An Evaluation of Presence and Source of Fecal Contamination at Golden Gardens Park

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Abstract

Golden Gardens is an 87.8 acre Park in Seattle, WA that has come under recent scrutiny over concerns about water quality in the streams and at the beach. Located just north of Shillshole marina in Seattle it has a swimming beach which is 1439 m in length, as well as a forested region directly east of the beach on a relatively steep hill with several drainages flowing into the sound. The purpose of this study was to assess the levels of indicator bacteria in beach water and in the fresh water drainages and to determine if the bacterial contamination is related to human or animal inputs. Specifically, we were interested in determining if the off-leash area for dogs, located on the hill above the beach, was contributing to the contamination. Water was collected five times from June-August 2010 at 4 locations at two depths along the beach and as many as 20 locations in the drainages on the hill. One-hundred ml aliquots of water were analyzed in duplicate for each site sampled via membrane filtration for fecal coliforms, and enterococci. DNA from Enterococci isolates were amplified by PCR targeting the *esp* gene in enterococci and DNA extracted from 200ml of fresh water was amplified for the 16S rRNA gene in *Bacteroides* to determine the source of contamination. Fecal coliform levels for marine beach water at Golden Gardens typically meet WA State bacteriological criteria for secondary contact recreation. The stream water frequently exceeded the USEPA recommended level of 33 enterococci per 100ml. Preliminary PCR results showed that two of 24 samples from one sampling period were positive for human specific *Enterococcus*. PCR analysis for human specific *Enterococcus* and for human and canine specific *Bacteroides* is still underway for the other sampling periods

Background

• Golden Gardens is one of the most frequently used city parks because of its accessibility, amenities, and views of the sound
  • Amenities include a swimming beach, bathhouse, picnic/fire pit sites, large field and ponds on its western side with a forested hill, hiking trails, and a dog park on its eastern portion

• Numerous drainages flow off from the hill into the sound

• Specific Aims:
  • To assess the levels of indicator bacteria in beach water and in the fresh water drainages
  • To determine if the bacterial contamination is related to human or animal inputs

Experimental Methods

Sampling
• Both marine and freshwater samples were collected on four separate occasions; additional marine water sampling occurred
  • Four marine locations along the swimming beach at two different depths
  • 21 different fresh water sites

• Samples were collected in sterile containers, stored immediately on ice, and processed within 24 hours of collection

Processing of Samples
• 100 ml of each sample were filtered in duplicate with membrane filtration through 0.45 µm filters (Millipore)
  •Filters were placed on selective media for enumeration of fecal coliforms and *Enterococcus*

• 200 ml portions of water were filtered through 0.2 µm Supor filters (Pall) for collection of *Bacteroides*
  •filters placed in 500µl of GITC solution and frozen at -80 °C until extracted

Source Typing: *Enterococcus*
• Five *Enterococcus* colonies from each location at each sampling occurrence were pooled together in 100 µl of PBS.

• Nucleic acid was heat released at 95 °C for 5 min and immediately placed on ice

• *PCR run for* Human specific -HS-*Esp* (680 BP)
F primer: TAT GAA AGC AAC AGC ACA AGT T
R primer: ACG TCG AAA GTT CGA TTT CC

Source Typing: *Bacteroides*
• 0.2 µm filters cut up and processed using Mo-Bio Power Soil DNA extraction kit

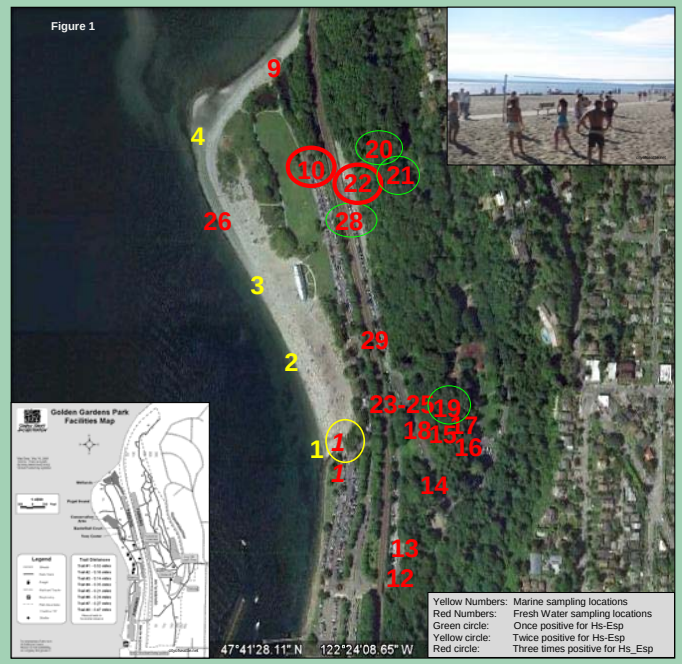
• 16S rRNA gene

• Human specific- HF183 (525 BP)
F primer: ATC ATG AGT TCA CAT GTC CG
R primer :CAA TCG GAG TTC TTC GTG

• Dog specific -DF475 (251 BP)
F primer:CGC TTG TAT GTA CCG GTA C
R primer:CAA TCG GAG TTC TTC GTG

Microbial Contaminant	Background Information	Criteria Fresh Water	Criteria Marine Water	Enumeration Method
Enterococcus 	• Sub group of fecal stretptococci • Minority *of subgroups cause infecections* • Used as Indicator for fecal contamination • May be source specific	• Primary contact: •33/100 ml (EPA recommended)	•Primary contact: 35/100 ml •70/100 ml • Less than 10% of samples exceeding 208/100ml	• Incubation on mEnt plates • Preliminary screening for VRE on mEnt plates with 6µg/ml vancomycin
Fecal Coliforms 	• Anaerobic, rod shaped bacteria living in intestines of warm-blooded animals • Most common is *E.coli* • Presence indicates fecal contamination • Not source specific	• Primary contact: 100 /100ml • Secondary contact: 200/100ml	• Primary contact: 14/100ml • Less than 10% of samples exceeding 43 /100ml	• Incubation on mFC plates with Rosilic Acid • Plating on on nutrient agar with MUG to identify *E. coli*

Figure 1



Sampling Sites

• Figure 1 shows marine and fresh water sampling locations from June 2010 to August.
• Locations 28, 29, and 18 are manholes
• Location 26 is a pipe outfall on the beach, visible only at low tide
• Note that locations 26, 27, 28, 29 were only sampled once

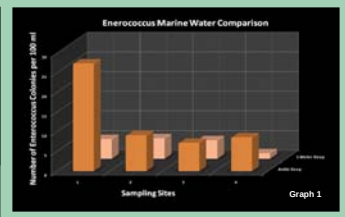
Source Typing

• Source typing is a group of tools which are used to help identify sources of microbial contamination
  •Library dependent methods require the development of host-origin database of phenotypic or genotypic attributes from suspected host groups
  •Library independent methods involve tracking sources using organism specific genes (PCR) or phenotypic methods

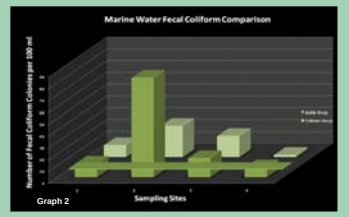
• The two methods used are library independent

• 12 out of 101 samples were positive for HS-*Esp*

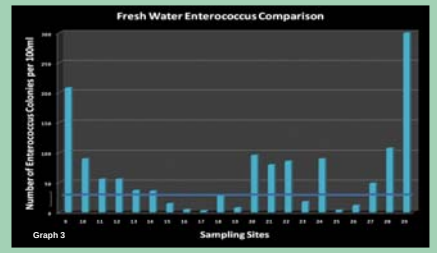
• *Bacteroides* source typing using the HF183 and DF475 primers is currently underway



• Graph 1: geometric mean of *Enterococcus* counts from marine sampling locations over all sampling periods
• The Washington State primary contact criteria is 35/100 ml



• Graph 2: geometric mean of fecal coliform counts from marine sampling locations over all sampling periods
• The Washington State primary contact criteria is depicted by horizontal bar at 14/ 100ml



• Graph 3: geometric mean of *enterococcus* counts from fresh water sampling locations over all sampling periods
• EPA recommended criteria is 33/100ml and depicted by horizontal bar

Conclusions/Future Work

• Marine water at Golden Gardens meets the secondary contact criteria for enterococcus

• Marine water at Golden Gardens generally meets the primary contact criteria for fecal coliforms, more testing is needed to confirm

• Fresh water drainages at Golden Gardens regularly exceed the EPA recommended standards for *Enterococcus*

• Source typing using HS-*Esp* gene showed possible human contamination highlighted by a clustered region, though further testing is needed to confirm

• Future work should include more sampling periods, analysis of screened VRE, and furthered source tracking

References/Aknowledgments

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