# Appendix C: Analysis of NEEAR culture data: combining marine and freshwaters

**Analysis of NEEAR culture data: combining marine and freshwaters**

*Bottom line: Marine and freshwater culture-derived data do not reflect a pattern suggesting that they are different. Based on our observations, marine and freshwater culture enterococci data are combinable.*

*Rationale and Evidence:*

- The main source of the fecal contamination (and thus, fecal indicators) - WWTP effluent - is the same at all NEEAR study beaches.
- The 1986 RWQC geometric mean (GM) indicator density, which is to be used as the reference point in the 2012 RWQC is (effectively) the same in freshwater and marine waters (GM 33 versus 35, respectively).
- The number of beaches evaluated in the NEEAR studies was a relatively small (4 freshwater, 3 temperate marine, and 1 tropical marine) subset of all waterbodies that the RWQC will apply to.
- The literature indicates that of the factors influencing enterococci fate in the environment, there is more evidence that sunlight, temperature and predation are more important in controlling enterococci concentrations than the effects of salinity.

*Analytical Approach for Comparing NEEAR Beaches:*

To determine if beaches should necessarily be grouped by salinity (grouping beaches as freshwater versus marine) we conducted several analyses. First, the observed range of culturable enterococci for each NEEAR beach was plotted in a box-and-whisker plot (Figure 1). As shown, there is substantial overlap in the observed densities across all beaches. There is also substantial intra-beach variability.

Next, we used an ANOVA analysis of the NEEAR culturable enterococci to test for equality of GMs between beaches. This test indicated that the means (Refer to Table 1) between beaches were significantly different ($p<0.001$). Since more than two beaches were compared, a statistically significant effect in the ANOVA only indicates that the GMs are different between the beaches, but not which beaches are different and how.

Given the overlap of observed culturable enterococci among all beaches (Figure 1) we examined the variability between individual beaches. A post-hoc test (Tamhane's test) was used to ascertain which pairs of GMs are significantly different. This test is suitable in this situation because the variances are not equal and the number of beaches in groups is not constrained to be equal. The major advantage of this test is that it accounts for both intra- and inter- variability in GMs for beaches to inform the groupings; another advantage is that it is a conservative test, accounting for multiple comparisons between beach GMs (the type I error $\alpha$ is constrained to be less than 0.05). As shown in Table 1, these statistically derived groups are not aligned strictly by classification of whether the waterbody was a fresh or marine beach.

*Figure 1.*

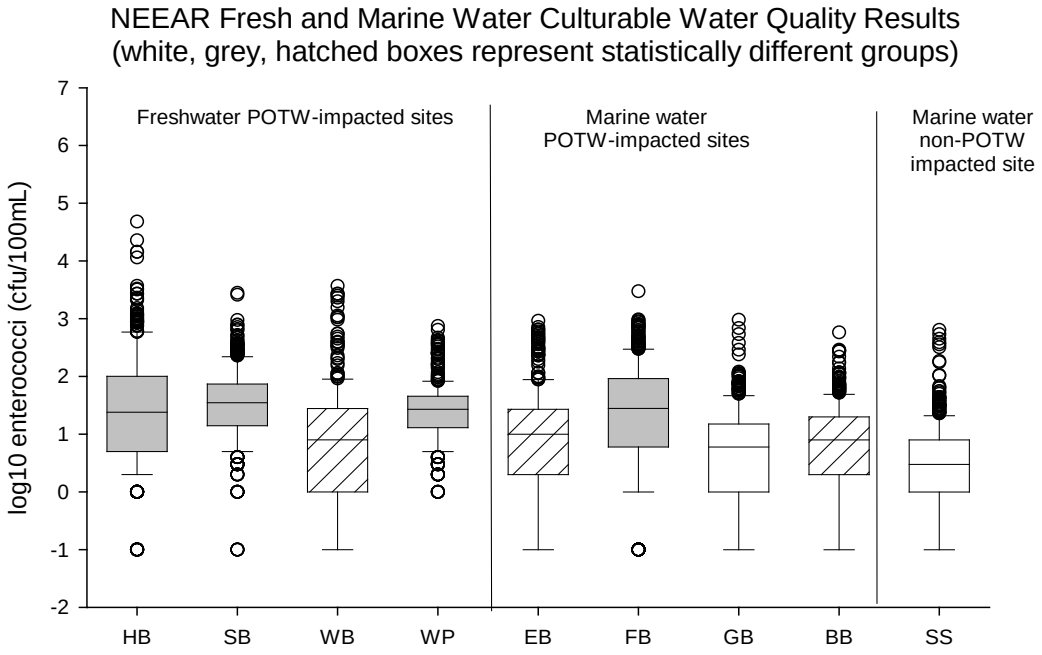


**Table 1. Homogenous subgroups of NEEAR beaches based on culturable enterococci results.**

(The $log_{10}$ means of each beach are listed under the column of the homogenous group to which it belongs. $Log_{10}$ standard deviations are shown in parentheses).

| Beach | Water body type | N | Group 1* (cfu per 100ml) | Group 2 (cfu per 100ml) | Group 3 (cfu per100ml |
|---|---|---|---|---|---|
| Surfside (SS) | marine | 530 | 0.48 (0.73) | | |
| Goddard (GB) | marine | 426 | 0.56 (0.92) | | |
| Boqueron (BB) | marine | 600 | | 0.75 (0.81) | |
| West Beach (WB) | fresh | 336 | | 0.83 (1.01) | |
| Edgewater (EB) | marine | 395 | | 0.88 (0.95) | |
| Fairhope (FB) | marine | 431 | | | 1.32 (0.95) |
| Washington Park(WP) | fresh | 421 | | | 1.39 (0.50) |
| Huntington (HB) | fresh | 420 | | | 1.4 (1.01) |
| Silver Beach (SB) | fresh | 423 | | | 1.49 (0.66) |

*Groups in the table are reflected by different colors and cross hatching in the figure above.

*Analysis Findings:*

- Extensive data analysis of NEEAR enterococci culture densities indicates that there is not a compelling distinction between marine and freshwater.
- The observed enterococci culture densities across all beaches are within the same range.
- While the means between individual beaches were significantly different, their differences are not aligned strictly by classification of whether the water body was a fresh or marine beach**.**

Background information

The literature indicates that of the factors influencing enterococci fate in the environment, there is more evidence that sunlight, temperature, and predation are more important in controlling enterococci concentrations than the effects of salinity. While numerous studies have documented these sunlight (Davies-Colley et al., 1999; Sinton et al., 1999; Boehm et al., 2002; Sinton et al., 2002; Noble et al., 2004; Liu et al., 2006; Boehm et al., 2009; Walters et al., 2009; Nevers and Boehm, 2010) and predation (Enzinger and Cooper, 1976; Chamberlin and Mitchell, 1978; McCambridge and McMeekin, 1981; Barcina et al., 2997; Menon et al., 2003; Noble et al., 2004; Boehm et al., 2005), there are overall fewer studies documenting temperature (Wait and Sobsey, 2001; Noble et al., 2004) and salinity (Hanes and Fragala, 1967; Noble et al., 2004) effects on enterococci persistence. While the results of all temperature studies illustrate that lower temperatures results in increased persistence, the results from the few salinity studies are conflicting – sometimes showing no effect of salinity, sometimes increased persistence at low salinities and sometimes increased persistence at high salinities. The most relevant study for the issue at hand on enterococci survival is Noble et al. (2004) who tested the persistence of enterococci from a variety of sources in fresh and seawater exposed to sunlight and various temperatures. They found that inactivation rates in did not differ amongst various treatments executed in seawater and freshwater. The Noble et al. (2004) paper is perhaps the most direct evidence of the lack of effect of salinity on the persistence of enterococci in the aqueous environment.

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