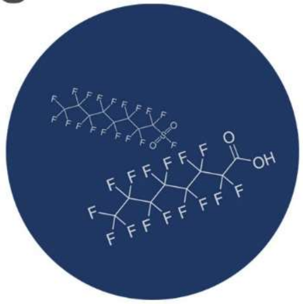
Maternal Exposure to Per- and Polyfluorinated Alkyl Substances (PFAS) in Drinking Water and Associations with Birth Outcomes



By

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Maternal Exposure to Per- and Polyfluorinated alkyl substances (PFAS) in Drinking Water and Associations with Birth Outcomes

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Executive Summary

Per- and polyfluoroalkyl substances (PFAS), used in firefighting foam and as water and oil-repellants in nonstick cookware, fabrics and other materials, are widely detected in watersheds across the United States, and globally. Epidemiological studies have found that PFAS are associated with adverse health effects, including thyroid disease, cancer, and adverse birth outcomes. In 2017-2018, high levels of PFAS were detected in both the Haw River and Cape Fear River in North Carolina, raising concerns for potential health effects in towns which draw drinking water from these rivers. This research sought to examine associations between exposure to PFAS (using watershed as a proxy for PFAS exposure) and birth outcomes in NC, focusing specifically on birth weight and gestational age at birth. A multiple linear regression model was used to compare outcomes in eleven regions of NC, defined by their drinking water source. After adjusting for potential confounders and stratifying analyses by infant sex, the largest difference in birth weight was observed in the Headwater of the Cape Fear River (serving the population of Eastern Chatham County and Goldston-Gulf District), where male infants were born 0.26 lbs (± 0.13) lighter on average, and were born 4.72 (± 1.93) days earlier, than the reference group (Falls Lake (Raleigh); $p<0.1$ for birth weight and $p<0.05$ for gestational age). Similar patterns were observed in populations drawing water from Lake Mackintosh, Jordan Lake, and the Cape Fear River, whereas no statistically significant differences in birth weight or gestational age were observed in the population drawing water from the Haw River (i.e., the Town of Pittsboro).

The first section, introduction, illustrated the basic information and manufacturing history of PFAS throughout the past few decades. It described toxicological findings of PFAS molecules in animal studies. The section also described the physico-chemical properties of PFAS molecules and their fate and transport in the environment, as well as ubiquitous human exposure to these molecules mainly through drinking water. Health effects of PFAS were shown through recent epidemiological and health cohort studies with an emphasis on adverse effects on birth outcomes. The section ended with the research rationale and objectives that this study accomplished: it aimed to explore the potential link between PFAS exposure through drinking water and the health effects in birth outcomes in North Carolina, with a focus in the population of Triangle Area which has been reported high levels of PFAS in one of its watershed, Haw River.

The second section, method and approach, includes the data source, data analysis, and how results were interpreted. The section laid out the basis for the analysis process using multiple linear regression model to compare the birth outcomes of population drawing water from different watersheds with the birth outcomes of a reference population receiving water of less or no known high level of PFAS. Selection of covariates that might confound the results was explained and justified. The section also discussed the expected forms of results.

The result section displayed the descriptive statistics of the population that was investigated. It showed results of the regression model produced in R and highlighted the main patterns observed. The statistically significant results were found in the population drawing water from a watershed, such as Headwater of Cape Fear River, where both birth weights and gestational age were smaller comparing to the reference group. It also highlighted the difference in effects seen between female and male infants, where male infants experienced a stronger effect in both outcomes. The heat map displayed the geospatial patterns of the effects on birth outcomes.

The discussion section interpreted the results and explored the possible other causes than PFAS contamination. The section also discussed limitations of the study including lack of data and confounding factors and provided direction for future research.

Overall, the research study concluded that there are spatial differences in adverse birth outcomes across NC, which may be due to exposure to contaminants in water such as PFAS; however, future studies are needed to specially examine PFAS exposures at the individual level.

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that share a chemical structure consisting of a carbon backbone with multiple fluorine atoms attached. Various functional groups are attached to different points along the backbone to provide desirable surface properties. PFAS have been manufactured and used since 1940s in the United States and encompass a large and complex family of more than 3000 fluorinated organic chemicals (Wang et al., 2017). Individual PFASs are named according to the number of carbon atoms in their backbone, as well as the type of functional group attached to the backbone. Long-chain PFAS refers to perfluorosulfonic acids with more than 6 carbons in their chain, including perfluorooctanesulfonic acid (PFOS), and perfulorocarboxylic acids with more than 8 carbons in their chain, including perflurooctanoic acid (PFOA) (FluoroCouncil).

PFAS are widely used as stain- and water-repellents in products like food packages, nonstick cookware, fire-fighting foams, polishes, and chrome plating. Due to their wide use over the last 60 years, PFAS their degradation products are globally detected (Picó, 2011). As research on PFAS and reports of human exposure have increased since 1970s, concerns regarding the impacts on human health have led to more attention from research scientists, and regulatory agencies. The main PFAS manufacturer, 3M, announced in 2000 that they would voluntarily phase out production of PFOA and PFOS globally as a precautionary measure. As a consequence, legacy PFAS, such as PFOA and PFOS, have subsequently been replaced by short-chain PFAS, which have similar chemical structures but fewer carbon atoms in the backbone compared to long-chain PFAS (ITRC, 2020).

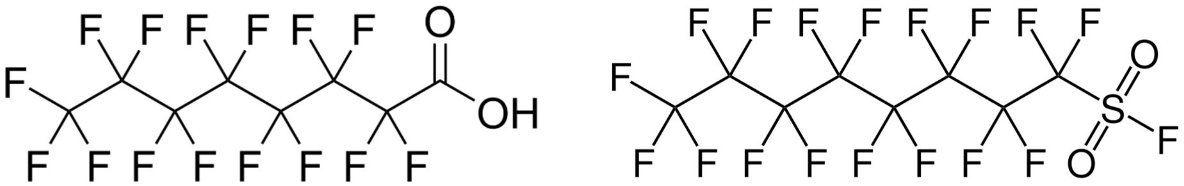

Figure 1. Chemical structures of perflurooctanoic acid (PFOA), on the left, and perfluorooctanesulfonic acid (PFOS), on the right.

PFAS Toxicity

Studies have examined both acute and chronic toxicity of PFAS. The 48-hour half-maximal effective concentration (EC50) for Daphnia magma is 5,251 mg/L for PFBA, 1,048 mg/L for PFHxA, and 239 mg/L for PFOA, indicating that acute effects decrease as carbon chain length decrease (Barmentlo et al., 2015). The EC50 of PFHxA is 12.84 mg/L for green algae, 4.72 mg/L for the diatom, and 3.18 mg/L for blue-green algae. In contrast, the EC50 is much lower for PFOA equating to 2.36 mg/L for green algae, 0.89 mg/L for the diatom, and 0.60 mg/L for blue-green algae (Latała et al., 2009). In Nordén et al, the LC50 for chicken embryos was found to be 8.5 µg/g egg for PFOS and 2.5 µg/g egg for PFOA, demonstrating the sensitivity of avian species to PFAS (2016). The EPA Health assessment workspace collaborative (HAWC) project estimated the Lowest Observed Effect Level (LOEL) of PFOA to be 5 mg/kg body weight for rats from an oral dose and 150 mg/kg for acute effects on rats through intraperitoneal injection. HAWC also estimated an LOEL of 4 mg/kg for PFOA for reproductive effects on mice (EPA HAWC). For PFOS, the LOEL through oral exposure is 0.5 mg/kg for chronic exposure in rats and the LOEL through oral exposure is 0.25 mg/kg for short-term exposure in mice (EPA HAWC). For short-chain PFAS, the toxic effects are relatively lower. For example, PFHxA has a no observable effect level (NOEL) of 10 mg/kg, and lowest-observed-adverse-effect level (LOAEL) of 200 mg/kg for subchronic exposure through an oral pathway in rats (EPA NCCT). Overall, animal toxicity tests as well as computational modeling suggest that PFAS can pose health risks to both humans and the ecosystem.

PFAS Exposure

In their chemical structure, PFASs are mainly made up of fluorine-carbon bonds that are highly stable and very difficult to break down, therefore forming a chain that does not easily degrade. Their physico-chemical properties are still poorly understood and widely debated. Based on the available data synthesized by Agency for Toxic Substances and Disease Registry, PFAS are generally soluble in water and most are present in their ionic forms when they are released into the environment (3M, 2000; Kwan, 2001; Zhao et al, 2014). Not much is known about the mobility of most PFAS molecules in soil, though PFOA was shown to be somewhat mobile in the soil (Prevedouros et al., 2006). Regarding their half-life, model predictions using the software EPI (Estimation Programs Interface) Suite estimated the half-life of long-chain PFAS to be around 2 years in both water and soil, and 8.45-31.3 years in air; the estimated half-life of short-chain PFAS is 0.65-2 years in water and soil, and 8.29~31.4 years in air (EPA EPI Suite). The US EPA defines a toxicant as persistent if it has a half-life of 60 days in water, sediment, and soil or a half-life of 2 days in air (Environmental Protection Agency, 1999). Therefore, PFAS are considered persistent chemicals in water because their half-life is greater than 60 days and their half-life in air is greater than 2 days.

The main sources of PFAS in the environment are industrial wastewater that contains PFAS molecules and the use of PFAS-containing products. PFAS detected in freshwater systems could be from direct industrial emissions or indirectly released via wastewater treatment plant (WWTP) effluents (Möller, et al. 2010). Conventional wastewater treatment methods cannot remove most PFAS molecules (Schultz et al., 2006). The biosolids applied to the ground could indirectly reach human through contamination of food and livestock (Lindstrom et al., 2011). Groundwater PFAS could be from the use of proprietary aqueous film forming foam (AFFF) which seeps into soils beneath former firefighting training areas into underground water systems (Keswani, 2013-2015, McGuire et al., 2014). A study showed that proximity to military site and civilian airports with the use of AFFF is significantly associated with the concentration of PFAS above the minimal reporting level (MRL) (Hu et al., 2016). These studies provide evidence that both freshwater and groundwater systems have been contaminated.

Due to the wide detection of PFAS in the environment, human exposure to PFAS is ubiquitous. Major pathways of human exposure include ingestion of contaminated drinking water and ingestion of food that is contaminated with PFAS during growth or during packaging. Inhalation and inadvertent ingestion of dust that contains PFAS chemicals in commercial products is also an exposure pathway, although minor in comparison to dietary pathways (D'Hollander, et al., 2010; Tittlemier et al., 2007). Hu et al. (2016), found that the levels of PFOS and PFOA in drinking water supplies serving 6 million Americans were higher than the US EPA's healthy advisory level (70 ng/L) and that many of the water supplies that were elevated were close to a military base, airport or waste water treatment plant, suggesting they are primary sources to drinking water. And in a study that analyzed serum levels of PFAS within the National Health and Nutrition Examination Survey (NHANES), the data suggested that more than 90% of the United States population has detectable levels of PFAS in their bodies and that levels of exposure vary substantially from person to person (Calafat, et al. 2007). Therefore, these studies demonstrate that PFAS exposure is ubiquitous and drinking water is a primary source of exposure.

Human Health Effects

Human PFAS exposure has been linked to cancer, immune suppression, and endocrine disruption, though majority of past studies have focused on a small number of PFAS compounds (e.g. PFOS, PFOA, PFNA and PFHxS). For example, Melzer et al. found that higher concentrations of serum PFOA and PFOS are associated with thyroid disease in the U.S. general adult population (2010) and PFOS and PFOA have also been associated with increased risk of breast cancer and prostate cancer (Bonefeld-Jorgensen et al. 2011; Eriksen et al., 2009).

There have been numerous studies examining prenatal and developmental exposure to PFAS and their potential health effects (Rappazzo et al, 2017). For example, prenatal exposure to PFAS has been associated with immunosuppression in early childhood (Granum et al., 2013). Maternal exposure to PFAS during pregnancy has also been also associated with adverse birth outcomes such as low birth weight and preterm birth in several studies (e.g. Apelberg et al., 2007; Chen et al., 2012). Similarly, both PFOS and PFOA were negatively associated with continuous measures of birth weight, after adjusting for covariates (Apelberg et al., 2007). Higher maternal PFOS exposure has also been associated with shorter gestational age (Chen et al., 2012). However, many studies suggest potential differences in the effect of exposure in male and female infants. For example, a study from China found a negative association between PFAS levels in cord blood and birth weight, which was stronger in male infants (Li et al., 2017) than female infants. Additionally, there are studies that found PFAS exposure is not significantly associated with birth outcomes (e.g. Manzano-Salgado et al., 2017). A number of factors, including differences in the measurement of exposure (e.g. material vs. cord serum) and the timing and level of exposure may have influenced the results of past studies. Thus, though generally supported by priori studies, the effects of PFAS exposure on birth outcomes remain to be fully explored.

Research Rationale and Objectives

PFAS contamination has been an ongoing issue in the state of North Carolina. A group of scientists of North Carolina State University and EPA sampled water along Cape Fear River in Wilmington, North Carolina. They detected high levels of PFAS molecules, particularly perfluoro-2-propoxypropanoic acid (PFPrOPrA, also named as GenX), in the water samples and identified the Chemours facility, a chemical manufacturer of PFAS, as the source (Sun et al., 2016). Cape Fear River is an important local source of drinking water for the residents in Wilmington, NC. PFAS contamination in the river has raised concerns and triggered ongoing scientific investigation regarding its health effects and the extent of human exposure. This research project focused on the state of North Carolina as part of the effort to investigate one aspect of the PFAS contamination and its health effects.

PFAS is an important issue for the quality of North Carolina drinking water. Since the detection of GenX in the Cape Fear River, more recurrent public discussions that involve government, researchers, environmental groups, industries, and affected communities have been ongoing. Community members are particularly interested in understanding the potential health effects of PFAS in North Carolina drinking water. A recent studies analyzed drinking water samples Raleigh, Cary, Durham, Pittsboro, and Chapel Hill, and found that the sum of 11 PFASs was as high as 760 ng/L in Pittsboro, NC, which is about 10 times of the level recommended by U.S. EPA health advisory level for PFOA and PFOS in drinking water (Herkert et al., 2020). However, total PFOA and PFOS were not >70 ng/L in any water tested. These high levels suggest a need to investigate health effects in communities receiving exposure to elevated PFAS through drinking water.

This project investigated the association between PFAS levels in drinking water and birth outcomes, specifically examining associations with gestational age and birth weight in infants. It was hypothesized that populations exposed to higher concentration of PFAS via drinking water may experience higher rates of adverse birth outcomes, expressed in shorter gestational age and lower birth weight in comparison to populations with low PFAS exposure. This project focused on the North Carolina's Triangle region, and in particular, the Town of Pittsboro, whose main source of drinking water is the Haw River. The project also included the Wilmington region due to ongoing discussion and public interest about PFAS contamination in the Cape Fear River and downstream drinking water sources. The results can be used to enhance our current understanding of PFAS exposure and health effects globally.

Method and Approach

Data Source and Collection

All protocols and procedures used in this project were reviewed and approved by the Duke University Institutional Review Board (IRB) prior to initiation. Data used in this study was provided by the North Carolina Department of Vital Statistics. For the purpose of the study, we used birth certificate data collected from 2012 to 2016. We selected births in Wake, Durham, Chatham, Orange, and Alamance counties for which drinking water PFAS measurements had previously been conducted for inclusion in these analyses. During the five year period of interest there were 77,161 individual births in these counties. We excluded plural births and any values that were likely coded incorrectly (e.g. a baby born at 25 weeks weighting 4000 g). In addition, based on sample size limitations, babies born in water districts that had less than 50 births across the 5-year period were excluded from analyses.

The residential location at birth was geocoded and was subsequently linked to its corresponding drinking water district Using ArcMap version 10.4. After identifying the drinking water districts, we matched the water districts with source water from which the drinking water was drawn. These "watersheds" were used as a proxy to reflect individual exposure to PFAS through drinking water.

Statistical analyses were performed in R (Rstudio Team, 2015). We first calculated descriptive statistics and then performed regression analyses. Multiple linear regression models were used to predict differences in gestational age and birth weight based on watersheds. Falls Lake, which is the main source of water for the City of Raleigh, and regions that purchase water from the city, was used as the reference group due to the fact that it supports a large, diverse population (e.g. Raleigh) and has no known prominent level of PFAS contamination (Herkert et al. 2020). Statistical significance was set at 0.01. Results of regression analyses were visualized in ArcGIS to present the birth outcomes as a heat map to identify regions that experience differences in gestational age and birth weight in comparison to the reference population.

Covariates

This research project also considered potential covariates that might influence associations between watersheds and birth outcomes and impact our statistical analyses. Analyses were stratified by infant sex. Besides the inherent difference in birth weight and gestational age between sexes, we expected to see a difference in the effects between male and female infants in both birth weight and gestational age based on the previous birth cohort studies (McGregor et al, 1992). We also selected confounders based on their relevance to birth outcomes and their potential influences on birth weights and gestational age. The cofounders that were included in this analysis were socioeconomic factors that might be associated with birth outcomes and exposure: education level (binary: high-education/non-higher education), race (binary: white/nonwhite; black/nonblack), marital status (binary: married/not married). Additional covariates included mother's age in years (continuous), body mass index (continuous), prenatal care (binary: below adequate level/adequate or above adequate level), and previous births (binary: gave birth before/never gave birth before).

Results

As shown in Table 1, there were 14 watersheds in total included in our analyses: 13 watersheds were in the triangle region, and 1 watershed represented the whole Wilmington region. After exclusion of plural births, and improperly coded values, and infants born in low population water districts, 74,751 infants were included in final

Table 1. Summary of watershed, water districts, and number of births between 2012-2016.

Watershed	**Number of Births**	**Water Districts**
Cape Fear River - Headwater	76	Chatham County - East
		Goldston-Gulf District
Cape Fear River	1,392	Town of Holly Springs
Corporation Lake, four wells, and Lake Mackintosh	613	Orange Alamance Water System
Eno River	328	Town of Hillsborough
Falls Lake	26,076	Town of Zebulon
		Town of Knightdale
		City of Raleigh
		Town of Wake Forest
		Town of Wendell
Graham-Mebane Lake	979	City of Graham
		Town of Green Level
		Town of Swepsonville
Haw River	185	Town of Pittsboro
Jordan Lake	9,428	Town of Apex
		Chatham County - North
		Town of Cary
		Town of Morrisville
Lake Mackintosh	3,295	Town of Gibsonville
		Village of Alamance
		Town of Elon
		Town of Ossipee
		City of Burlington
		Town of Haw River
Lake Michie & Little River Reservoir	16,322	City of Durham
Neuse River, Falls Lake & Lake Benson, Cape Fear River	1,123	Town of Fuquay-Varina
Rocky River	729	Chatham County - Southwest
		Town of Siler City
University Lake, Cane Creek Reservoir, Quarry Reservoir, and Jordan Lake	2,578	Orange County Water and Sewer Authority
Wilmington Region	11,627	Aggregated 29 water districts

Table 2. Descriptive statistics for the population under study.

		N
Total		74,751
Gender		
	Male	39,497 (52.8%)
	Female	35,254 (47.2%)
Mother's Marital Status		
	Married	53,491 (71.5%)
	Not Married	21,252 (28.4%)
	Unknown	8 (<1%)
Mother's Race		
	White	47,161 (63.0%)
	Black	14,958 (20.0%)
	Others	12,632 (17.0%)
Mother's Age		
	<= 25	24,953 (33.4%)
	26-30	23,797 (31.8%)
	31-35	17,476 (23.4%)
	> 36	8,525 (11.4%)
Mother's Education		
	Obtained Secondary Education or Below	48056 (64.3%)
	Obtained Post-Secondary Education or Higher	26,459 (35.4%)
	Unknown	236 (0.3%)
Mother's Pre-pregnancy Body Mass Index (BMI)		
	Underweight (< 18.5)	5,296 (7.1%)
	Normal (18.5-24.9)	28,706 (38.3%)
	Overweight (25.0-29.9)	23,387 (31.3%)
	Obese (> 30.0)	16,005 (21.4%)
	Unknown	1,357 (1.9%)
Mother's Prenatal Care		
	Inadequate	22,986 (30.7%)
	Adequate or > Adequate	50,886 (68.1%)
	Unknown	879 (1.2%)
Parity	Gave birth before	36,927 (49.4%)
	Never gave birth before	37,806 (50.6%)
	Unknown	18 (0.99%)
Smoking	Smoked during pregnancy	7,822 (10.5%)
	No smoking	66,915 (89.6%)
	Unknown	14 (0.01%)

analyses in this study. Table 2 provides information a summary of the demographic information for included infants. There were slightly more male infants than female infants. The majority (71.5%) of mothers were married and they were predominantly non-Hispanic white (63.0%). Most mothers gave birth before the age of 30 years and approximately one-third of the mothers had obtained an educational degree above college. Around two-thirds of the mothers were categorized as overweight or obese based on their BMI score before their pregnancy. A majority of the mothers (68.1%) had received adequate or above adequate prenatal care during their pregnancy and approximately half of the mothers had given birth at least once before. Approximately 10% of the mothers smoked before or/and during pregnancy.

Multiple linear regression models were conducted to investigate associations between drinking water and birth weight after adjusting for confounding variables. Results, shown in Table 3, suggest that babies born in most watersheds had lower average birth weights compared to the reference population in all three stratified groups (i.e. both sexes, male infants, and female infants). For example, the largest difference was observed in the region of the Headwater of the Cape Fear River. Infants were born 0.261 lbs (± 0.136, $p < 0.1$) lighter than the reference population when adjusting for confounding variables. However, some water districts had higher average birth weights compared to the reference population, including the Cape Fear River, Lake Michie, Neuse River, and University Lake, though the differences were not statistically significant. There was also an apparent difference in the relationship between water sources and birth weight between female infants and male infants. In general, male infants experienced a larger reduction in birth weights compared to female infants. For instance, in the population living in the districts receiving water from Lake Mackintosh, male infants were born 0.145 lbs (± 0.029, $p < 0.01$) lighter than the reference population. In contrast, female infants were born 0.075 lbs (± 0.032, $p < 0.05$) lighter than the reference population.

The results also suggested that infants born in most watersheds had a shorter gestational duration compared to the reference population, except University Lake and Eno River. For example, the largest difference (compared with the reference population) was observed in the region of the Headwater of the Cape Fear River. Male infants were born 0.674 weeks (±0.276, $p<0.05$) earlier than the reference population. We also observed a difference in patterns of association between males and females: for 11 out of 13 population effect estimates were greater in males compared to females, suggesting that male infants in these populations experienced a greater decrease in gestational age than female infants. For instance, in the Wilmington region, male infants were born 0.854 days (±0.189, $p<0.01$) earlier than the reference population, whereas female infants were born 0.406 days (±0.217, $p<0.1$) earlier than the reference population.

In Figure 2, top and bottom panels display maps highlighting the magnitude of the differences in birth outcomes spatially, compared to the reference population.

Discussion

Overall, we found statistically significant differences in birth outcome parameters, including birth weight and gestational age, among the various watersheds investigated. For example, the Headwater of Cape Fear River population had the lowest adjusted birth weight and gestational age compared to the reference population. However, the population was small, and these results should be interpreted with caution. It is possible that the population experienced a shift in birth outcomes due to other regional or extraneous factors. Importantly, we were unable to find any specific PFAS measurements for this water system and it is unclear if PFAS levels are detectable in this watershed. For both outcomes of interest, the average adjusted birth weight and adjusted gestational age in Town of Pittsboro, which drew water from Haw River, was smaller comparing to reference population across both sexes; however, these results were not statistically significant compared to the reference population. This might be due to a lack of statistical power given the small sample size of births in the Town of Pittsboro compared to the size of the reference population.

Infants born in towns where the water is served from Lake Mackintosh were also significantly smaller and born earlier compared to the reference population. This includes the City of Burlington and adjacent cities and towns. Environmental monitoring in Lake Macintosh did not find elevated levels of PFAS (source: PFAST Network

Table 3. Change in Birth Weight and Gestational Age for Each Watershed in Comparison to the Falls Lake, NC Watershed. All models were adjusted for race, age, education, BMI, marital status, smoking, prenatal care quality and parity. (Numbers in parentheses represent the standard error of the mean estimate)

	Birth Weight Pounds			***Gestational Age Days***		
Watershed	***Both***	***Female***	***Male***	***Both***	***Female***	***Male***
Cape Fear River	0.007	0.045	-0.036	-1.099***	-0.917*	-1.12**
	(0.032)	(0.048)	(0.043)	(0.350)	(0.539)	(0.462)
Cape Fear River - Headwater	-0.261*	-0.254	-0.249	-3.01**	-1.358	-4.718**
	(0.136)	(0.201)	(0.181)	(1.484)	(2.268)	(1.932)
Corporation Lake, Four Wells, and Lake Mackintosh	-0.021	-0.022	-0.018	-0.469	-0.14	-0.525
	(0.048)	(0.070)	(0.064)	(0.518)	(0.791)	(0.686)
Eno River	-0.088	-0.065	-0.115	-0.007	-0.077	0.399
	(0.066)	(0.095)	(0.090)	(0.721)	(1.071)	(0.966)
Graham-Mebane Lake	-0.043	-0.019	-0.079	-1.176	-1.05*	-1.12**
	(0.038)	(0.056)	(0.051)	(0.420)	(0.630)	(0.546)
Haw River	-0.071	-0.108	-0.064	-0.287	-0.119	-0.231
	(0.088)	(0.124)	(0.122)	(0.959)	(1.393)	(1.302)
Jordan Lake	-0.034**	-0.031	-0.043**	-0.595***	-0.336	-0.735***
	(0.014)	(0.021)	(0.020)	(0.154)	(0.238)	(0.210)
Lake Mackintosh	-0.114***	-0.075**	-0.145***	-1.75***	-1.449***	-1.813***
	(0.022)	(0.032)	(0.029)	(0.238)	(0.364)	(0.315)
Lake Michie, Little River Reservoir	-0.016	-0.046***	0.008	-0.196	-0.035	-0.231
	(0.012)	(0.017)	(0.016)	(0.126)	(0.196)	(0.168)
Neuse River, Falls Lake, Lake Benson, Cape Fear River	-0.003	0.012	-0.018	-0.336	-0.133	-0.574
	(0.036)	(0.052)	(0.049)	(0.392)	(0.581)	(0.525)
Rocky River	-0.064	-0.103	-0.002	-1.078**	-1.547**	-0.567
	(0.046)	(0.065)	(0.063)	(0.497)	(0.735)	(0.672)
University Lake, Cane Creek Reservoir, Quarry Reservoir, and Jordan Lake	0.008	-0.026	0.023	0.833***	1.267***	0.602*
	(0.025)	(0.036)	(0.033)	(0.266)	(0.406)	(0.350)
Wilmington Region	-0.048***	-0.015	-0.091***	-0.756***	-0.406*	-0.854***
	(0.013)	(0.020)	(0.018)	(0.147)	(0.217)	(0.189)

	Birth Weight Pounds			Gestational Age Days		
Covariates	**Both**	**Female**	**Male**	**Both**	**Female**	**Male**
Infant Sex (reference = male)						
Female	-0.493***			0.504		
	(0.009)			(0.098)		
Maternal Race and Ethnicity (reference = other race or ethnicity)						
non-Hispanic white	0.300***	0.130***	0.473***	2.261***	2.611***	2.247***
	(0.013)	(0.019)	(0.018)	(0.147)	(0.210)	(0.196)
non-Hispanic black	-0.345***	-0.394***	-0.260***	-2.289***	-2.856***	-1.645***
	(0.015)	(0.021)	(0.021)	(0.168)	(0.245)	(0.224)
Maternal Age (continuous years)	-0.017***	-0.005***	-0.022***	-0.294***	-0.448***	-0.196***
	(0.001)	(0.001)	(0.001)	(0.007)	(0.014)	(0.014)
Maternal Postsecondary Education	0.230***	0.432***	-0.063***	-0.448***	-0.0007	-0.749***
	(0.011)	(0.016)	(0.016)	(0.126)	(0.175)	(0.175)
Pre-pregnancy BMI (continuous)	0.030***	0.026***	0.020***	-0.084***	0.035***	-0.133***
	(0.001)	(0.001)	(0.001)	(0.007)	(0.014)	(0.014)
Maternal Marital Status	0.132***	-0.045**	0.301***	2.835***	3.388***	2.415***
	(0.012)	(0.018)	(0.016)	(0.133)	(0.196)	(0.175)
Adequate Prenatal Care	-0.148***	-0.174***	-0.014	-0.126	-1.344***	-0.049
	(0.010)	(0.015)	(0.013)	(0.105)	(0.168)	(0.140)
Maternal Smoking During Pregnancy	-0.389***	-0.199***	-0.596***	1.323***	-2.128***	2.52***
	(0.015)	(0.027)	(0.019)	(0.168)	(0.301)	(0.203)
Previous Birth Parity	0.283***	0.382***	0.159***	-0.266**	-1.155***	0.077
	(0.010)	(0.015)	(0.014)	(0.112)	(0.161)	(0.147)
Note:	*p<0.1; **p<0.05; ***p<0.01					

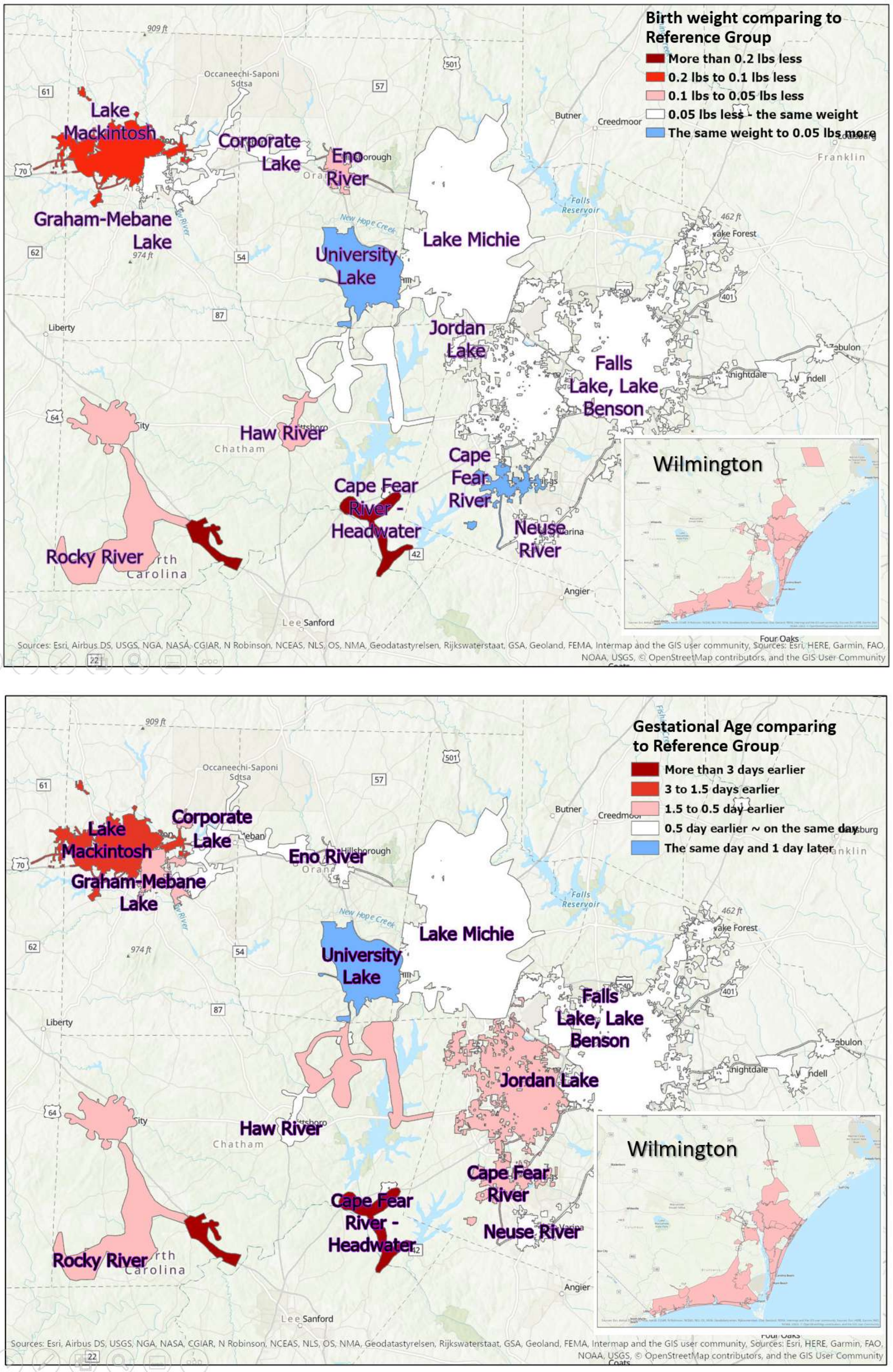

Figure 2. Change in birth weights (top panel) and gestational age (bottom panel) among populations comparing to the Falls Lake watershed.

reporting), suggesting that there might be other sources of contamination or factors contributing to these differences observed in this population. Interesting, in this area, patterns were similar in males and females; there was a significant decrease in both birth weight (-0.114 lbs ± 0.022, $p<0.01$, for both sexes) and gestational age (-1.75 days weeks ± 0.24, $p<0.01$, for both sexes) compared to the reference population. Previous research evaluating exposure to PFAS primarily observed differences by sex. Studies suggested that male infants were at a greater disadvantage and at higher risk of morbidity and mortality when experiencing adverse birth outcomes such as preterm birth (Naeye et al, 1971; Roy et al, 2014). Therefore, these observations suggest that PFAS may not be a factor.

Additionally, the outcome of this research could provide insights into spatial differences in birth outcomes that were not a result of the variability among demographic characteristics, such as race, age, educational status, and body mass index, among the different populations. The populations that showed significant differences in birth outcomes compared to the reference could be investigated further to study the causes of such difference. There might be other factors or exposures to other chemicals that contributed to such difference.

This research study had several important limitations. First, this study used watersheds as a proxy for human exposure to PFAS contamination. Though it distinguished populations based on their drinking water source, and likely reflects some differences in PFAS exposure via drinking water, we did not have data on individual water consumption or direct measures of exposure. Future in-depth examination at the individual level is essential to identify specific PFAS exposures that might be contributing to differences in birth outcomes. For example, future studies could investigate serum levels of PFAS in different populations to further validate the trends observed in this study. Moreover, there were other potential confounding factors that could be present, such as access to nutritious food and household income, that this research project could not adjust for due to lack of data. Therefore, there were other factors that might influence results. Lastly, there might also be other contaminants in the water systems that have a different source and exposure profile compared to PFAS, and, therefore, we could not account for these exposures in our analyses.

Taken together, these results can be used to provide a better understanding of differences in birth outcomes in NC. Although differences in birth outcomes were relatively small in these analyses, and perhaps not clinically significant at the individual level, small shifts in birth weight and gestational age have large implications at the population level, particularly as preterm birth and low birth weight are associated a number of chronic health conditions. There are other populations across the nation whose drinking water sources are impacted by PFAS contamination. The approach employed in this study could be applied to other regions under certain assumptions to explore whether these patterns could be observed anywhere else in the U.S. The results of the study can also provide supporting evidence to implement regulations on PFAS in drinking water and to reduce the overall use of PFAS to minimize further exposure.

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