Sampling Methodology

What we will measure:

Water samples will be tested for phosphates, nitrites, and bacteria. These are the three measurement criteria that are the main indicators of pet waste or fertilizer in the water.

Sampling sites:

Stream sampling will be done at 3 sites, the two sites where the stream enters the neighborhood (one at each of the two tributaries) and one site where it exits the neighborhood.

Frequency and timing of sampling:

- In general, monthly chemical sampling and twice-yearly biological sampling are considered adequate to identify water quality changes over time.
- Biological monitoring will be done in April and in August.
- Phosphates, nitrites and bacteria will be measured approximately 4 times over the 6 month period March through August.
- Since a program designed to determine whether polluted runoff is a problem should monitor after storms and heavy rainfalls, we plan to do sampling 48 hours after a heavy rain when possible.

Sampling equipment:

- The choice of equipment proposed for these measurements is based on recommendations from the Chesapeake Monitoring Cooperative and the Patapsco Heritage Group (PHG). The Hanna Checkers used by PHG for measuring phosphates and nitrites have the advantage of being used at the stream site and give results in a few minutes.
- Phosphates will be measured using a **Hanna Phosphates Colorimeter Low Range** (0-2.5 ppm, accuracy +-0.04 ppm). The threshold values for Phosphates are .05 ppm – SUSPECT and 0.10 ppm – PROBLEM.
- Nitrites will be measured using a **Hanna Nitrite Colorimeter Low Range** (0-600 ppb, accuracy +-20 ppb). The thresholds for Nitrites are 250 ppm – SUSPECT and 370 ppm – PROBLEM.
- Bacteria will be measured using a **Coliscan EasyGel Water Monitoring kit**. The bacteria testing is done after 24-48 hours of incubation. Arrangements have been made to borrow an incubator from the Greater Patapsco Community Association (GPCA).
- Biological measurement will be done using a seine net to collect macroinvertebrates and a guide to identify them and recording the numbers. Two members of the team will be doing training through PHG on March 31.

The sampling and testing procedures are described in detail below.

Stream sampling and testing procedures

Phosphates - Hanna Phosphate Colorimeter Low Range (HI713)

- Collect samples in 2 bottles
- Add packet of reagent to one bottle
- Put untreated bottle in Hanna Checker (C1)

- Wait for it to display C2
- Put treated bottle in Hanna Checker
- Wait 3 minutes (it has timer on it)
- Record value on Data Sheet

Nitrates - Hanna Nitrite Colorimeter Low Range

- Collect samples in 2 bottles
- Add packet of reagent to one bottle
- Put untreated bottle in Hanna Checker (C1)
- Wait for it to display C2
- Put treated bottle in Hanna Checker
- Wait 15 minutes (it has timer on it)
- Record value on Data Sheet

Bacteria - Coliscan Test kit

- Storage of materials: Freeze Coliscan MF medium (product may be thawed and re-frozen). All other items should be stored at room temperature.
- Before going out into the field:
    - Prepare a cooler with ice or freezer packs
    - Pre-label sample bottles with site ID and date,
- Sample collection:
    - Wade into the main flow of the stream, then take a few steps upstream with minimal disturbance. Sampling point should be where the main flow of the water is.
    - Un-cap the sterile and pre-labeled bottle without touching the inside of the lid
    - Fill and dump bottle once downstream
    - Fill again without getting any bubbles: using a U motion dip the bottle into the water down and away from yourself to the depth of about 0.3 m allowing the bottle to fill ¾ full.
    - Exit the stream and cap the bottle
    - Put bottle on ice in a cooler bag
- Sample plating:
    - Write the site ID, date ,and time on the Petri dish lid with permanent marker. It is best to use small lettering on the outer rim of the dish.
    - Open pipette packet bulb-side first so that you do not contaminate the tip.
    - Gently mix the water in the bottle.
    - Pipette the desired volume (1.- to 5.0 mL) of sample water directly into Coliscan media bottle and recap the bottle. Be careful not to let the bottle lid touch anything to prevent sample contamination. Get a total of 4-5 mL and record the number of mL.
    - Gently mix (do not shake) bottle of Coliscan media containing the sample water, and then pour the entire contents into a Petri dish. Only open the Petri dish long enough to pour in the sample.
    - Gently swirl Petri dish so the Coliscan media covers the entire bottom.
    - Allow the media to solidify for approximately 60 minutes prior to incubation.

    - Put plates in incubator upside down (media on the top) and maintain at 37 degrees C for 24 hours.
- Reading the plates:
    - Place the Petri dish on a whilte background or in natural sunlight. Count the number of dark blue (NOT TEAL) to purple (NOT PINK) colored colonies (disregard any light blue, blue-green or white colonies), larger than a pinprick size on each plate. Do not pay attention to halos around the dots, but only the center color.
    - Refer to the color guide to help identify colonies.
    - Record the number of colonies on the data form.
    - Calculate the number of E. coli per 100 mL of water:
        1. Divide 100 by the number of mL that you used for your sample.
        2. Multiply the count in your plate by the result obtained from #1. This will be the number of E. coli per 100 mL of water.
- Cleanup:
    - Throw used pipettes and emptied and rinsed Coliscan bottles in the recycling.
    - Add bleach to each Petri dish to completely cover the solid media. Allow dishes to stand for at least 10 minutes to ensure all bacteria have been killed. Place plates in a zip-lock bag and dispose of in the trash.

Biologicals

Historical data found: 2011 BIBI score of 1.29 on stream where it crosses Lightning View Rd (West of Thunder Hill Rd). Stream Health Index Map (arcgis.com)