S3).

3.3. Association between sampling frequency requirements and absolute difference from PSR standards

Discrepancies between MWQP data and PSR standards were calculated in log CFU/100 mL, where the GM and STV thresholds are 2.1 and 2.6 log CFU/100 mL, respectively. If the GM_{Total} and STV_{Total} diverged by only ±0.71 and ± 2.20 log CFU/100 mL E. *coli*, respectively, from the PSR thresholds, 19 samples were as good as 20 samples in reaching the same conclusion of passing or failing PSR standards (Fig. 2). Ninety-nine percent agreement with compliance was achieved between GM_{Total} and GM_{sub} (n = 5) and between STV_{Total} and STV_{sub} (n = 5) if the absolute difference between GM_{Total} and STV_{Total} and the PSR thresholds was at least 2.6 and 4.5 log CFU/100 mL E. *coli*, respectively (Fig. 2). As noted earlier, sites MA03 and MA07 had GMs close to the PSR standard of 126 CFU/100 mL, at 126.98 CFU/100 mL and 126.57 CFU/100 mL, respectively. Because the GM calculated for *E. coli* at these sites were close to the PSR standard, it was not possible to reduce the number of samples used for GM calculations to reach the same PSR standard determination, as was found with the total sample size (Fig. 2a). For STV, an $R^2 = 0.55$ ($p = 0.006$) indicated that, for sites with larger absolute differences from the STV standard (410 CFU/100 mL), a lower number of samples can be used to reach the same PSR compliance determination as was reached with the full sample set (Fig. 2b).

Water types for which GM_{Total} and STV_{Total} diverged considerably from the PSR standards of ≤126 CFU/100 mL and ≤410 CFU/100 mL E. *coli* (in either direction) required a smaller number of sub-samples to obtain a congruent GM_{Sub} and STV_{Sub} (Fig. 3), compared to sites with a GM_{Total} or STV_{Total} close to the PSR standards. When GM_{Sub} and GM_{Total} and STV_{Sub} and STV_{Total} were congruent, the same conclusion on whether the water source was compliant with the PSR could be reached with fewer than 20 samples. When we regressed the number of samples required to achieve a binomial proportion greater or equal to 99% on the absolute difference between GM_{Total} and GM_{Sub}, we obtained an $R^2 = 0.67$ ($p = 0.09$) (Fig. 3a). The GM_{Total} for non-tidal fresh river water had the smallest absolute difference from the PSR standard of 126 CFU/100 mL E. *coli* and required 100/126 samples to achieve a binomial proportion greater or equal to 99% congruency between GM_{Total} and GM_{Sub}. For STV, the R^2 value was 0.82 ($p = 0.03$), and pond water and reclaimed water had STV values closest to the PSR standard of 410 CFU/100 mL, and therefore required the most samples to achieve at least 99% binomial proportion congruency between GM_{Total} and GM_{Sub} (Fig. 3b).

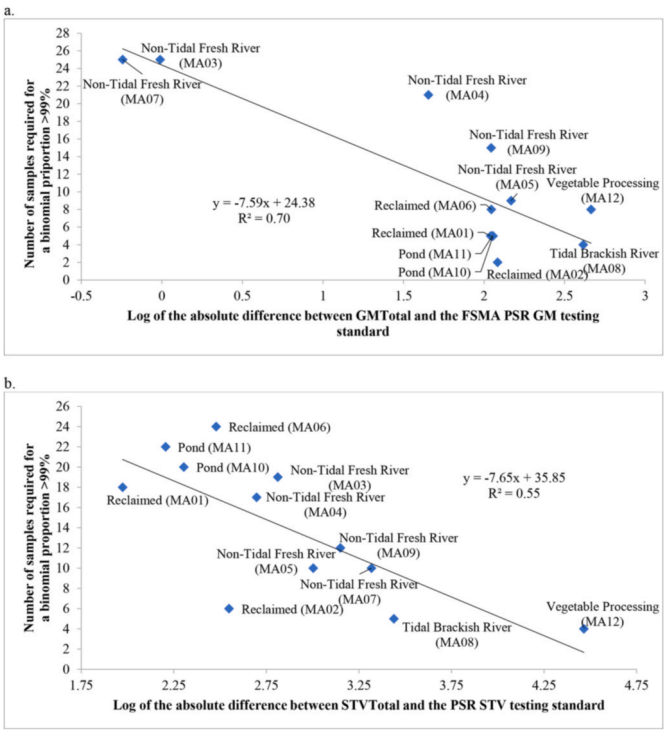

Fig. 2. Relationship between the sample size required for a binomial proportion >99% and the absolute difference between the a) geometric mean (GM_{Total}) and b) statistical threshold value (STV_{Total}) calculated for each site using all available measurements and the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) water testing standards (GM ≤ 126 CFU/100 mL; STV ≤410 CFU/100 mL).

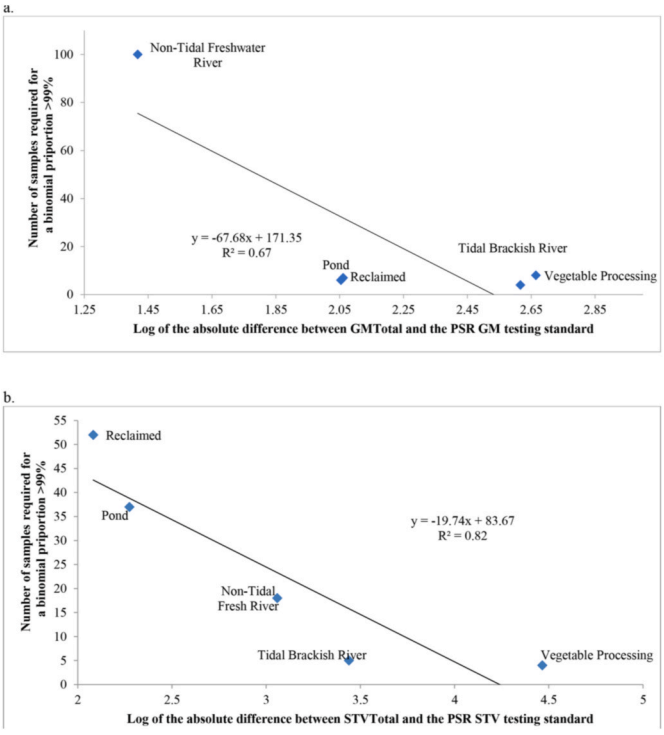

Fig. 3. Relationship between the sample size required for a binomial proportion >99% and the absolute difference between the a) a) geometric mean (GM_{Total}) and b) statistical threshold value (STV_{Total}) calculated for each water type and the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) water testing standards (GM ≤ 126 CFU/100 mL; STV ≤410 CFU/100 mL).

4. Discussion

4.1. Impact of water type on PSR compliance

Farmers use a variety of water sources including surface water, groundwater, rainwater, and reclaimed water (Agricultural Water, 2016). In our previous survey of farmers in the Mid-Atlantic (D.C., Delaware, Maryland, Pennsylvania, Virginia, and West Virginia) and Southwest (Arizona, California, Colorado, Nevada, New Mexico, and Utah) regions of the U.S., we found that the most commonly used source of water in the Mid-Atlantic is groundwater (59%) while surface water is the most commonly used water source in the Southwest (61%) (Suri et al., 2019). The results from the current study found that when looking at water types in the Mid-Atlantic region, pond water and reclaimed water met PSR standards for GM and STV, whereas tidal brackish river water, non-tidal fresh river water and vegetable processing water did not meet PSR standards for GM or STV (Table 2). These results suggest that water quality differs depending on the specific type of surface water (pond, tidal brackish, or non-tidal fresh river water) or reclaimed water used. Tidal brackish river water, vegetable processing water, reclaimed water and pond water required a sub-sample size of five or less for agreement with the PSR standard determination for GM at least 90% of the time. For STV, a sub-sample size of two to seven was required for tidal brackish river water, non-tidal fresh river water and vegetable processing water for agreement with the PSR standard determination 96%, 90%, and 91% of the time, respectively. Future research with additional samples and sites representing each water type would be needed to confirm the patterns observed in this study.

4.2. Implications for PSR water testing sampling frequency requirements

The agricultural community has questioned if the current number of samples (n = 20 over 2–4 years) required in the PSR are necessary, especially given the economic burden on farmers. Once initial data for building the MWQP is obtained, there is the potential to lower the number of samples required for the remainder of and past the 2–4 years that encompass the baseline period. In Florida, Havelaar et al. (2017) used water samples from six agricultural ponds to compare the GMs and STVs using all 90 samples from each pond to subsets of 20 samples each. They determined that 20 samples were not sufficient to characterize the bacteriological quality of pond water due to the variability of *E. coli* levels and stated that updating the GM and STV with five samples per year would hinder the ability to notice shifts in water quality (Havelaar et al., 2017). In our study using data from the Mid-Atlantic region in a Monte Carlo simulation with up to 50,000 replicates, we found that as the absolute difference between the GM_{Total} and STV_{Total} of a site and PSR threshold values increased, the number of sub-samples required to achieve high agreement with GM_{Total} and STV_{Total} decreased. Due to the association between the number of samples required for high congruency between GM_{Total}/STV_{Total} and GM_{Sub}/STV_{Sub} and the absolute difference between the site and PSR regulation values, a sub-sample size of 19 was sufficient to reach agreement if values fell within a defined range of *E. coli* counts beyond the PSR thresholds. At this level, ±0.71 and ± 2.20 log CFU/100 mL from the GM and STV in the PSR standards, respectively, meant the sub-sample number could be reduced by one. These findings suggest that a MWQP could be constructed from 19 samples instead of 20 if the data fall within the defined range. By contrast, larger differences between MWQP data and the PSR standards resulted in a dramatic reduction in sub-samples needed to attain agreement. Sub-samples of only five were sufficient when the absolute difference between MWQP parameters and the PSR thresholds were at least ±2.6 and ± 4.5 log CFU/100 mL for GM and STV, respectively. Subsequently, our findings suggest that if the initial 2–4 year period and establishment of a MWQP shows significant deviation from the GM and STV PSR standards, as defined here, the PSR could be updated with four instead of five samples annually. Requiring less than 20 samples for a MWQP and four samples to be taken per year, thereafter, would reduce the financial and logistical burden on farmers.

Farmers who have periodically tested their water for the purposes of compliance requirements for Good Agricultural Practices (GAP) programs or other similar programs, may be able to use recent data already collected on *E. coli* levels in their water sources to determine how many samples are required for PSR water testing. As of July 2021, twenty-one farms in Maryland met USDA GAP and GHP criteria (Companies that meet USDA GAP & GHP acceptance criteria, n.d.). A 2013 survey found that 23% of Maryland farmers used surface water for irrigation some of the time and 76% of Maryland farmers did not perform annual testing of irrigation water for fecal contamination (Marine et al., 2016). As of 2013, about 40% of farmers surveyed (n = 266) in Georgia, South Carolina, and Virginia used well water for irrigation that had been tested for *E. coli*, and 31% of farmers used untested well water, surface water, or rainwater for irrigation (Harrison et al., 2013). If farmers have water sources with *E. coli* values that diverge at least 2.1 and 2.6 logs from the PSR values for GM (126 CFU/100 mL) and STV (410 CFU/100 mL) respectively, then fewer samples could potentially be used to determine whether their water source continues to exceed or comply with the PSR standards. Therefore, if the FDA were to reduce the number of annual samples required by farmers with existing MWQP data showing the defined deviations from GM and STV PSR standards, farmers would save time and money while preserving a reliable understanding of water quality (United Fresh Produce Association, 2013; Wall et al., 2019).

4.3. Impact of reduced sampling frequency on farmers

Compliance with the PSR is estimated to cost U.S. farmers and the foreign produce sector $460 million and $171 million annually, respectively (Costs to Farmers and Consumers – Produce Rule). The FDA estimates the cost of compliance with the Produce Rule will be $4477 per year for very small farms ($25,000 to $250,000 in annual sales), $12,384 per year for small farms ($250,001 to $500,000 in annual sales), and $29,545 per year for large farms (over $500,000 in annual sales) (Costs to Farmers and Consumers – Produce Rule). Although the rule includes exemptions for farms with average annual food sales less than $500,000 per year, the FDA has the ability to revoke an exemption if there is significant risk of a foodborne illness outbreak or if an outbreak can be directly linked to a farm (FSMA Final Rule on Produce Safety - 2016). If a very small or small farm has their exemption revoked, the cost of complying with the PSR would take a significant portion of their profits, potentially up to over half of the profits from a very small farm.

A 2019 systematic review found financial difficulty was one of the four most cited influences on farmers' mental health, referenced in 21% of studies on the mental health of U.S. farmers (Daghagh Yazd et al., 2019). Government regulation was the fifth most cited risk factor for farmers' mental health (Daghagh Yazd et al., 2019). Due to the expected financial burden of PSR on farms, especially small farms (making less than $500,000 annually) which make up about 90% of farms in the U.S., many farmers could experience an increase in stress as a result of complying with PSR regulations (ERS, USDA. Farming and Farm Income.).

The cost of collecting and processing twenty samples ranges from $1746 to $2400 depending on the source of the cost estimate (United Fresh Produce Association, 2013; Wall et al., 2019). Allowing farmers to utilize previous, recent water quality data to determine whether the water meets or exceeds the PSR standard determination would result in cost savings for the farmers. If farmers were allowed to collect four samples annually rather than five to update the MWQP, there would be an annual savings of $175 to $240 for the farmer per water source, resulting in savings of $875 to $1200 over 5 years, a substantial amount for many farmers (United Fresh Produce Association, 2013; Wall et al., 2019).

4.4. Potential interacting factors

The results of this study only used growing season samples due to the seasonality of *E. coli* levels (Anderson et al., 1983; Blaustein et al., 2013; Faust et al., 1975). *E. coli* levels tend to be lower in surface waters during winter months (Solaiman et al., 2020). Therefore, if samples taken outside of the growing season had been included, it could have lowered the GM and STV for the time of year when farmers are using agricultural water, leading to an erroneous increase in the number of sites and water types that comply with PSR regulations. Confounding factors such as temperature, rainfall, sunlight, and the surrounding soil type from each site could have influenced *E. coli* levels (Anderson et al., 1983; Blaustein et al., 2013; Faust et al., 1975; Pachepsky and Shelton, 2011; Tassoula, 1997; Whitman et al., 2004). Due to regional differences in environmental factors, differences in *E. coli* levels throughout the U.S. and the variety of water sources used throughout the country, these findings may not be generalizable to areas outside the Mid-Atlantic U.S. where the samples were collected. Characterization of water sources in different regions would help determine applicability of these data.

For the Monte Carlo simulation, the simulated samples were all pulled from data collected from the same site over two years and the number of repetitions for each sample size was capped at 50,000. Preliminary calculations with substantially larger sample sizes for a subset of the sites indicated that the overall conclusions reached in this manuscript would not change with more than 50,000 repetitions. For some sites, the total number of samples was less than the required 20 data points collected over 2–4 years that are needed to generate a MWQP in compliance with the PSR, so the total number of samples within two growing seasons was used as reference. Results from these sites with less than 20 total samples were similar to those from sites with the required 20 or more total samples.

5. Conclusions

The FSMA PSR was written with the goal of improving food safety in the U.S., and water quality monitoring is an important component of a preventative approach. However, the regulation has placed an increased responsibility and economic burden on farmers due to the high frequency of water quality testing. As financial difficulties and government regulations are some of the most cited influences on farmers' mental health, it is important to evaluate if fewer water samples would be sufficient to reach the same PSR compliance decision to reduce the cost of sample collection and processing. If the GM and STV from the full sample size are close to the PSR standards (126 CFU/100 mL and 410 CFU/100 mL *E. coli*), the current recommendations of adding five yearly data points to the MWQP would seem adequate. On the other hand, our results show that as a water source's GM and STV diverge from PSR standards (either higher or lower), smaller sub-sample sizes were sufficient to reach agreement regarding the determination on PSR compliance as with the full sample size. This means that an MWQP of less than 20 samples would be sufficient under certain conditions and once a MWQP is generated, four samples or less each year thereafter would be adequate to maintain a robust profile. Reducing the number of samples required per year would provide economic and logistical relief to farmers during the growing season when farming and harvesting activity is at its peak, especially benefitting small-scale growers who are disproportionately impacted.

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Declaration of competing interest

All of the authors have no competing interests or conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112480.

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