Using Caenorhabditis Elegans As An Environmental Indicator for Impaired Urbanized Watersheds

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Research

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Using *Caenorhabditis elegans* as an Environmental Indicator for Impaired Urbanized Watersheds

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ABSTRACT

Background: Third Fork Creek is a historically impaired urban stream that flows through the city of Durham, North Carolina. Caenorhabditis *elegans* (*C. elegans*) are non-parasitic, soil and aquatic dwelling nematodes that have been used frequently as a biological and ecotoxicity model. We hypothesize that exposure to Third Fork Creek surface water will inhibit the reproduction and chemotaxis of *C. elegans*. Using our ring assay model, nematodes were enticed to cross the impaired water samples to reach a bacterial food source which allowed observation of chemotaxis. The total number of nematodes found in the bacterial food source and the middle of the plate with the impaired water source was recorded for three days. **Results:** Our findings suggest a reduction in chemotaxis and reproduction on day three in nematodes exposed to Third Fork Creek water samples when compared to the control (p-value<0.05). These exploratory data provide meaningful insight to the quality of Third Fork Creek located near a Historically Black University. **Conclusions:** Further studies are necessary to elucidate the concentrations of the water contaminants and implications for human health. The relevance of this study lies within the model C. *elegans*, that has been used in a plethora of human diseases and exposure research but can be utilized as an environmental indicator of water quality impairment.

Background

C*aenorhabditis elegans* are a useful nematode model for genotoxcity, molecular biology (Leung et al., 2008), and neurological disorders such as Alzheimer's and Parkinson's disease (Harrington et al., 2010; Link, 2006). C. *elegans* are effective eukaryotic models because a large portion of their genome is evolutionary conserved and 83% of their proteome is homologous to humans (Lai et al., 2000). C. *elegans* also can be found naturally in soil and water and have been identified in leaf litter and gastropods (Caswell-Chen et al., 2005; Hope, 1999; Schulenburg & Félix, 2017). These nematodes have been utilized in several environmental toxicology studies to evaluate toxicity of soils (Black & Williams, 2001; Harmon & Wyatt, 2008; Höss et al., 2009; Huguier et al., 2013), sediments (Höss et al., 1999; Tejeda-Benitez & Olivero-Verbel, 2016; Traunspurger et al., 1997) and water (Hitchcock et al., 1997; Mutwakil et al., 1997; Turner et al., 2013). C. *elegans* assist to maintain soil health by regulating bacteria populations and by indirectly supporting biodiversity. Studies concluded C. *elegans* are a representative model for ecotoxicity (Boyd et al., 2000; Hägerbäumer et al., 2015). In the study conducted by Hitchcock et al., nematodes were exposed to several composite water samples from five points from within the wastewater treatment plant system. Using a 72-hour nematode mortality test, nematodes experiences increased mortality when exposed to wastewater entering the wastewater treatment plant. In the study conducted by Mutwakil et al., transgenic nematodes were exposed to five water samples collected from the River Carnon in England, which is known to have ancient mining history. Transgenic expression was observed in nematodes exposed to all five samples, with the least amount of expression found in nematodes exposed to water

samples containing less contaminants. C. *elegans* are a prodigious model due to the ease of culture in laboratory settings; can be maintained at 25 °C; consume bacteria; and have a short well-studied lifespan (Leung et al., 2008). In this study, C. *elegans* are used to investigate toxicity of a historically impaired urbanized watershed in Durham, North Carolina.

Third Fork Creek (TFC) watershed drains 16.6 square miles of the city of Durham, North Carolina before reaching Jordan Lake and eventually the Atlantic Ocean (Figure 1). The predominate land use is a combination residential, commercial and vacant unmanaged space (Stormwater and GIS Services Division, 2019). TFC watershed is classified as protected upstream (WS-V) and nutrient sensitive waters (NSW) (Stormwater and GIS Services Division, 2019), which means TFC drains into a drinking water source (Jordan Lake); is protected for wading, boating, fishing, wildlife, fish consumption; and requires additional nutrient management to reduce the excessive growth of aquatic vegetation (Division of Water Resources, 2021). Common sources of pollutants are sediment and erosion, private and public sanitary sewer spills, petroleum and cooking grease spills (Stormwater and GIS Services Division, 2019).

TFC has a history of suffering from pollution. Of the 13.5 miles assessed, the water quality index is fair (35%) or poor (19%) (Stormwater and GIS Services Division, 2019), yet the number of sampling locations throughout the watershed has declined from 7 locations in 2016 (Spence, 2015; Stormwater & GIS Services, 2017) to one location in 2019 (Stormwater and GIS Services Division, 2020). In 2019, the City of Durham tested one location within the TFC watershed and reported a water quality index value of 76 due to poor bacteria levels, fair nutrient and

turbidity levels, and poor aquatic life (Stormwater and GIS Services Division, 2020). The significant reduction in sampling locations within the TFC watershed is concerning, because TFC flows into the Jordan Lake which serves as a drinking water source for several communities located in Wake, Durham and Orange counties (Division of Water Resources, 2021). Since TFC runs adjacent to North Carolina Central University (NCCU), a site located near the campus was selected for this exploratory study. We hypothesized the water collected from this site along TFC would inhibit *C. elegans* chemotaxis and reproduction. For this study, a ring assay was utilized to measure the chemotactic behavior of the C. *elegans* and the reproduction of nematodes exposed to the highly urbanized TFC surface water samples.

METHODS

Water Collection and Sampling

Grab water samples were collected from one sampling location along TFC (Figure 2) during the summer of 2019 on June 24 and July 8 and during the winter of 2020 on February 17, February 24, March 2, and March 9. Plastic 50 ml conical tubes were used to collect surface water samples from TFC. Samples were immediately placed on ice in a cooler for travel to the laboratory and stored at 4 ˚C. The winter 2020 sampling period was halted after March 9, 2020 due to the COVID-19 pandemic.

C. elegans Inoculation and Culture

C. elegans (N2 strain), *E.coli* (K-12 strain) and Nematode Growth Agar (NGA) were purchased from Carolina Biological Supply Company (Burlington, NC). E.coli was aseptically inoculated

onto NGA plates and incubated overnight at 37 °C to create bacteria lawn plates to support and maintain nematodes until time of experiments. Nematodes were inoculated onto bacteria lawn plates and maintained at 25° C.

Nematode Ring Assay

A ring assay model (Worku & Gerald, 2009) was used to analyze nematode chemotaxis and reproduction. An NGA plate was inoculated with *E. coli* around the ring of the plate to entice nematodes to cross the NGA plate (Control), sterile water or TFC sample. The plate was incubated overnight at 37 °C. The following day, a 100 microliter volume of sterile water or TFC water sample (water sample was refrigerated immediately after sample collection and used within 24 hours for nematode exposure) was added to the middle of the plate. Immediately following the addition of sterile water or TFC water sample, nematodes were inoculated with a sterile toothpick from nematode bacteria lawn growth plates onto the designated ring assay plates. A range of 20-30 nematodes were seeded on each plate. Nematodes in the middle, ring and total number of live nematodes were counted each day post-exposure for three days. Chemotaxis shown in the results as nematodes present in the *E. coli* ring and reproduction was shown as total amount of live nematodes on the entire plate.

Statistical Analysis

The assays were conducted in triplicate in independent times for reproducibility assessment. Each ring assay plate was inoculated with nematodes. Three ring assay plates were used for the control group which consist of no water or TFC sample. Sterile water was used on another

three ring assay plates. Three ring assay plates were used for the treatment group which consisted of the TFC water sample.

The Control (no water in the middle of the Ring Assay plates), sterile water (sterile water in the middle of the Ring Assay plates) and TFC water sample (Collected surface water from TFC in the middle of the Ring Assay plates) were assessed each week following grab water sample collection. A Two-Way ANOVA and Tukey's Multiple Comparisons post-hoc test was employed via GraphPad Prism version 9 to analyze nematode chemotaxis (nematodes present in the *E. coli* ring) and reproduction (total number of live nematodes on the entire plate).

RESULTS

The data from summer 2019 water sampling and collection indicates chemotaxis increased by Day 3 as expected, however no differences between treatment groups were observed (Figure 4A). Generally, nematodes in the control group are expected to reach the bacterial ring as well as reproduce over the three-day life cycle. These nematodes were capable of reaching the food source without difficulty which lead to the increased number of nematodes on day three. Nematodes reproduced significantly when comparing day 1 to day 3 in all treatment groups (Figure 4B). TFC did not alter nematode chemotaxis and reproduction (Figure 4C and 4D). Overall nematode chemotaxis and reproduction were not affected by the TFC exposure.

Table 1. Comparison of Nematode Chemotaxis Post-Exposure to Third Fork Creek Water Samples

Date	Tukey's Multiple Comparison's Test	Mean Difference	P value
17-February-2020	Day 2-Control vs. TFC	-43.67	0.0260
	Day 2-Sterile Water vs. TFC	-53.00	0.0070
	Day 3-Control vs. TFC	-148.0	<0.0001
	Day 3- Sterile water vs. TFC	-196.3	<0.0001

24-February-2020	Day 3-Control vs. Third Fork Creek	76.00	0.0120
	Day 3-Sterile Water vs. Third Fork Creek	264.3	<0.0001
2-March-2020	Day 3-Control vs. Third Fork Creek	75.33	0.0015
	Day 3-Sterile Water vs. Third Fork Creek	216.3	<0.0001
9-March-2020	Day 3-Control vs. Third Fork Creek	69.33	<0.0001
	Day 3-Sterile Water vs. Third Fork Creek	209.7	<0.0001

By day 3, the number of C. *elegans* present in the food source was significantly higher than the control and sterile water (Figure 5A). Nematodes successfully crossed the chemical gradient, TFC sample and reached the food source (E. coli) (Table 1). Similarly, in figure 5B, nematodes exposed to TFC water sample experienced significant increases in reproduction compared to the control and sterile water. The TFC water sample did not prevent reproduction of nematodes. Nematodes in the middle of the plate were also counted and no significant differences were found between treatment groups (data not shown). By day 3, TFC water samples decreased chemotaxis when compared to the control and sterile water treatments shown in figure 5C. Similarly, by day 3, TFC water samples significantly decreased reproduction (figure 5D) when comparing control and sterile water (table 2). Nematodes in the TFC treatment were inhibited from reaching the E. coli ring (food source) and did not reproduce as much as the controls.

Nematodes exposed to TFC water collected on March 2 and 9 were inhibited from reaching the bacteria ring when compared to the controls (Figure 6A and Figure 6C). TFC not only prevented chemotaxis for both weeks but also significantly decreased reproduction (Figure 6B and Figure

6D) by day 3 (Table 2). TFC water collected the first weeks of March inhibited chemotaxis as well as nematode reproduction.

Table 2. Comparison Nematode Reproduction Post-Exposure to Third Fork Creek Water Samples

Date	Tukey's Multiple Comparison's Test	Mean Difference	P value
17-February-2020	Day 2-Control vs. TFC	-51.67	0.0196
	Day 2-Sterile Water vs. TFC	-54.67	0.0135
	Day 3-Control vs. Third Fork Creek	-182.0	<0.0001
	Day 3-Sterile Water vs. Third Fork Creek	-234.7	<0.0001
24-February-2020	Day 3-Control vs. Third Fork Creek	77.67	0.0084
	Day 3-Sterile Water vs. Third Fork Creek	267.7	<0.0001
2-March-2020	Day 3-Control vs. Third Fork Creek	77.33	0.0048
	Day 3-Sterile Water vs. Third Fork Creek	215.0	<0.0001
9-March-2020	Day 3-Control vs. Third Fork Creek	79.33	<0.0001
	Day 3-Sterile Water vs. Third Fork Creek	212.3	<0.0001

Discussion

Our preliminary data show a decrease in nematode chemotaxis and reproduction when exposed to TFC winter 2020 water samples. By day 3, 75% of water samples inhibited C. *elegans* from reaching the food source (E.*coli*) and reproducing. The impaired water samples are thought to contain various contaminants such as pesticides from stormwater runoff that could reduce the nematodes' ability to reach the food source. Pharmaceuticals such as caffeine, antibiotics, fire retardants and pesticides were found in the TFC watershed (Rhodes et al., 2013). C. *elegans* exposed to caffeine experience food aversion behavior (Min et al., 2017) and decreased larval development (Min et al., 2015). Nematodes feed off bacteria commonly found

in the soil and water and antibiotics could decrease the levels of this food source. Also, certain pesticides are broad spectrum and can have nematicidal effects which could explain the decrease of nematode chemotaxis and reproduction.

Unfortunately, the COVID-19 pandemic paused the water collection and nematode analysis in winter 2020. We anticipate commencing weekly collections during the summer 2021. This study will be expanded to elucidate genetic and proteomic differences in nematodes exposed to grab water samples from TFC watershed. In addition, to the nematode analysis, we will also investigate common water quality parameters such as pH, dissolved oxygen and analysis of common nutrients and pollutants. The water samples collected in this study are not of a pure sample. We do not know the internal concentrations of the constituents in collected samples. The volume used in the ring assay is very small in comparison to the whole sample collected. Since this is an exploratory study, we used 100% of the sample. For future observations we will seek to establish ECx values regarding the nematode experiments.

Increasing the number of sampling locations, frequency of sample collection and using C. *elegans* as an environmental indicator species will assist in evaluating seasonal differences observed from our current TFC dataset. Therefore, future sampling locations will be selected upstream and downstream of the current NCCU water sampling location (Figure 2) to focus on traditionally underserved minority communities near North Carolina Central University, a Historically Black University. Future nematode observations will include counting eggs to better understand the reproduction and analyzing locomotion to observe chemotaxis more efficiently.

Evidence provided by this study will supplement the sampling efforts conducted by the city of Durham and can be used to educate residents about potential health implications.

Conclusions

The sampling locations in this preliminary study are limited, but the information provided in this short article supports the need for including additional sites along TFC in future studies. Even though this study is focused on a local stream, our methods can be used to study water quality impairment in freshwater systems at larger scales and in different locations using a very inexpensive model organism.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not Applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

Not applicable.

Funding

Not applicable.

Authors' contributions

Carresse Gerald developed the experimental design, oversaw the research details and wrote the manuscript.

Boris DeShazo and Hayden Patterson conducted experiments and commented on final manuscript.

Porche` Spence developed map figures and edited the manuscript.

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Figure 1. Jordan Lake Watershed including Third Fork Creek

Figure 2. Third Fork Creek land use and NCCU current and potential sampling locations

Figure 3. Ring Assay Plate

Figure 4. Effect of TFC grab samples on Nematode Chemotaxis and Reproduction during summer 2019.

Figure 5. Nematode Chemotaxis and Reproduction Decreased Post-Exposure to TFC Surface Water Collected in February 2020. n=3. Data are presented as mean ± SD. * represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.0001$

Figure 6. Nematode Chemotaxis and Reproduction Decreased Post-Exposure to Third Fork Creek Surface Water Collected in March 2020. n=3. Data are presented as mean ± SD. * represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.0001$

Figures

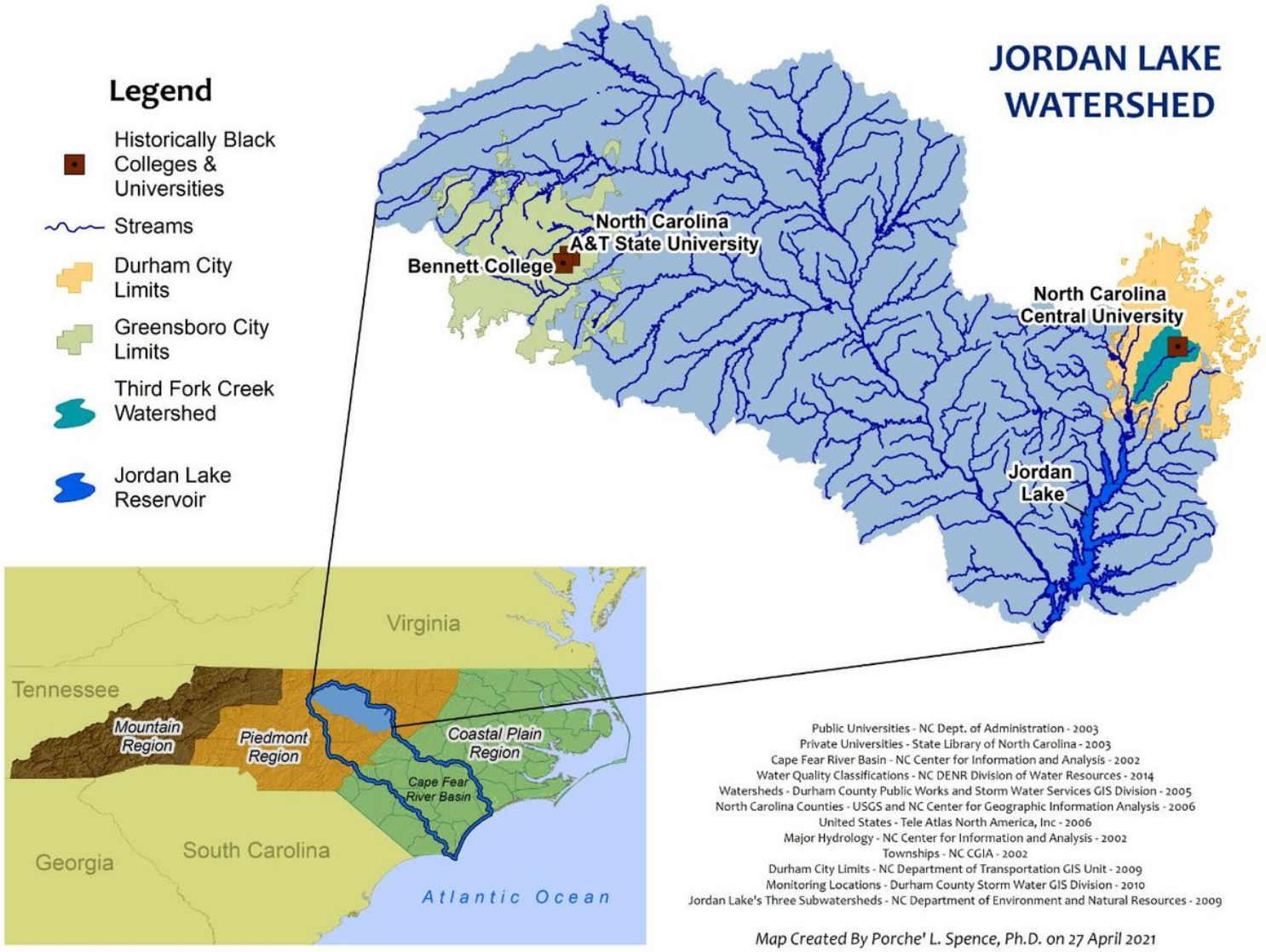

Figure 1

Jordan Lake Watershed including Third Fork Creek

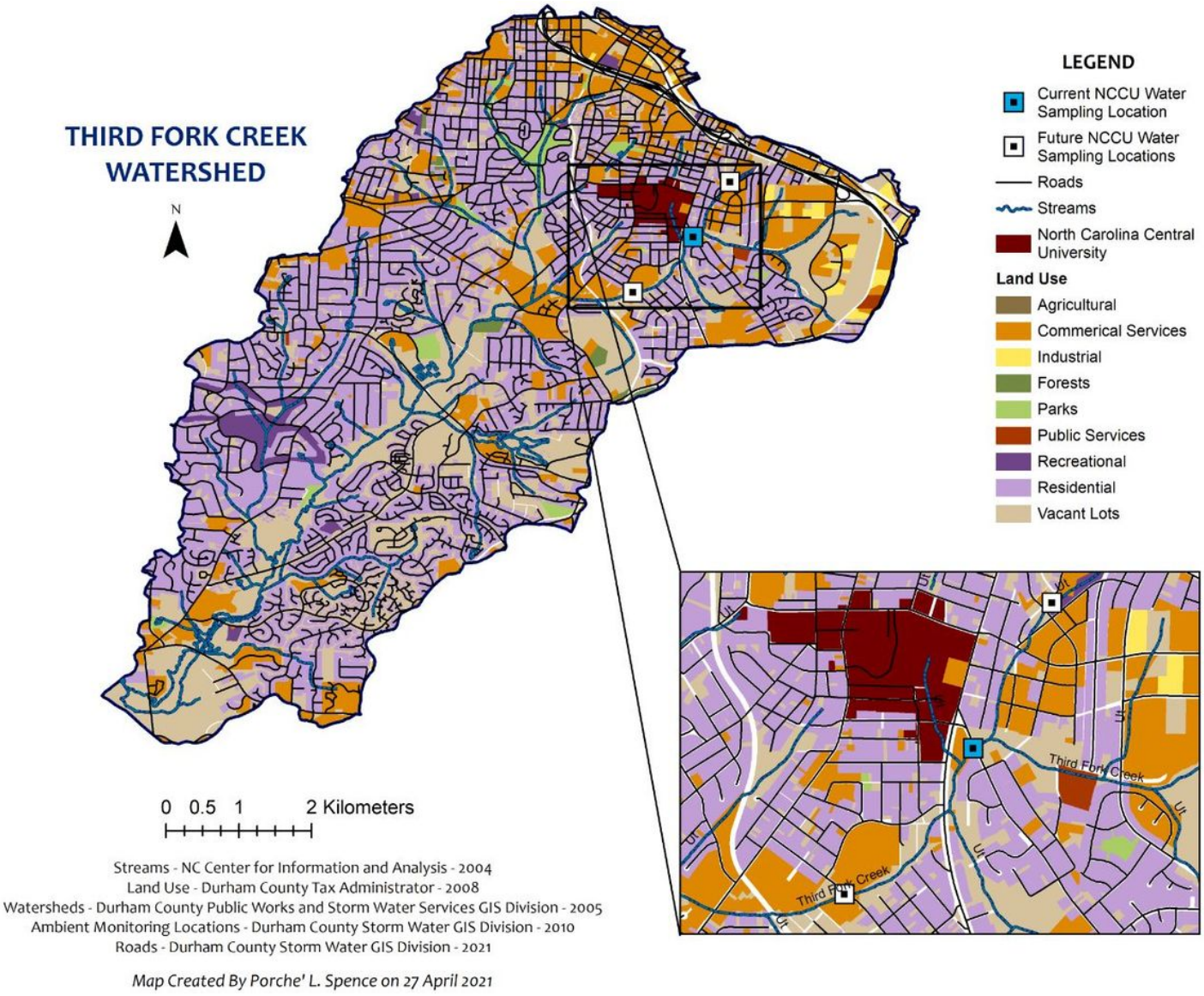

Figure 2

Third Fork Creek land use and NCCU current and potential sampling locations

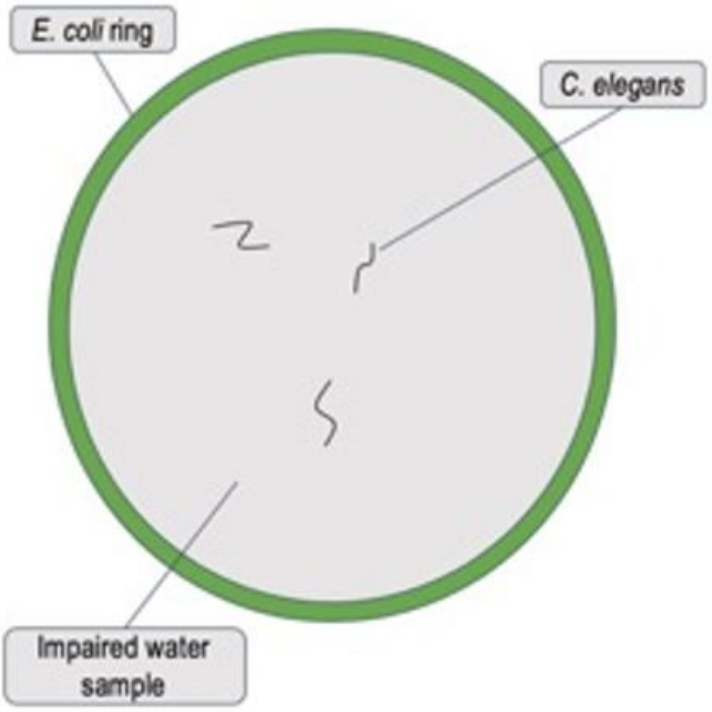

Figure 3

Ring Assay Plate

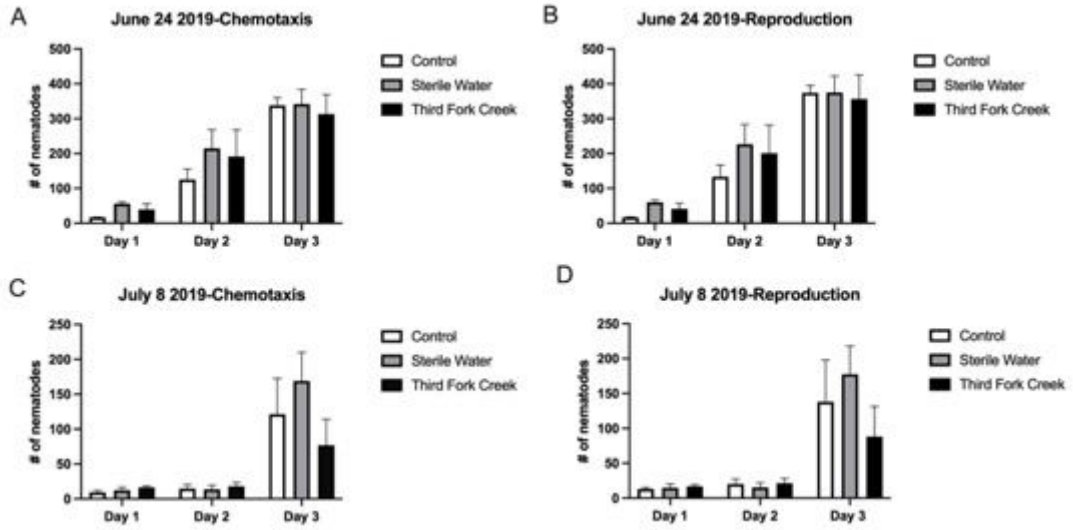

Figure 4

Effect of TFC grab samples on Nematode Chemotaxis and Reproduction during summer 2019.

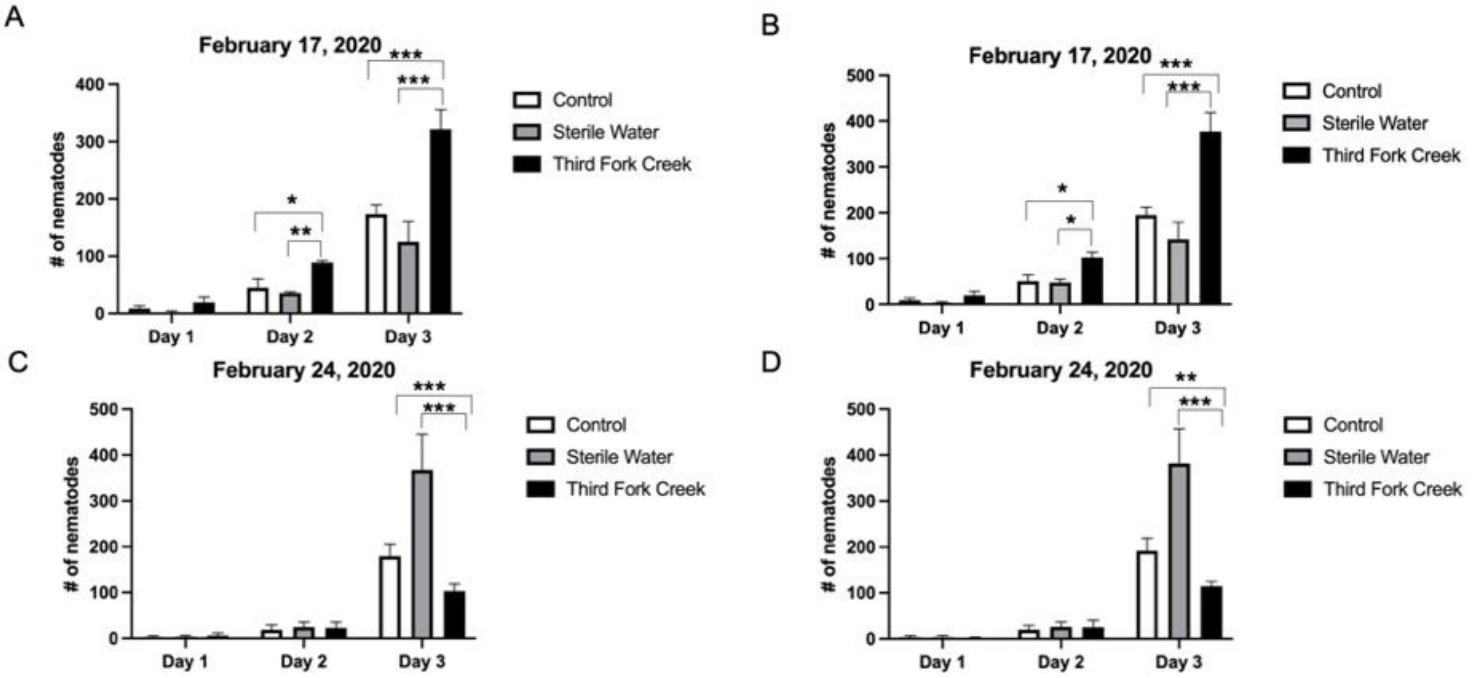

Figure 5

Nematode Chemotaxis and Reproduction Decreased Post-Exposure to TFC Surface Water Collected in February 2020. n=3. Data are presented as mean ± SD. * represents p<0.05, ** represents p<0.01, *** represents p<0.0001

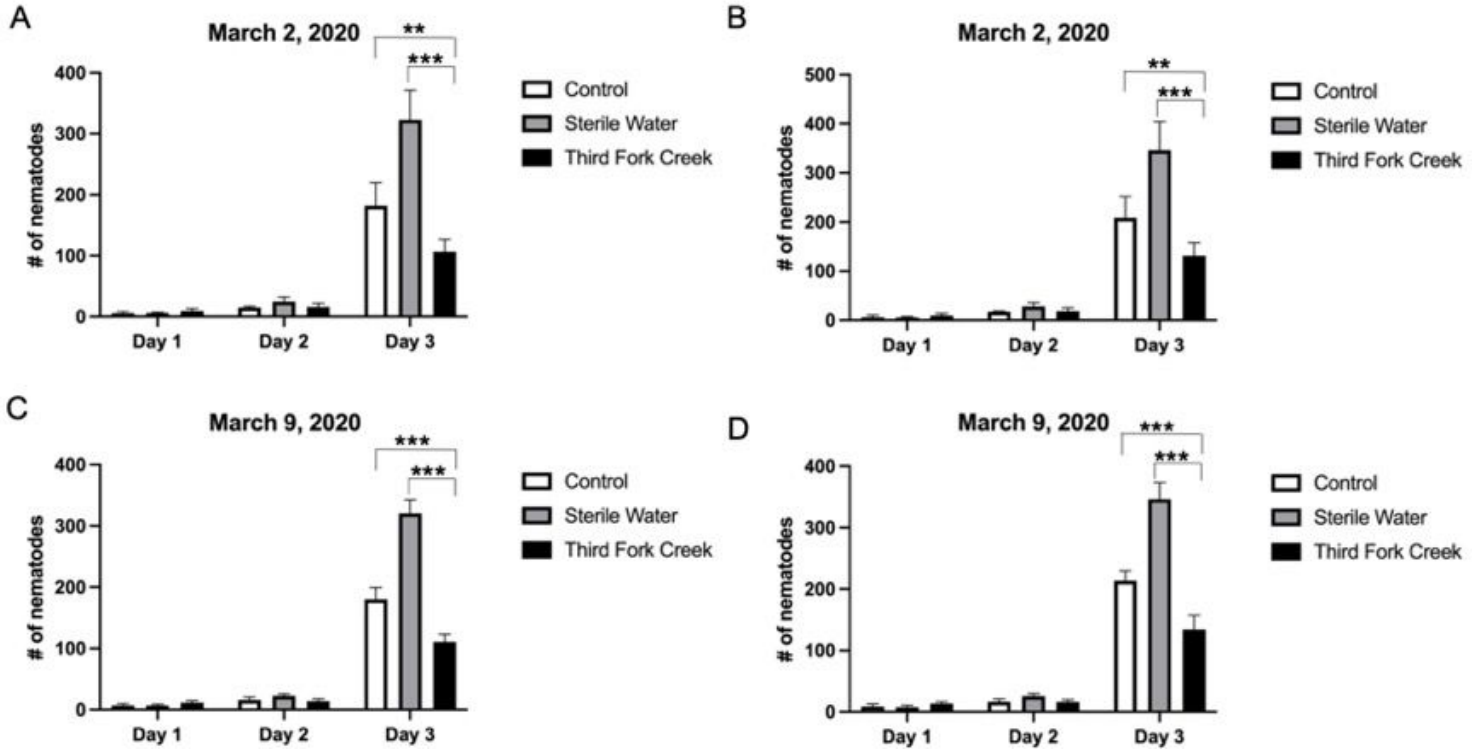

Figure 6

Nematode Chemotaxis and Reproduction Decreased Post-Exposure to Third Fork Creek Surface Water Collected in March 2020. n=3. Data are presented as mean ± SD. *represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.0001$