

Spatial and Temporal Variation in PFAS Concentrations in Smallmouth Bass

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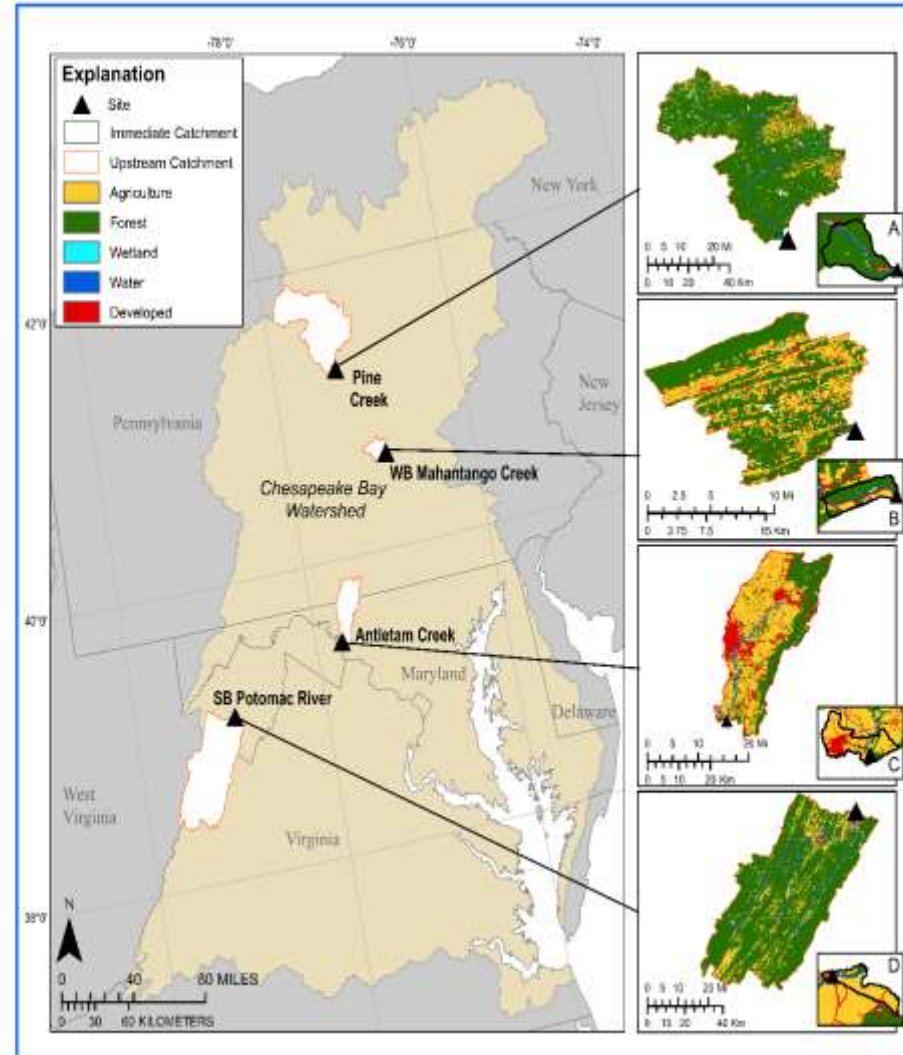


Smallmouth Bass Health Assessments

- In response to fish kills, observed endocrine disruption (intersex) and population declines in certain watersheds of the Chesapeake, samples were collected from 2013-2019
 - Visible abnormalities, condition factors, age, sex documented
 - Blood was collected for plasma vitellogenin and plasma aliquots frozen for future analyses
 - Tissues were preserved for histopathology
 - Select tissues were frozen for chemical analyses
 - Pieces of liver and gonad preserved for molecular analyses
 - Anterior kidney (2017-2019) collected for immune functional analyses

Long Term Integrated Monitoring

- 2013-2019
- Biological monitoring of adults in spring and fall and young-of-year in early summer
- Surface water sampling for hormones, pesticides, phytoestrogens monthly (bimonthly in spring)
- Two in the Potomac (mouth of Antietam Creek and South Branch Potomac near Moorefield)
- Two in the Susquehanna (mouth of Pine Creek and West Branch Mahantango Creek)



Preliminary Analyses of Perfluoroalkyl Chemicals in Plasma

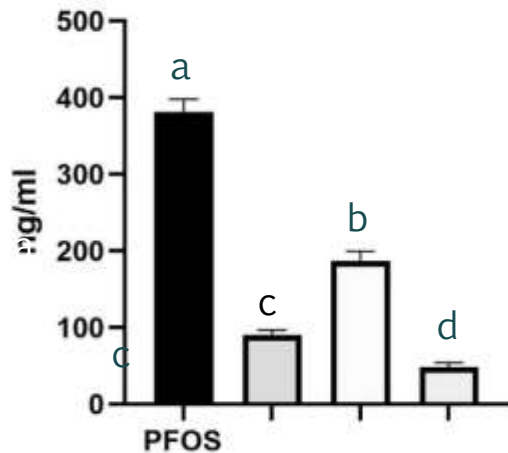
- Used the 2018 archived plasma samples
- 13 PFAS including 9 perfluorocarboxylic acids and 4 perfluorosulfonates
- Analyzed by SGS-AXYS, Sidney, British Columbia by LC-MS/MS
- Concentrations in ng/ml plasma

Compounds Detected in Smallmouth Bass Plasma

- Four compounds were detected in every plasma sample
 - PFOS (perfluorooctane sulfonate) – 8 carbon sulfonate
 - Ranged from 20 – 574 ng/ml
 - PFUnA (perfluoroundecanoate) – 11 carbon carboxylic acid
 - Ranged from 3 – 55 ng/ml
 - PFDA (perfluorodecanoate) – 10 carbon carboxylic acid
 - Ranged from 2 – 37 ng/ml
 - PFDoA (perfluorododecanoate) – 12 carbon carboxylic acid
 - Ranged from 1 – 34 ng/ml
- Two additional compounds were detected in some samples
 - PFOSA (perfluorooctane sulfamide) – 8 carbon sulfonate
 - Ranged from BD – 1.7 ng/ml
 - PFNA (perfluorononanoic acid) – 9 carbon carboxylic acid
 - Ranged from BD – 1.3 ng/ml

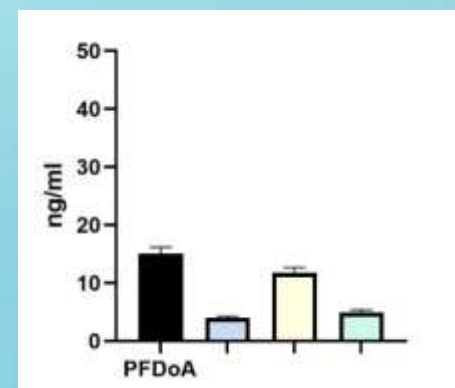
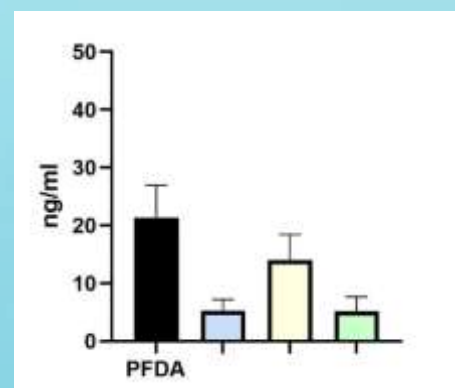
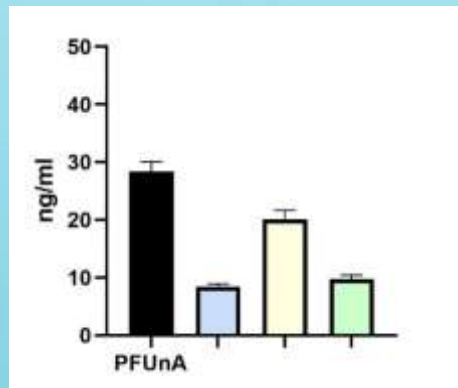
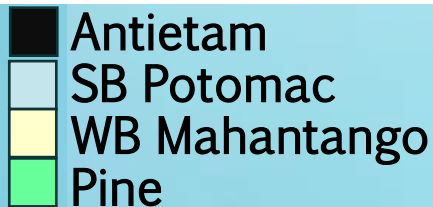
Significant Site Differences

- Compared spring and fall and found no seasonal differences so seasons were combined for the site comparisons



PFOS concentrations were significantly higher than other compounds and all four sites were significantly different.

Other three compounds showed the same pattern but South Branch, Potomac and Pine Creek were not significantly different

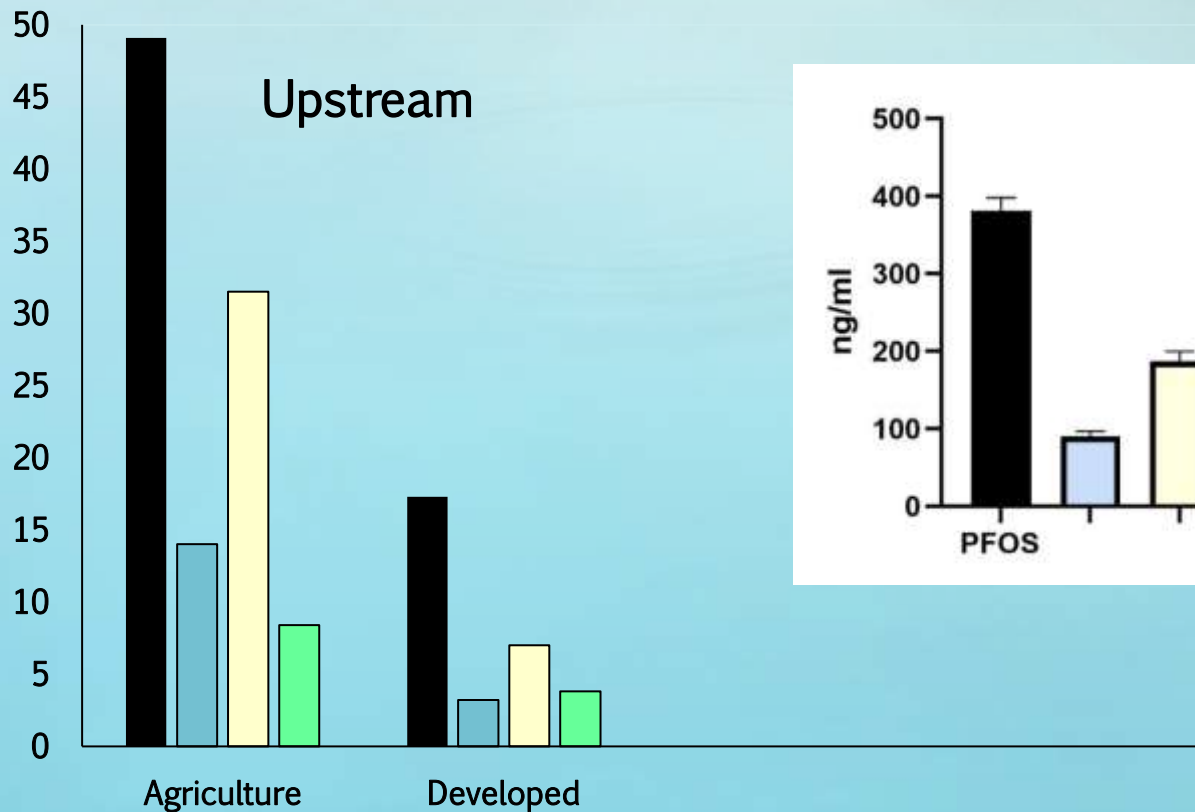


Sex Differences

- Combining sites and seasons
 - No difference in age between male (n = 66) and female (n = 64)
 - No difference in PFOS or PFDA plasma concentrations of females and males
 - Both PFDoA and PFUnA concentrations were significantly higher in males than females
- Analysis by site
 - Similar findings – no difference between sexes at any site for PFOS
 - PFDoA and PFUnA concentrations were higher in males than females at all sites
 - PFDA concentrations were higher in males only at Pine Creek

Associations with Land-Use Attributes Potential Sources

- Land-use characteristics were calculated for both the upstream cumulative drainage and the immediate catchment scale



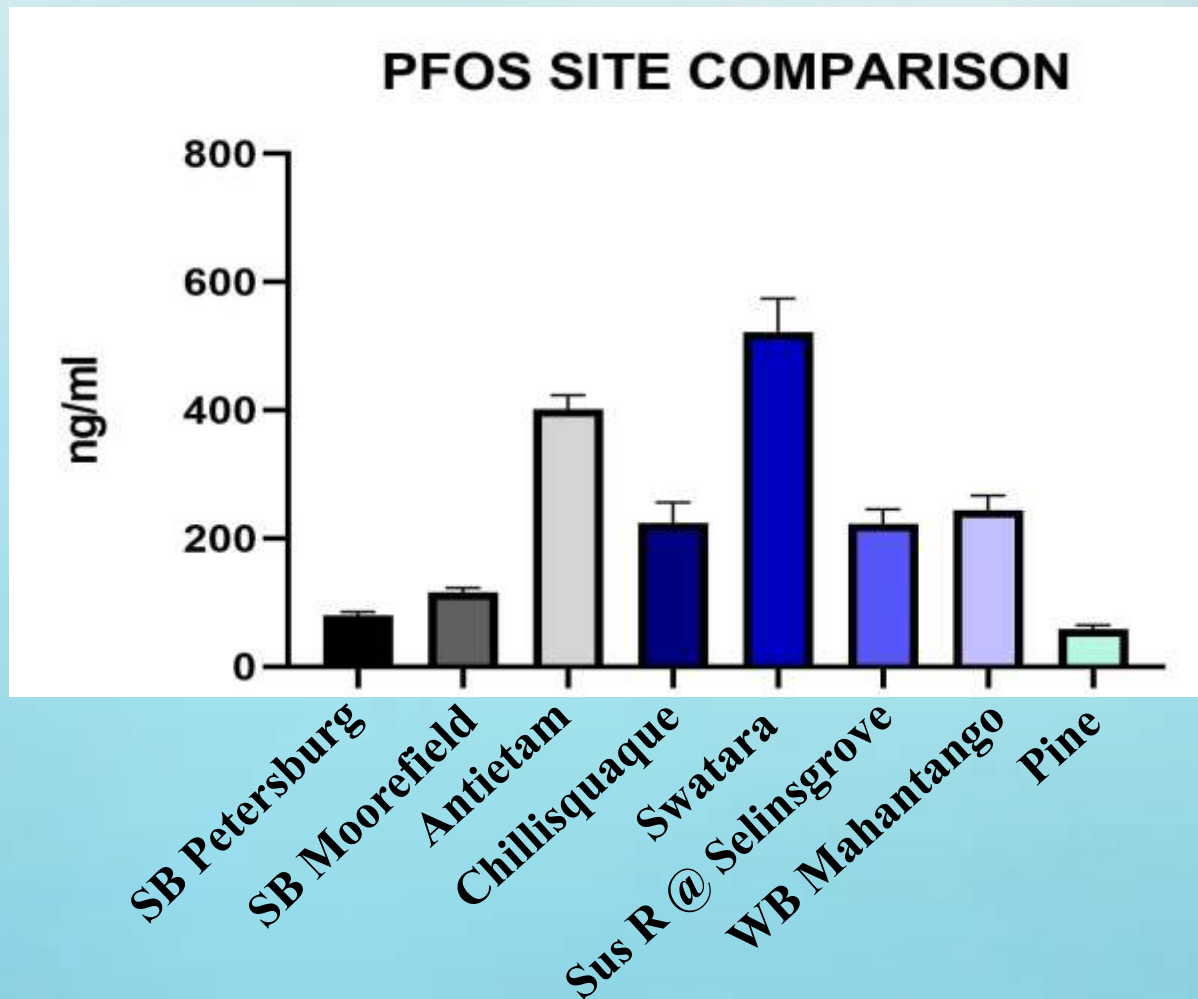
Comparison with Other Studies

- Difficult to compare to many other studies as most analyze PFAS in muscle (fillets) or liver
 - Most species studied find plasma/blood > liver > muscle
 - For studies worldwide that have analyzed blood/plasma our results are some of the highest
 - Striped bass from Cape Fear downstream of a PFAS production plant were higher
 - Highest concentration in Antietam smallmouth was 574 ng/ml PFOS
 - Highest concentration in Cape Fear striped bass was 977 ng/ml PFOS

Additional Analyses

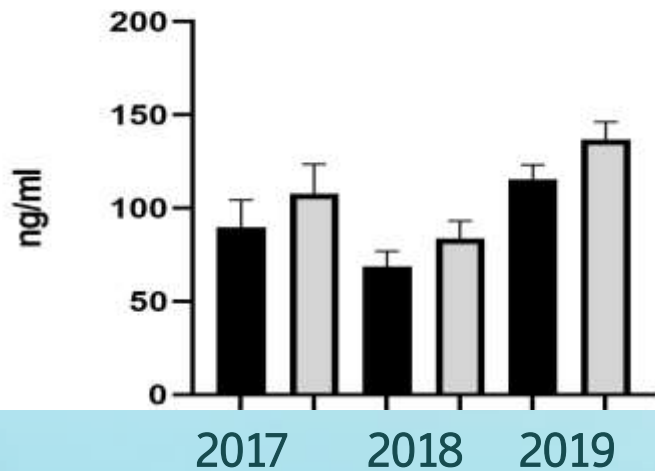
- Archived plasma samples from 2017 and 2019 from these same sites were submitted for analyses to determine if temporal differences occurred and increase sample sizes to evaluate potential effects.
- Submitted plasma from additional sites for a larger spatial analysis and to understand sources better.

Site Comparisons

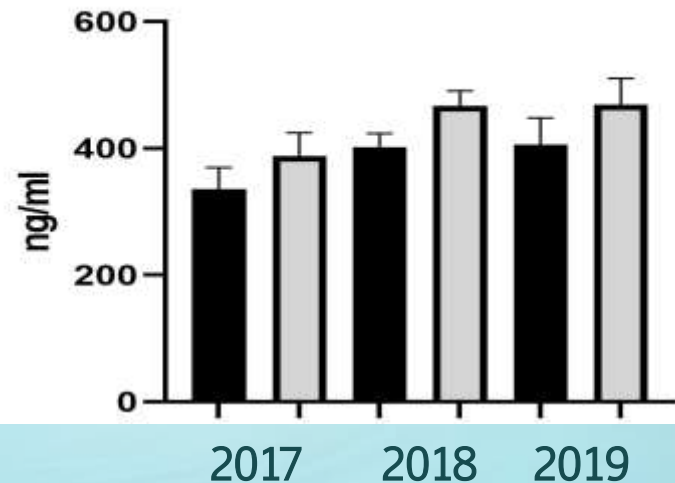


Temporal Comparisons

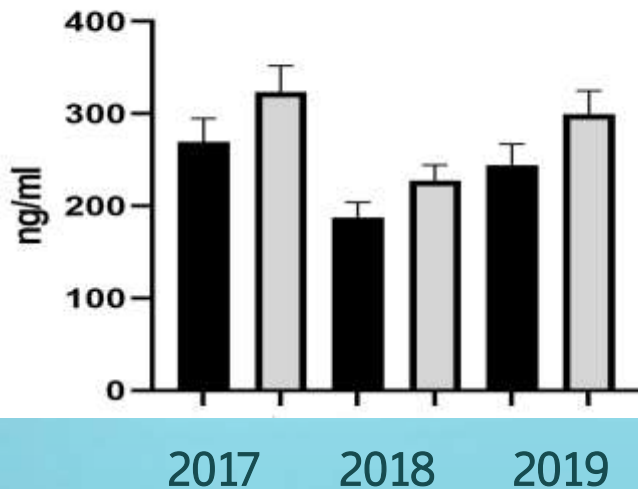
South Branch Potomac



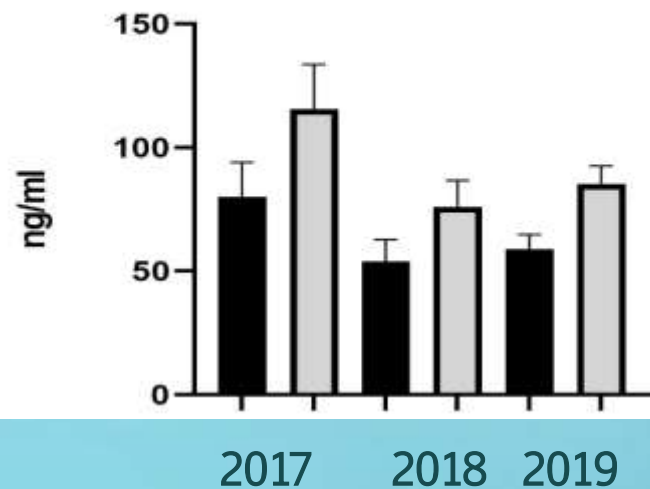
Antietam Annual



West Branch Mahantango



Pine Creek Annual



■ PFOS
■ Total PFAS

Associations with Landuse Attributes

- Combining all years/seasons/sites positive correlations were:
- PFOS and Total PFAS
 - Total pesticide application in the immediate catchment
 - #TRI (EPA Toxic Release) facilities in upstream catchment
- PFDA and PFUnA
 - Nitrogen, phosphorus and total nutrients from biosolid application in upstream catchment
- PFDoA
 - No significant associations

Ongoing Studies

- For more recent analyses SGS-AXYS has a method for 40 compounds
- No current data from Potomac, sites in PA, including Swatara Creek, show numerous other compounds with the expanded list
- Tissue concentrations – in general plasma/blood is highest, liver and then muscle (fillet), however, not true for all compounds
- This Fall, in conjunction with MD DNR population sampling, nonlethal collection of blood along the upper Potomac for PFAS as well as potential blood and plasma biomarkers

Associations with Biological Indicators

- Begun to examine look for correlation of PFAS with biological endpoints measured in wild smallmouth bass
- Using the plasma concentrations measured in wild bass, exposing isolated hepatocytes and (anterior kidney) immune cells to evaluate gene transcript abundance and immune function in laboratory-held smallmouth bass

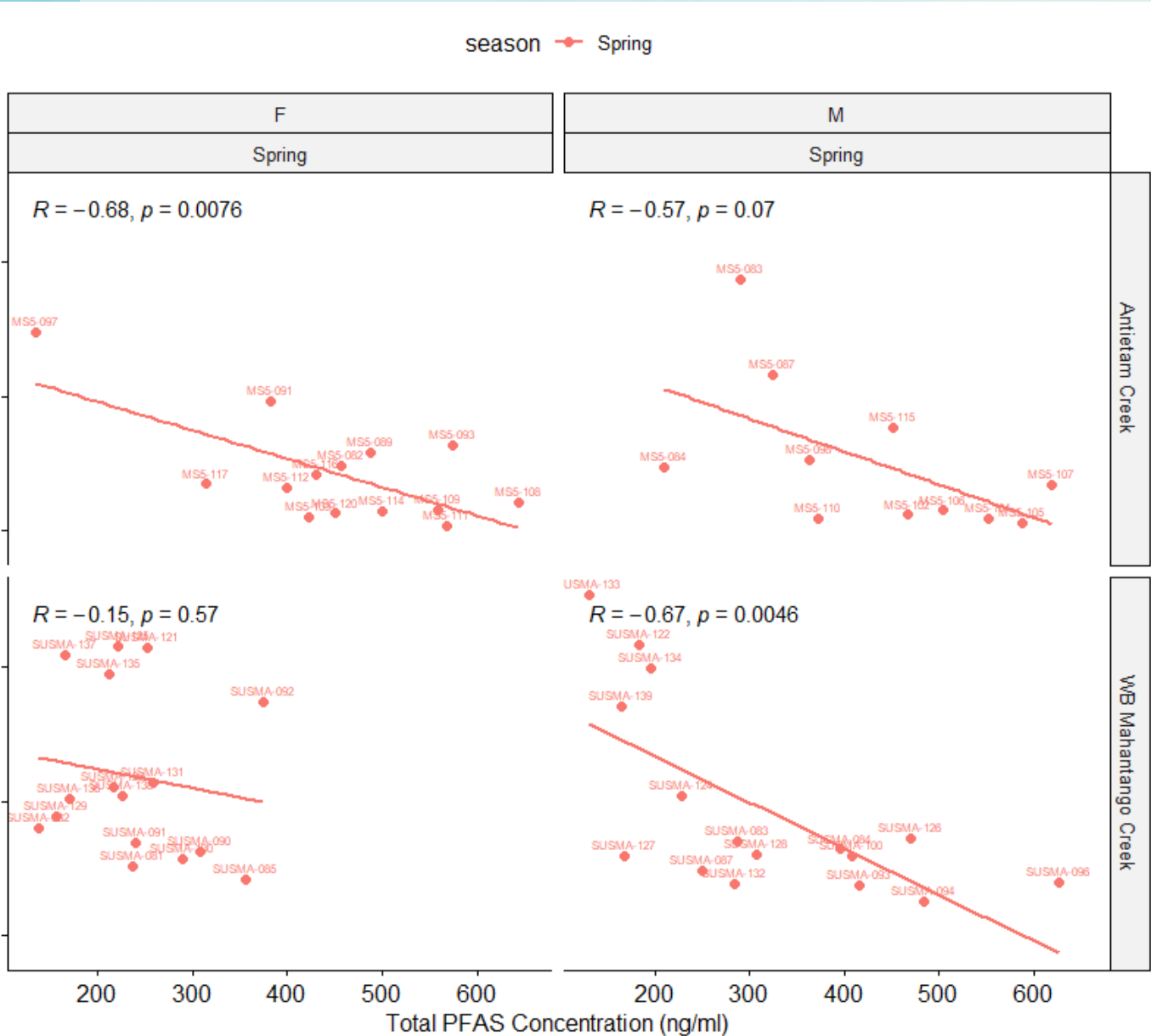
Some Effects of PFAS

- Immunomodulation
 - Increased trematode infections in amphibians
- Oxidative damage
- Chronic inflammation
- Reproductive endocrine disruption
 - Estrogen receptor-mediator effects
- Dysregulation of thyroid function
- Possible carcinogens – testicular and kidney cancer in humans

Immune System of Fish

- Innate responses or nonspecific – do not need prior priming (exposure) – macrophage and neutrophil bactericidal activity, numerous soluble factors
- Adaptive (acquired) or specific immunity – enhanced response if have prior exposures – antibody response
 - B lymphocytes – IgM+ - produce antibodies
 - T lymphocytes – IgM- - cytotoxic T cells involved in killing virus-infected cells

Immunomodulatory Effects

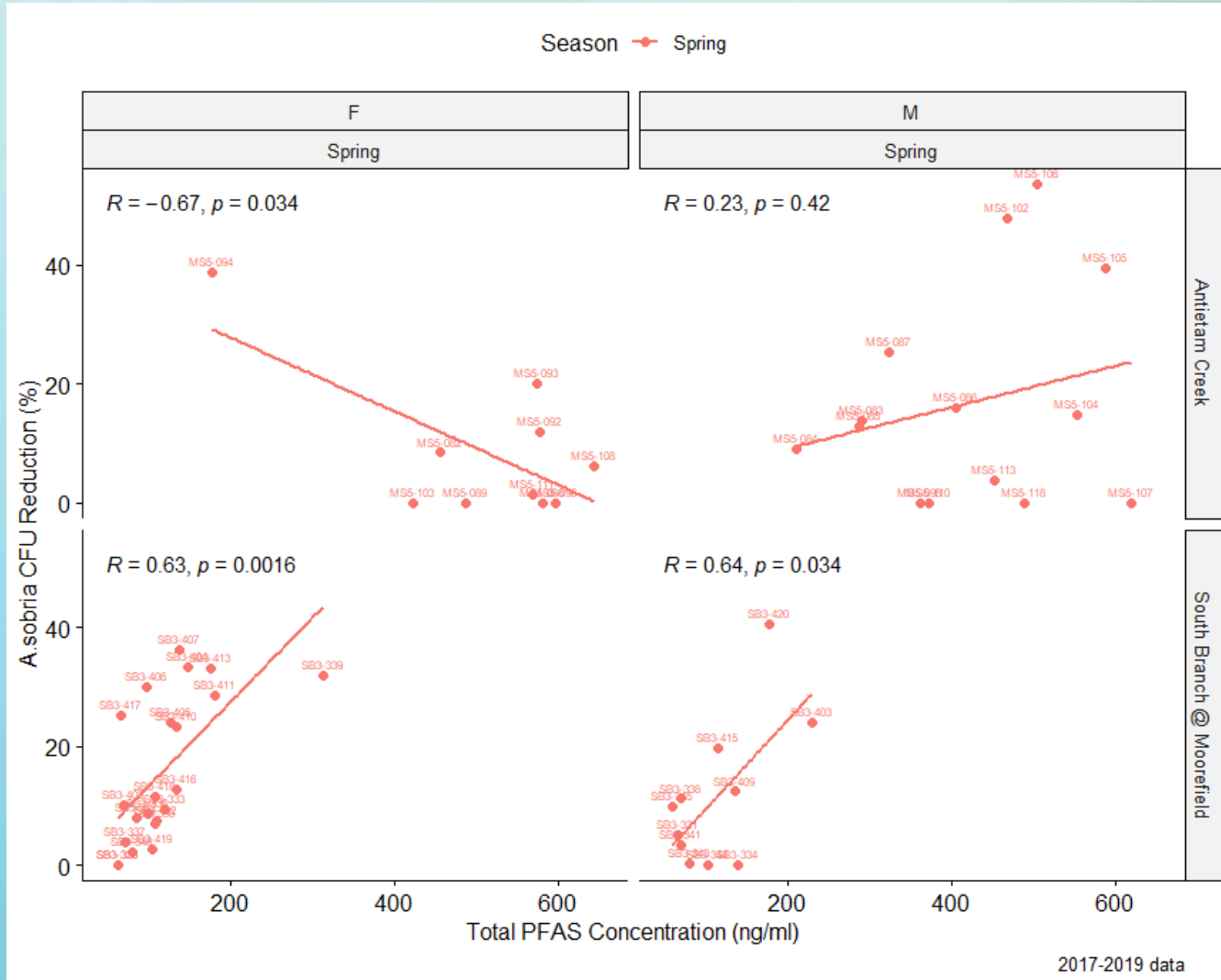


Unstimulated lymphocyte proliferation (2017-2018)

Adaptive response

Total PFAS

An Innate Response Bactericidal Activity



Summary

- Much more data analysis to understand associations and the role of PFAS in any adverse effects
- Wild fishes are exposed to complex mixtures of numerous contaminants, as well as other environmental stressors, parasites and pathogens
 - Data from these same fish documented mercury and other legacy contaminants as well as exposure to peaks of surface water contaminants, particularly in the spring
 - We hope by utilizing numerous fish health indicators from organismal to molecular we can begin to tease out the most important risk factors for adverse health effects
- Further research is needed to identify sources and effects

Acknowledgements

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