# Seasonality of *Enterococcus* levels in Deer Island effluent

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## **1.0 Introduction**

The Massachusetts Water Resources Authority's (MWRA) Deer Island Treatment Plant (DITP) provides secondary wastewater treatment to 2 million people in the metropolitan Boston area. Under the National Pollutant Discharge Elimination System (NPDES) permit issued jointly by the U.S. Environmental Protection Agency (EPA) and the Massachusetts Department of Environmental Protection (DEP), MWRA monitors effluent fecal coliform bacteria levels against permit limits through daily sampling and testing. Rex (2011), updated by Codiga *et al* (2016), highlighted the MWRA's monitoring of *Enterococcus* and fecal coliform in DITP's receiving water, Massachusetts Bay. This report summarizes the results of *Enterococcus* monitoring in the effluent, including a comparison between effluent and receiving water bacteria levels. Although the current DITP NPDES permit limits only fecal coliform, the analysis presented in this report examines *Enterococcus*. *Enterococcus* was added to the effluent monitoring program at DITP in the early 2000s in anticipation of its inclusion in revised EPA recreational water quality criteria and Massachusetts state water quality standards.

## 2.0 Background

The completion of the deep water outfall for DITP in September 2000 transferred the effluent discharge from the shallow waters of Boston Harbor to the deeper waters of Massachusetts Bay. Public concern resulted in an extremely comprehensive NPDES permit, which not only included the standard effluent monitoring, but also extensive ambient – or receiving water – monitoring.

One of the advantages of a deep water outfall is the high level of dilution it provides. Studies conducted in support of outfall design and permitting (Roberts *et al* 1993a, 1993b) indicated a minimum of 70-fold dilution. These studies were validated by dye studies conducted in April and July of 2001 (Hunt *et al* 2002a, 2002b; Roberts *et al* 2011). The NPDES permit incorporates this dilution factor by assigning a geometric mean fecal coliform bacteria limit of 14,000 CFU/100mL in final effluent. This was calculated by taking the Massachusetts water quality standards at the time of permit issuance – July 2000 – for Class SA waters (waters suitable for shellfishing and primary contact recreation) and applying the minimum dilution factor of 70:

 Class SA fecal coliform standard for primary contact recreation at the time of permit issuance of 200 CFU/100mL \* dilution factor of 70 = NPDES effluent limit of 14,000 fecal coliform CFU/100mL In recent years, EPA and the public health community has moved away from the use of fecal coliform as a pathogen indicator in recreational waters in favor of the indicator bacteria *Enterococcus*, particularly in marine waters. Following a recommendation made in 1986 for bacterial water quality criteria, EPA promulgated rules in 2004 that lead to the use of *Enterococcus* as the indicator of choice for marine waters, and *Enterococcus* has been adopted as the primary pathogen indicator in the latest Massachusetts water quality standards (USEPA 2004; 314 Code of Massachusetts Regulations [CMR] 4.00). Future NPDES permits for marine dischargers such as DITP may include *Enterococcus* as either a limit or a "report only" parameter, the latter meaning that a parameter is not subject to a defined limit, but results are reported to the regulatory agencies. To prepare for these anticipated regulatory changes, MWRA has monitored effluent *Enterococcus* levels for more than 10 years in conjunction with the permit-required fecal coliform monitoring.

Currently, the Class SA standards for *Enterococcus* in Massachusetts are a geometric mean of 35 colonies/100mL calculated over a specified time period and a single sample maximum of 104 colonies/100mL (314 CMR 4.05(4)(a)[4][b]).

For the purposes of this report, the potential *Enterococcus* geometric mean limit, assuming the minimum dilution factor of 70 is unchanged, is calculated as:

 Class SA geometric mean limit of 35 colonies/100mL \* dilution factor of 70 = potential limit of 2,450 colonies/100mL for a geometric mean over a specified time period (e.g., daily, weekly, monthly)

The potential single sample limit is calculated similarly:

• Class SA single sample limit of 104 colonies/100mL \* dilution factor of 70 = potential limit of 7,280 colonies/100mL for a single *Enterococcus* sample

The potential geometric mean limit of *Enterococcus* of 2,450 colonies/100mL and the potential single sample limit of 7,280 colonies/100mL will be referenced throughout the remainder of this report. They are projected potential limits based on a minimum dilution factor of 70, and current state water quality standards. While actual future limits remain unknown, these potential limits are useful for the purpose of the present analysis to assess the consequences on compliance with a change in bacterial indicators.

#### **3.0 Methods**

Effluent samples for bacteria analysis (both *Enterococcus* and fecal coliform) are collected from a sampling site (MWRA location code DEFF) located after the weirs at the end of the chlorine disinfection basins, before the effluent drops into the shaft to the outfall tunnel. Samples are therefore subject to chlorination but not dechlorination. Since the injection site for the sodium bisulfite used in dechlorination is located in the outfall tunnel itself and inaccessible to samplers, dechlorination is simulated in the sample bottle. At the time of sample collection, the sampling staff calculates the effluent travel time from DEFF to the dechlorination point using PI Process Book™ (OSIsoft, LLC; San Leandro, CA) software available at the lab. The effluent sample is set aside for the calculated time, simulating the additional contact time with chlorine between DEFF and the sodium bisulfite injection site. Dechlorinating agent is then added manually to the sample bottle. *Enterococcus* and fecal coliform samples are collected simultaneously three times a day, 365 days a year, at the DEFF site (MWRA DLS 2013a).

The samples are analyzed using the IDEXX Enterolert<sup>®</sup> method by MWRA's Department of Laboratory Services (DLS) as described in the MWRA DLS Standard Operating Procedure (SOP) for *Enterococcus* testing (MWRA DLS 2013b). Results are reported in Most Probable Number (MPN) per 100mL, equivalent to colonies/100mL.

DLS began to use the Enterolert<sup>®</sup> method exclusively in late March 2007. In order to ensure maximum comparability of data both within and between years, only data from the full years 2008 to 2014 were used (n=7,669 individual *Enterococcus* samples).

## 4.0 Results and Discussion

For the effluent analysis in this report, samples that were non-detects – reported in the DLS Laboratory Information Management System (LIMS) as <10 MPN/100mL – are treated as 1 MPN/100mL.

Table 1 below provides descriptive statistics for individual samples. The numbers are not aggregated by day, month, or year. NPDES permits generally require the use of geometric means to report effluent bacterial data. Arithmetic means are not typically used for effluent bacteria, but are a more conservative measure than the geometric mean, and are included for comparison to results in Codiga *et al* (2016), which are partially based on arithmetic means.

| Count | Non-             | Samples >   | Arithmetic  | Geometric   | Min*        | Max*        |
|-------|------------------|-------------|-------------|-------------|-------------|-------------|
|       | detects          | 104         | Mean        | mean        | (MPN/100mL) | (MPN/100mL) |
|       | (%)              | MPN/100mL   | (MPN/100mL) | (MPN/100mL) |             |             |
|       |                  | (%)         |             |             |             |             |
| 7669  | 1,210<br>(15.7%) | 3,837 (50%) | 718         | 78          | 4           | 24,200      |

| Table 1. | <b>Descriptive statistics for</b> | Enterococcus single | e samples, 2008-2014 |
|----------|-----------------------------------|---------------------|----------------------|
|          |                                   |                     |                      |

\* Excludes non-detects, which are reported as 1. 24,200 MPN/100mL is the maximum detection limit for the current method and dilution used by DLS, which allows for a minimum detection limit of 10 MPN/100 mL. Some results may be lower than the standard detection limit of 10 MPN/100mL due to the use of differing dilutions.

Table 2 shows a breakdown of the single sample data by month. The number of non-detects is highest during the summer-fall months, and drops from December to April, when high *Enterococcus* counts are the most common. Note the total number of single samples exceeding 104 MPN/100mL was 3,837 (50%). When the dilution factor of 70 is applied, the total number of samples exceeding the potential 7,280 MPN/100mL limit is considerably less – 124 samples, or 1.6%.

| Table 2. | Effluent Enterococcus | s single sample data | by month, 2008-2014 |
|----------|-----------------------|----------------------|---------------------|
|----------|-----------------------|----------------------|---------------------|

|                | Jan    | Feb    | Mar    | Apr    | May    | Jun    | Jul    | Aug    | Sep    | Oct    | Nov    | Dec    | Total  |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Samples        | 654    | 591    | 652    | 629    | 650    | 630    | 651    | 651    | 629    | 651    | 630    | 651    | 7,669  |
| Samples > 104  | 589    | 578    | 621    | 524    | 367    | 252    | 76     | 42     | 40     | 109    | 186    | 453    | 3,837  |
| (%)            | (90.1) | (97.8) | (95.2) | (83.3) | (56.5) | (40.0) | (11.7) | (6.5)  | (6.4)  | (16.7) | (29.5) | (69.6) | (50)   |
| Samples > 7280 | 21     | 31     | 44     | 10     | 0      | 1      | 1      | 0      | 1      | 1      | 4      | 10     | 124    |
| (%)            | (3.2)  | (5.2)  | (6.7)  | (1.6)  | (0)    | (0.16) | (0.15) | (0)    | (0.16) | (0.15) | (0.63) | (1.5)  | (1.6)  |
| Nondetects     | 6      | 0      | 2      | 9      | 29     | 52     | 199    | 260    | 313    | 231    | 94     | 15     | 1,210  |
| (%)            | (0.9)  | (0)    | (0.3)  | (1.4)  | (4.5)  | (8.3)  | (30.6) | (39.9) | (49.8) | (35.5) | (14.9) | (2.3)  | (15.8) |

The following series of figures presents effluent *Enterococcus* data from DITP. To avoid repetition, the figures are briefly described here and analysis of the figures will follow.

Figure 1 shows the distribution of the single samples collected and analyzed between 2008 and 2014, by month. The horizontal lines on the boxes are, from top to bottom, the 75<sup>th</sup>, 50<sup>th</sup>, and 25<sup>th</sup> percentiles. The whiskers are the 90<sup>th</sup> (top) and 10<sup>th</sup> (bottom) percentiles. "Outlier" samples greater than the 90<sup>th</sup> percentile and less than the 10<sup>th</sup> percentiles are represented by dots, with the absolute highest and lowest values as open circles. Note that this and most other graphs in this report are plotted on a logarithmic y-axis. For Figures 1 and 2, the horizontal line is the potential single sample limit of 7,280 MPN/100mL.

Figure 2 shows a time series of the individual samples from the entire 2008-2014 study period.

Figures 3 through 7 show *Enterococcus* data that has been aggregated into daily (Figures 3 through 5), weekly (Figure 6), and monthly geometric means (Figure 7), to which the potential

geometric mean limit of 2,450 MPN/100mL is applicable. The current DITP permit requires evaluation of fecal coliform daily geometric means and the maximum weekly geometric mean in a given month against the fecal coliform limit of 14,000 CFU/100mL. While the maximum weekly geometric means for the month were not calculated, all the weekly means during the 2008-2014 period were calculated and are included in Figure 6.

Figure 3 shows the daily geometric means in a time series format. Figure 4 shows the same data by year, with each year being overlaid over the other years in the study period. Figure 5 is a distribution plot of the daily geometric means by year in the same format as Figure 1, although individual outlier means are not included.

Figures 6 and 7 show the weekly geometric means and the monthly geometric means, sorted by year, and overlaid over one another – similar to Figure 4 above.

Weekly geometric means in Figure 6 are calculated using MWRA's standard NPDES reporting protocol – with the week running from Sunday to Saturday. Although monthly geometric means are not currently a permit limit for DITP, Figure 7 shows the monthly geometric means for *Enterococcus* against the potential permit limit of 2,450 MPN/100mL.



Figure 1. Distribution of *Enterococcus* single samples, by month, 2008-2014 Horizontal line is the potential single-sample limit of 7,280 MPN/100 mL.



**Figure 2.** Time series of single sample *Enterococcus* data, 2008-2014 Horizontal line is the potential single-sample limit of 7,280 MPN/100 mL.



Figure 3. Time series of daily effluent *Enterococcus* geometric means, 2008-2014 Horizontal line is the potential limit of 2,450 MPN/100 mL.



Figure 4. Yearly comparison of daily effluent *Enterococcus* geometric means, 2008-2014 Horizontal line is the potential limit of 2,450 MPN/100 mL.



**Figure 5.** Distribution plot of daily *Enterococcus* geometric means, by year Outliers above the 90<sup>th</sup> and below the 10<sup>th</sup> percentile are not displayed, although the absolute maximum and minimum means are, as open circles. Horizontal line is the potential limit of 2,450 MPN/100 mL.



Figure 6. Yearly comparison of effluent *Enterococcus* weekly geometric means, 2008-2014 Horizontal line is potential limit of 2,450 MPN/100 mL.



Figure 7. Yearly comparison of effluent *Enterococcus* monthly geometric means, 2008-2014 Horizontal line is potential limit of 2,450 MPN/100 mL.

There is a clear seasonal trend apparent in all of the preceding figures (excepting Figure 5, which is an annual comparison). *Enterococcus* counts rise considerably in the winter and early spring, with counts peaking in March. The rise begins in December and high counts continue into April and sometimes May. The summer and fall months consistently show low counts and high numbers of nondetects relative to the "peak season" of December to April. This pattern holds true over both the single sample and the aggregated geometric mean data.

For both single sample and aggregated data, nearly all of the samples or geometric means that exceeded the potential limit(s) happened in the winter or early spring. As mentioned previously, 124 of the single samples were above the potential single sample limit of 7,280 MPN/100mL (Figs. 1 and 2). For daily geometric means, there were 169 days over the potential 2,450 MPN/100mL limit (Figs. 3 and 4). For both the single samples and the daily geometric means, most of exceedences were in the winter or early spring.

The period 2008-2014 contained 366 NPDES permit weeks; of those, 14 weeks were above the potential limit of 2,450 MPN/100mL. Weekly geometric means ranged from 10 MPN/100mL to 6,943 MPN/100mL. As Figure 6 shows – and as should be expected given the previous discussion – the high values were in the weeks of year corresponding to the winter and spring, and were low in the summer and fall.

Surprisingly, there was only one exceedence of the potential monthly geometric mean limit during the 2008-2014 timeframe (Fig. 7). Unsurprisingly, however, that exceedence occurred during March 2010. Not only is March typically a wet month in the Boston area, March 2010 saw a record amount of rainfall (14.9 inches) since the outfall tunnel was first opened in 2000.

Figure 5 shows a relatively low inter-year variability in the distribution of daily geometric means. Therefore, seasonal changes seem to be the major driving force in changes in *Enterococcus* counts.

The seasonal pattern of late winter and early spring high effluent bacteria levels is thought to result from two main factors. The first factor is that the efficacy of chlorine-based disinfection decreases with temperature (CSU-Sacramento 1989, USEPA 1999). Virus studies in drinking water indicate that contact time needs to be increased two or three times to compensate for an 18 degree Fahrenheit (deg F) decrease in temperature. The lowest wastewater temperatures at DITP occur from December to April, where average effluent temperatures from 2008 to 2014 were below 60 deg F. In August and September, the months with the highest numbers of nondetects, the effluent temperature averages close to 71 deg F.

The second factor is that chlorination at DITP occurs in a fixed-length basin so the contact time, and thus disinfection efficiency, decreases when effluent flow rates are high. Effluent flow rates tend to be highest in winter and spring due to rainfall and snowmelt.

At DITP, the factors most impacting disinfection effectiveness – high flow, short disinfection contact time, and low wastewater temperatures occur at the same time – the winter and early spring. The result seems to be the seasonal trend of high counts during that part of the year that are seen in the effluent *Enterococcus* results – both single sample and aggregated geometric means. Table 3 summarizes the flow and disinfection contact time data along with the temperature data, by month. Figure 8 plots monthly means of flow, contact time, and temperature with monthly geometric means of *Enterococcus*. Note how the curves fit well against each other. Figure 9 is a scatter plot of contact time versus temperature, with the *Enterococcus* counts colormapped against each point. Again, high counts cluster in the areas where either contact time is short or effluent temperature is low, or a combination of the two.

|          | Jan   | Feb   | Mar   | Apr   | May   | Jun   | Jul   | Aug   | Sep   | Oct   | Nov   | Dec   |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Effluent | 334.6 | 372.4 | 457.2 | 379.7 | 306.8 | 311.5 | 275.4 | 270.2 | 270.4 | 298.2 | 306.0 | 378.9 |
| flow     |       |       |       |       |       |       |       |       |       |       |       |       |
| (MGD)*   |       |       |       |       |       |       |       |       |       |       |       |       |
| Contact  | 1.00  | 0.95  | 0.80  | 0.91  | 1.07  | 1.08  | 1.22  | 1.27  | 1.26  | 1.17  | 1.15  | 0.96  |
| time     |       |       |       |       |       |       |       |       |       |       |       |       |
| (hrs)*   |       |       |       |       |       |       |       |       |       |       |       |       |
| Effluent | 55.9  | 54.3  | 53.7  | 57.0  | 61.3  | 65.4  | 69.2  | 70.8  | 70.7  | 67.7  | 63.8  | 59.0  |
| temp     |       |       |       |       |       |       |       |       |       |       |       |       |
| (deg F)* |       |       |       |       |       |       |       |       |       |       |       |       |

 Table 3. DITP average effluent flow, disinfection contact time, and temperature by month, 2008-2014

\*Data are from DITP PI at the time of *Enterococcus* sample collection and are not subject to strict QA/QC procedures. Data were accessed through PI-Datalink. Effluent flow, contact time, and temperature are from tags EFF-TFLOW, DACTTOS.C, and DA8T502A, respectively. Table revised 12/8/16.





Process data (temperature, contact time, and flow) are from PI-Datalink. Note inverted right hand yaxes of temperature and contact time. Figure revised 12/8/16.



#### Figure 9. Colormapped scatter plot of disinfection contact time versus effluent temperature for each Enterococcus sample collected from 2008-2014

Points are then mapped to the color scale on the right indicating *Enterococcus* counts. Note that high counts cluster in the lower left corner where effluent temperatures are low and contact time is short. Process data (temperature, contact time, and flow) is from PI-Datalink. Figure revised 12/8/16.

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#### 5.0 Effluent Measurements vs. Receiving Water Measurements

Codiga et al (2016) summarized the bacteria samples taken in DITP's receiving water, Massachusetts Bay, by MWRA field teams and consultants between 1999 and 2014. As with the effluent samples, these receiving water samples were analyzed by the MWRA's Central Lab. This section compares results from the 2008 to 2014 effluent samples with the receiving water samples collected in the same time period from stations N16 and N20, located near the eastern and western ends of the outfall (referred to as the "Outfall" areas in Figure 1 and Table 1 in Codiga *et al*). These stations are located where high counts attributable to MWRA effluent would be expected, if they occurred. Unlike effluent samples, which are collected three times a day, the frequency of receiving water sampling is much lower, typically one survey per month. The counts of samples collected from the surface and sub-pycnocline depths were aggregated (Codiga *et al* covers the receiving water sampling methodology; the *Enterococcus* analysis method used is IDEXX Enterolert<sup>®</sup>, the same used for effluent samples). For the receiving waters, the geometric means were computed by adding 1 to the raw values (because nondetect values were <1, rather than the <10 for the effluent values, as minimum detection limits are lower for seawater samples), calculating the geometric mean, then subtracting 1 from the result.

*Enterococcus* numbers in the receiving water are a small fraction (between about 1/10,000 and 1/10) of the counts seen in the effluent. The first table (Table 4) shows the descriptive statistics of the "Outfall" area samples. Note both the low number of samples and the high percentage of nondetects (91.4% of the samples were nondetects; compare to Table 2, which shows that only 15.8% of the effluent samples were nondetects). The three highest *Enterococcus* counts seen in the period were 30, 30, and 98 MPN/100mL, collected in June 2014, December 2014 and September 2012, respectively. Even these three highest counts were not above the state water quality standard single sample limit of 104 MPN/100mL. Table 5 is a basic comparison of effluent and receiving water data when aggregated by monthly geometric mean.

|               | Jan    | Feb   | Mar    | Apr    | May    | Jun    | Jul    | Aug    | Sep    | Oct   | Nov    | Dec    | Total  |
|---------------|--------|-------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|
| Samples       | 14     | 14    | 20     | 20     | 26     | 28     | 28     | 32     | 28     | 28    | 16     | 14     | 268    |
| Samples > 104 | 0      | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0     | 0      | 0      | 0      |
| (%)           | (0)    | (0)   | (0)    | (0)    | (0)    | (0)    | (0)    | (0)    | (0)    | (0)   | (0)    | (0)    | (0)    |
| Nondetects    | 13     | 14    | 19     | 18     | 23     | 25     | 27     | 28     | 23     | 28    | 15     | 12     | 245    |
| (%)           | (92.9) | (100) | (95.0) | (90.0) | (88.5) | (89.3) | (96.4) | (87.5) | (82.1) | (100) | (93.8) | (85.7) | (91.4) |

#### Table 4. Receiving water Enterococcus single sample data by month, 2008-2014

Table 5. Comparison of effluent and receiving water *Enterococcus* monthly geometric means, 2008-2014

|             | Jan | Feb | Mar  | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|-------------|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Effluent    | 504 | 895 | 1029 | 393 | 115 | 60  | 12  | 7   | 5   | 12  | 38  | 247 |
| geometric   |     |     |      |     |     |     |     |     |     |     |     |     |
| mean        |     |     |      |     |     |     |     |     |     |     |     |     |
| (MPN/100mL) |     |     |      |     |     |     |     |     |     |     |     |     |
| Receiving   | 0.2 | 0   | 0.1  | 0.2 | 0.3 | 0.3 | 0.1 | 0.4 | 0.9 | 0   | 0.8 | 0.4 |
| water       |     |     |      |     |     |     |     |     |     |     |     |     |
| geometric   |     |     |      |     |     |     |     |     |     |     |     |     |
| mean        |     |     |      |     |     |     |     |     |     |     |     |     |
| (MPN/100mL) |     |     |      |     |     |     |     |     |     |     |     |     |
| Effluent    | 654 | 591 | 652  | 629 | 650 | 630 | 651 | 651 | 629 | 651 | 630 | 651 |
| samples (n) |     |     |      |     |     |     |     |     |     |     |     |     |
| Receiving   | 14  | 14  | 20   | 20  | 26  | 28  | 28  | 32  | 28  | 28  | 16  | 14  |
| water       |     |     |      |     |     |     |     |     |     |     |     |     |
| samples (n) |     |     |      |     |     |     |     |     |     |     |     |     |



Figure 10. Comparison of effluent and receiving water *Enterococcus* geometric means by month, 2008-2014

Note that *Enterococcus* was not detected in the receiving waters in February and October during the 2008-14 time period. The log y-axis exaggerates monthly variations in the receiving water *Enterococcus* counts, which range from 0-0.9 MPN/100mL, compared to 5-1,029 MPN/100mL in the effluent.

*Enterococcus* sample sizes from the receiving waters are small, especially compared to the effluent sampling program. Nonetheless, the vast majority of samples are non-detects (91.4%). These results from 2008-2014 are broadly representative of all years sampled since 1999 (as

shown in Codiga *et al* 2016). Neither the relatively *Enterococcus* high counts in the effluent nor the strong seasonal effluent signal is seen in the receiving waters (Tables 4 and 5, Figure 10).

While such a seasonal signal might not be expected in some of the study regions further from the outfall – the "Nearfield" and "Coastal" regions referenced in Codiga *et al* (2016) – one would expect to see a greater correlation with the effluent levels in the "Outfall" region, as the samples are collected very near the outfall discharge area. It is possible that the travel time in the outfall tunnel reduces *Enterococcus* counts – as described above, the collected sample is held to simulate the additional contact time to the dechlorination point. After dechlorination, at the average flow of 361 MGD for the 2008-2014 study period, effluent still has approximately 11.1 hours to travel through the outfall tunnel to the discharge point into Massachusetts Bay. Bacterial die-off may occur this time. This could explain why that even in the spring, when daily effluent *Enterococcus* geometric means frequently exceed 1,000 MPN/100mL, elevated counts are not detected in the receiving water. This supports the conclusion that the seasonably variable effluent counts at the treatment plant have very little influence on the very high rate of compliance with *Enterococcus* standards in the outfall receiving waters.

#### 6.0 Operational and Permit Compliance Implications

The results and discussion section touched on the three most likely factors that influence the seasonality of the *Enterococcus* counts: flow, temperature, and contact time. Figure 8 emphasizes the relationship between those three factors and the actual *Enterococcus* counts, while Figure 9 emphasizes the relationship between contact time (which is a proxy for flow), temperature, and *Enterococcus* counts. As flows increase and contact times and wastewater temperatures decrease, *Enterococcus* counts increase and the number of nondetects becomes negligible.

An obvious process change to address these issues would be to increase chlorine dosing. Tree *et al* (2003) show that while *Enterococcus* is more resistant than *E. coli* to chlorine, 100% kill still occurred within 15 minutes under laboratory conditions in primary effluent, where greater amounts of solids than exist in DITP's secondary-treated effluent would shield bacteria from disinfection. In this scenario, costs for chemical purchases for both additional chlorine and sodium bisulfite (used for dechlorination) needed for higher dosing would increase. Currently, DITP has budgeted for the additional costs.

Higher chlorine dosing at DITP, while likely effective, could result in effluent toxic to marine organisms, even after dechlorination. The DITP NPDES permit requires whole effluent toxicity (WET) testing monthly at DITP. WET testing involves exposing test organisms to varying

dilutions of effluent to assess both acute (death) and chronic (growth and reproduction) effects. Chlorine, interactions between chlorine and ammonia, and compounds formed as byproducts of the disinfection reactions are a known contributor to toxicity effects on organisms used for WET testing (Szal *et* al 1991, Wang *et al* 2007).

Another feature of the current NPDES permit is the inclusion of the mixing zone and the 70:1 dilution factor afforded to the fecal coliform limits. This is a key item to retain in the next permit. Without the dilution factor, fully half of the *Enterococcus* single samples collected from 2008-2014 were over the single sample limit of 104 MPN/100mL. However, with the dilution factor, only 124 samples (or 1.6%) were over the potential limit of 7,280 MPN/100mL. Figures 11 and 12 dramatically illustrate the need for a mixing zone-based dilution factor for *Enterococcus*. Since the opening of the outfall tunnel, compliance with the fecal coliform limit with the dilution factor has been excellent, but without the dilution factor, single sample *Enterococcus* counts would be in violation of a likely water quality standard-based permit limit for nearly nine months of the year at current levels of chlorine dosing.

Figures 11 and 12 also graphically illustrate seasonal trends in nondetects (highest number in the low flow/high wastewater temperature months of summer and early fall) and high *Enterococcus* counts (highest numbers in the high flow/low wastewater temperature months of winter and early spring).



Figure 11. Percent effluent *Enterococcus* samples in three categories if the 70:1 dilution factor *is not* applied

The three categories are: nondetects, detected but below the 104 MPN/100mL single sample limit, and samples equal to or above the 104 MPN/100mL limit.



Figure 12. Percent effluent *Enterococcus* samples in three categories if the 70:1 dilution factor *is* applied

The three categories are: nondetects, detected but below the 7,280 MPN/100mL single sample limit, and samples equal to or above the 7,280 MPN/100mL limit.

Given that non-compliance with *Enterococcus* limits are most frequent during the winter months when primary recreation is unlikely, this argues against the need for additional treatment measures at this time of year. Fecal coliform limits and effluent sampling would likely remain in place, as shellfishing standards continue to use fecal coliform and not *Enterococcus* limits; in any case, Codiga *et al* (2016) found that fecal coliform levels in Massachusetts Bay are very low and do not show any seasonal effects.

An alternative would be to retain fecal coliform as a bacterial indicator in a new DITP NPDES permit, and have *Enterococcus* be a report-only or a seasonally limited parameter to limit increased chlorine use. Given an *Enterococcus* report-only alternative in a NPDES permit, MWRA would report *Enterococcus* for permit-required reporting but would not be held to a specific *Enterococcus* limit. For the seasonal limit, *Enterococcus* would be limited in the summer and fall months but report-only in the winter and spring. Since permit limits are based on water quality standards and one of the rationales of the Massachusetts water quality standards is to protect public health (314 CMR 4.01(4)), there is no need to have an *Enterococcus* limit in the winter months where minimal – if any – recreational activity would be occurring in Massachusetts Bay around the outfall. Precedents also exist for seasonal limits – Clinton Treatment Plant has seasonal permit limits in its NPDES permit for ammonia and phosphorus. Ammonia is limited throughout the year but the limit fluctuates by month. For phosphorus, it is limited from May to October but is a report-only parameter for the balance of the year. Something analogous could be put into place at DITP for *Enterococcus*.

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