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Indian River Microbial Source Tracking

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Background

The Indian River is a 303(d) impaired tidal tributary of the Elizabeth River's Eastern Branch. In a 2014 State of the Elizabeth River scorecard, the Indian River received an 'F' health score based on the enterococci fecal indicator bacteria (FIB), dissolved oxygen, nitrogen, phosphorus, and phytoplankton (ERP, 2014). A deeper understanding of FIB dynamics in the river was needed to understand potential public health impacts from chronically elevated bacteria levels. In addition Virginia Department of Environmental Quality (VA DEQ) routine monitoring data has shown elevated bacteria in the river and a bacteria total maximum daily load (TMDL) is being developed for the lower Eastern Branch, which includes the Indian River.

Hampton Roads Sanitation District (HRSD), in partnership with the Elizabeth River Project and the City of Chesapeake conducted a comprehensive microbial source tracking study of Indian River upstream of the Indian River Bridge from August 2016 through July 2017. The overall goal of the study was to characterize and partition sources of FIB during dry weather. Specific objectives were (1) to identify obvious human fecal contamination sources using human-associated molecular markers, (2) determine the RWQC exceedance frequency adjacent to a VA DEQ routine monitoring station, and (3) identify non-human fecal contamination sources based on a hypothesis-driven approach (Reicher et al., 2011).

Methods

Sample sites are shown on Figure 1. The sampling plan was designed to evaluate stormwater infrastructure inputs into the river during all tidal stages and throughout multiple seasons. Samples within stormwater outfalls or at the intersection of stormwater discharges and receiving waters are less affected by dilution, which allows for more confidence in detecting positive molecular marker signals there. Sampling sites were located at either the farthest downstream location of a stormwater collection system or in the receiving waters surrounding a neighborhood serviced by septic tanks. Positive identification of human fecal contamination would have been followed by adaptive, in-stormwater collection system sampling in conjunction with in-pipe sanitary surveys to identify infrastructure issues. HRSD collected 90 water samples from 29 sites around, and upstream of the VA DEQ monitoring site (Figure 1). Enterococci concentrations in dry weather grab samples (8/19/2016, 8/23/2016, 10/21/2016, 1/17/2017, 2/7/2017, 5/16/2017, 7/21/2017) were quantified by IDEXX define substrate technology. In addition, all samples were filtered and analyzed for enterococci, fecal *Bacteroides* spp., HF183, HumM2, BacCan, and Goose molecular markers using droplet digital PCR (Cao et al., 2015; Converse et al., 2009; Shanks et al., 2009; Kildare et al., 2007; Fremaux et al., 2010). The enterococci assay is based on the 2012 Recreational Water Quality Criteria (RWQC) and US EPA method 1611. Fecal *Bacteroides* spp., HF183, and HumM2 molecular markers are associated with recent fecal contamination. While the more general fecal *Bacteroides* spp. marker is indicative of fresh mammalian fecal contamination, HF183 is the most frequently used human-associated fecal contamination marker. A recent study suggested a HF183 threshold of 4200 copies/100 mL to represent a benchmark illness rate of 30 gastrointestinal illnesses per 1000 swimmers (the current recreational acceptable risk level; Boehm et al., 2015). For reference HF183 is found in the range of $10^6 – 10^7$ copies/100 mL in Hampton

Roads raw sewage, but can be lower in the collection system. HumM2 is US EPA's licensed human-associated assay; Boehm et al. (2015) suggested a threshold of 2800 copies per 100 mL. BacCan and goose marker are a dog and goose-specific fecal contamination marker, respectively. Samples that did not detect the presence of a molecular marker were documented as below detection limit.

Results

Human-Associated Fecal Markers

Human fecal contamination was not detected at significant levels—there were no obvious signs of a compromised sewer collection system during dry weather. HF183 was detected in 8.9% (8/90) of samples, with only 1 sample detected over the 4200 copies/100 mL threshold. Overall, the 8 positive samples were sporadically distributed—only 2 samples were from the same site. Any sites that were positive for the human-associated HF183 marker were targeted in follow-up sampling events. During these events no evidence (no positive HF183 detection) was found to indicate a compromised collection system. There were no positive detections of the US EPA HumM2 human-associated marker during the study.

Enterococci Concentrations

While there was little evidence of human fecal contamination in the Indian River samples, enterococci concentrations were elevated throughout the sampling locations. While only 72 samples were run for culture enterococci, 64% (46/72) of samples exceeded the single sample maximum of 104 MPN/100 mL RWQC adopted by Virginia. For sites adjacent to the VA DEQ monitoring location 27% (3/11) exceeded the limit. The 2012 RWQC guidelines recommend the use of US EPA method 1611, a molecular enterococci quantification method for use in recreational waters as an alternative to culture enterococci quantification. Note that Virginia has not adopted the 2012 RWQC or the acceptable alternative methods. For interpretation of molecular enterococci concentrations, US EPA recommends a threshold of 2000 calibrator cell equivalents/100 mL for an acceptable illness rate of 36/1000 primary contact recreators. For the purposes of this study we interchange calibrator cell equivalents with copies. Six out of 90 samples exceeded the 2000 copies/100 mL molecular enterococci threshold. While there were less exceedances if the alternative molecular indicators was used, caution should be taken in interpreting all enterococci (culture and molecular) data in this study since the RWQC is meant to be applied to recreational waters, not stormwater outfalls or stormwater collection systems (e.g. ditches, BMPs, stormwater pipes, receiving waters).

Non-Human Microbial Source Tracking Markers

Given the ubiquitous elevated enterococci in the Indian River with no apparent human fecal contamination, non-human host-specific molecular markers were run on the samples. Using the hypotheses-driven approach described by Reicher et al. (2011), the following non-human bacteria sources were identified in the watershed: environmental (naturalized from fecal sources), domestic pets,

geese, and wildlife. A combination of fecal *Bacteroides* spp., dog- and goose-specific markers were chosen to partition fecal sources. Recent mammalian fecal contamination was detected in 82% (74/90) samples. These positive detections were distributed throughout the entire watershed (Figure 2). While mammal fecal contamination was ubiquitous throughout, 17 samples negative for fresh mammalian fecal contamination provide evidence for environmental sources of enterococci constituting a portion of fecal bacteria to the river. The dog fecal marker was detected in 77% (69/90) of total samples (Figure 3). While this high detection frequency is evidence for dog fecal contamination contributing to elevated bacteria in the river, only 77% (57/74) of positive fecal *Bacteroides* spp. detections were also positive for dog fecal marker. This suggests 23% of positive mammalian fecally contaminated samples could be due to wildlife. It should also be noted that percentage is likely an underestimation since dog fecal markers are likely masking the wildlife signals in other samples. Goose fecal detection occurred at a 10% (9/90) rate in samples (Figure 4). This is likely due to the low selectivity of the goose-specific assay and the fact that the marker is found in low concentration in goose feces (data not shown). Dilution likely quickly dilutes this signal. It should be understood that the above values represent frequencies of detection for each respective marker, rather than indications of the relative proportion that each source (human, dog, etc.) contributes to the detected fecal load of a given sample. Note when interpreting non-human marker dot distribution maps (Figures 2-4) that dilution plays a role in concentrations. Samples were taken in varying locations (e.g. stormwater outfalls, ditches, open water) with varying levels of water flow and volume.

Human fecal contamination was not a dominant contributor of fecal bacteria during dry weather to the Indian River. In addition, evidence of dog, wildlife, environmental, and goose sources of contamination exist but these results do not suggest that any particular non-human source is contributing more than another.

Key findings

- Evidence of human fecal contamination was not found in the Indian River during dry weather
- Elevated enterococci concentrations were ever-present
- Dogs, wildlife, environmental, and goose fecal markers were positively identified in samples
- Dog fecal markers had the highest rate of detection in samples

Recommendations

- Conduct a wet weather stormwater outfall screening to determine if there are any collection system infrastructure issues that are only seen during wet weather events
- Use a loading-based sampling scheme to determine host-specific marker loads
- Public education program to eliminate dog fecal contamination entering the Indian River

Figure 1. Site map of Indian River. Samples sites collected by HRSD identified by yellow circles.

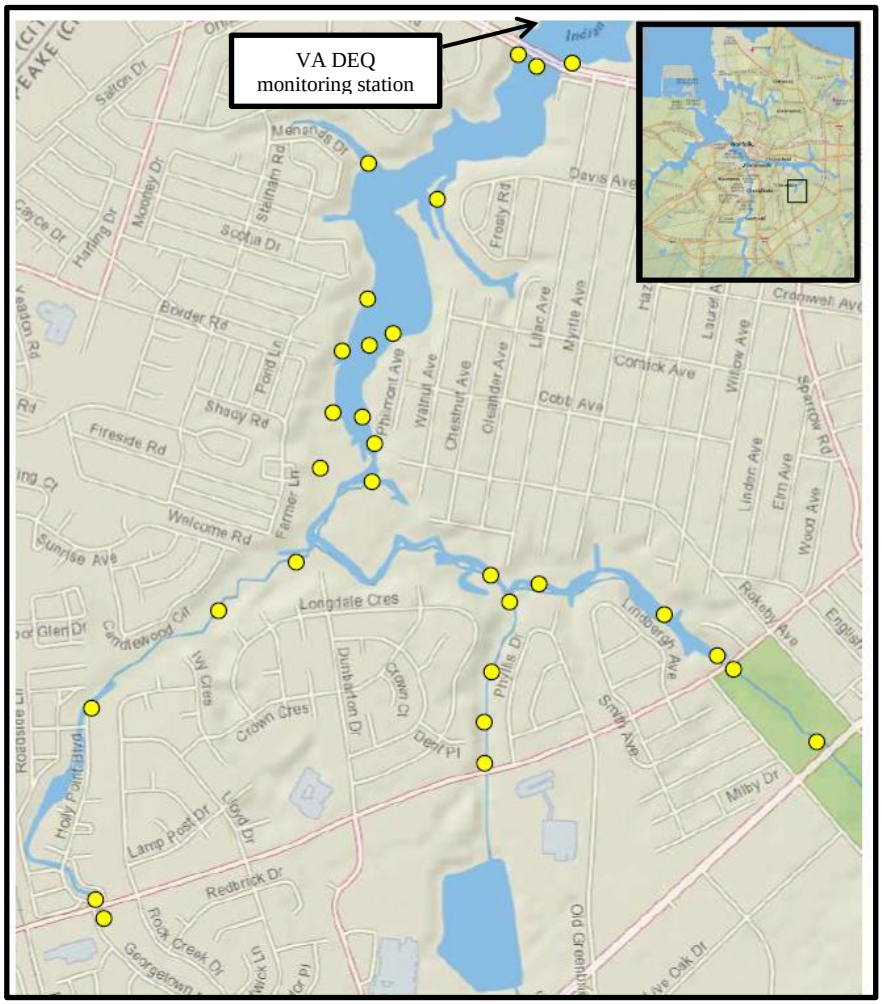


Figure 2. Fecal *Bacteroides* spp. marker dot distribution map for Indian River. Study marker concentrations were averaged for each site.

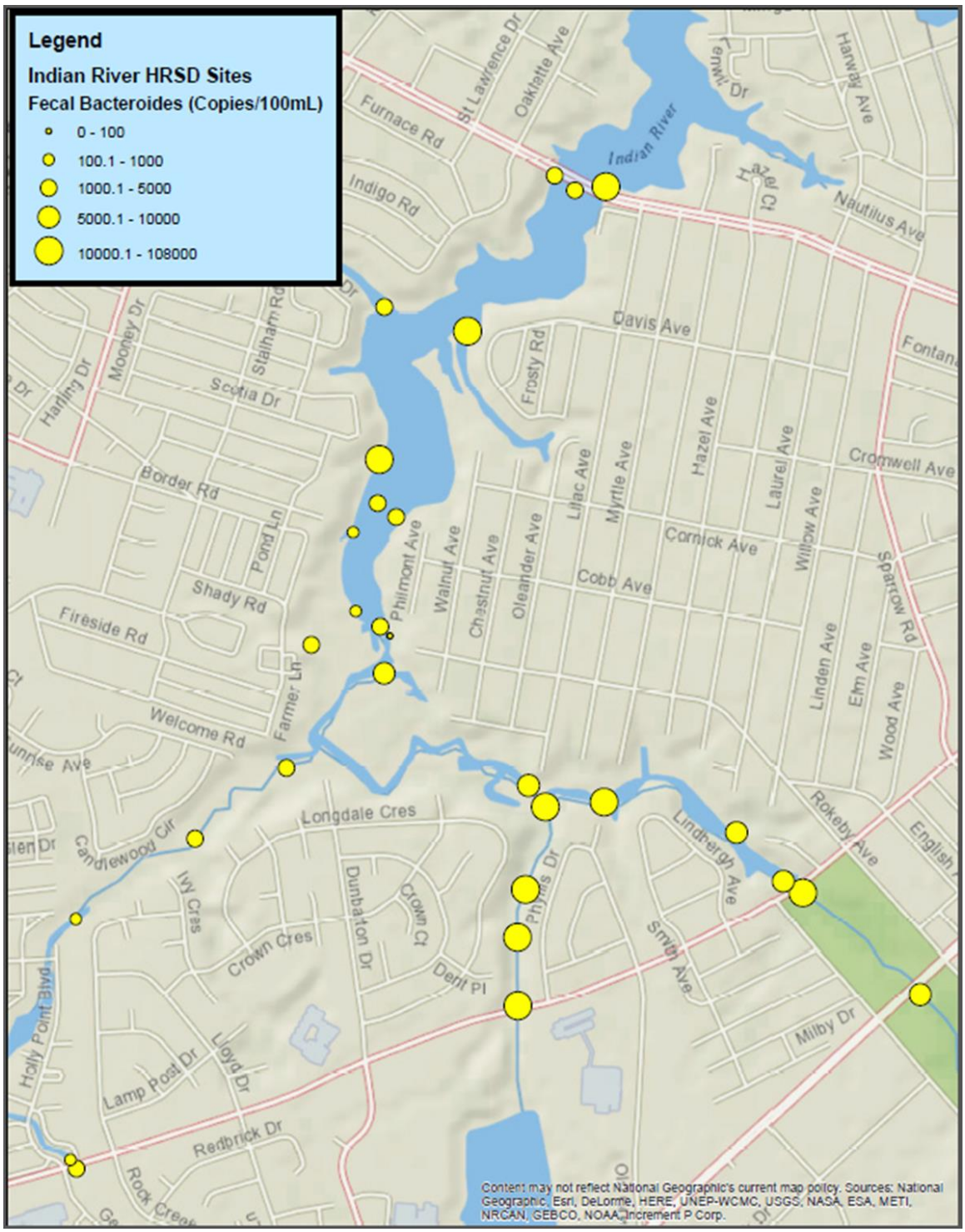


Figure 3. Dog fecal marker dot distribution map for Indian River. Study marker concentrations were averaged for each site.

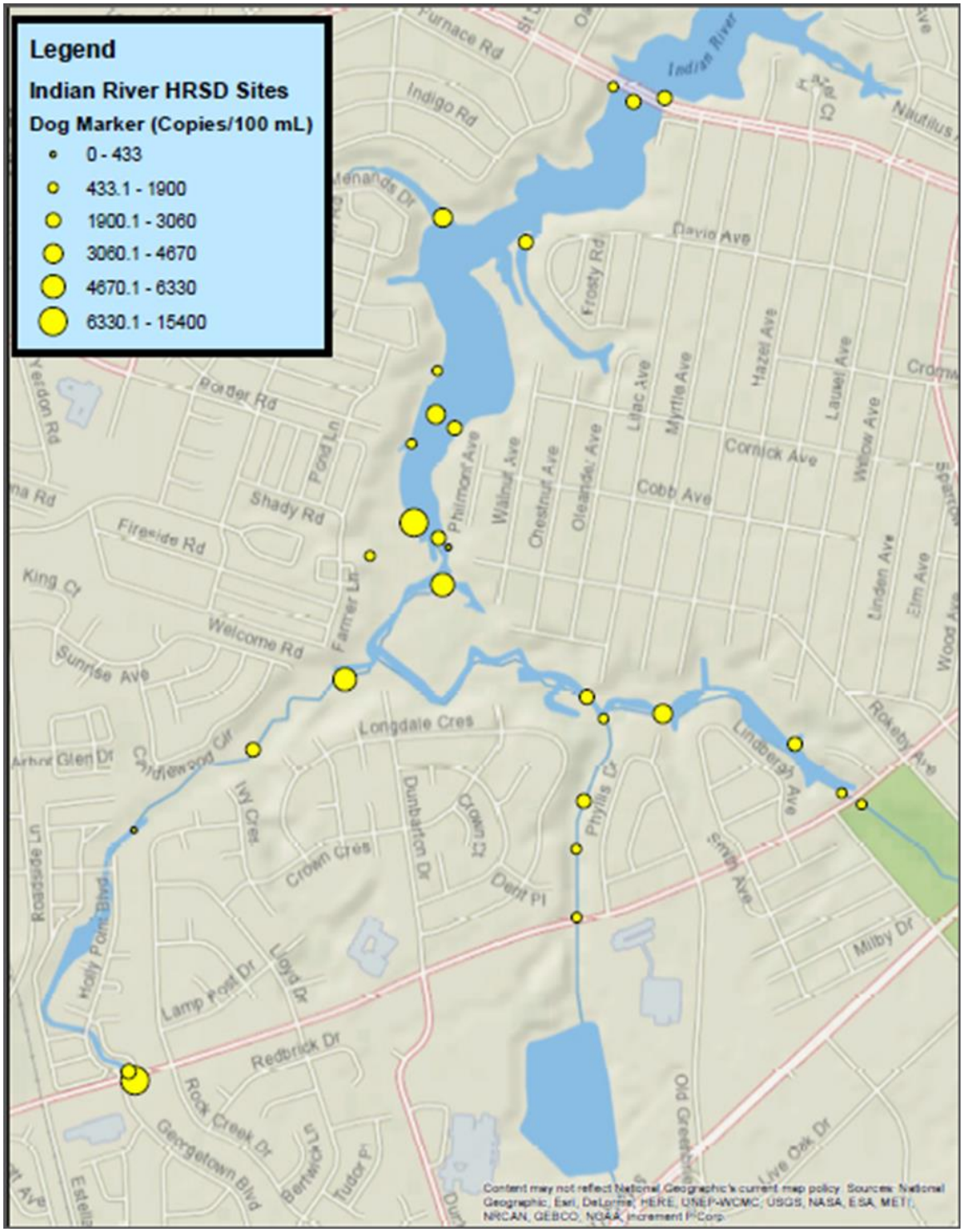
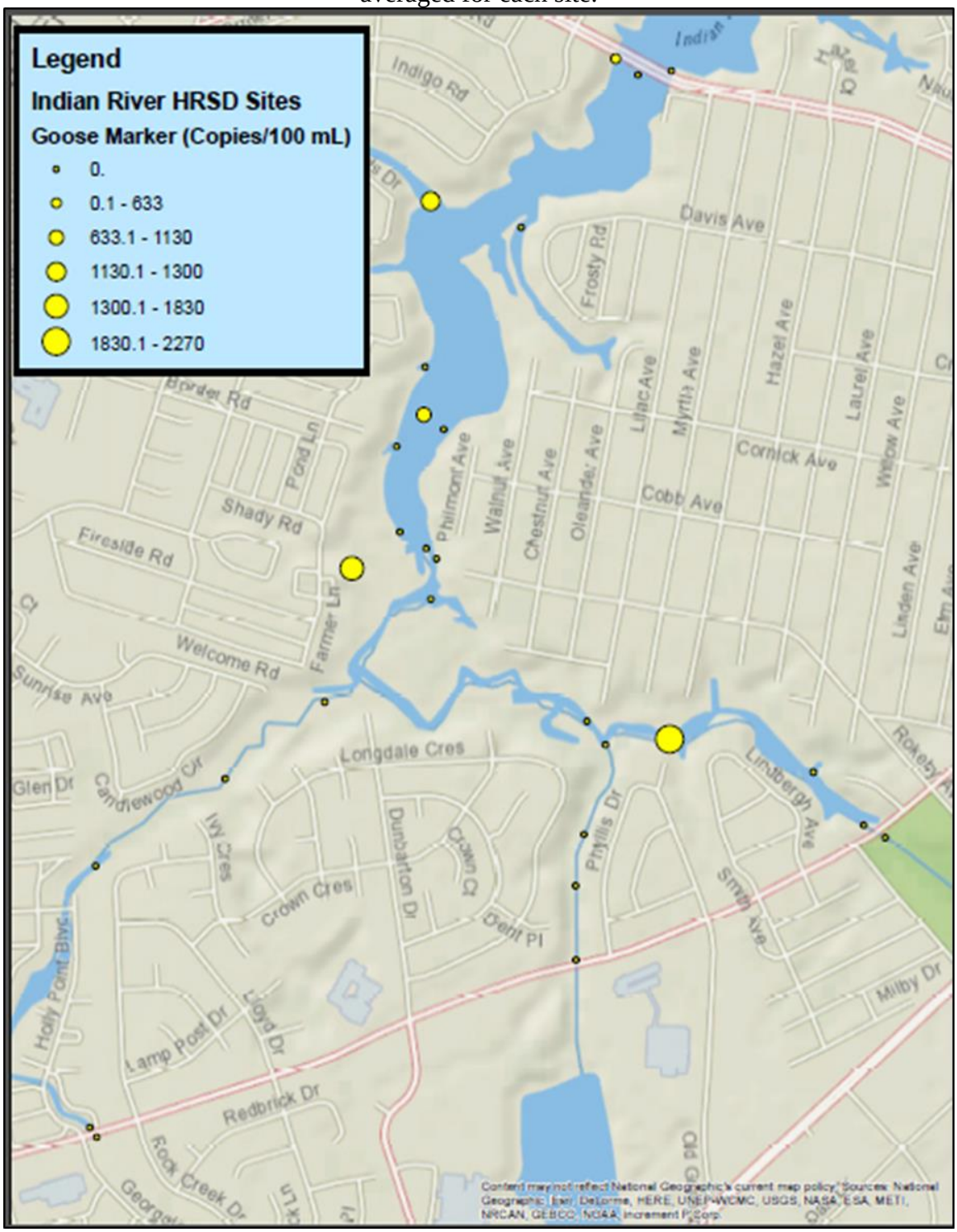


Figure 4. Goose fecal marker dot distribution map for Indian River. Study marker concentrations were averaged for each site.



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