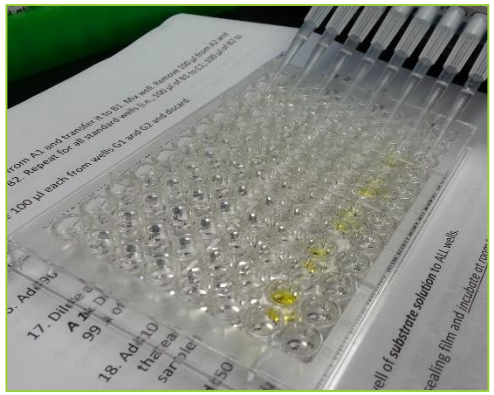
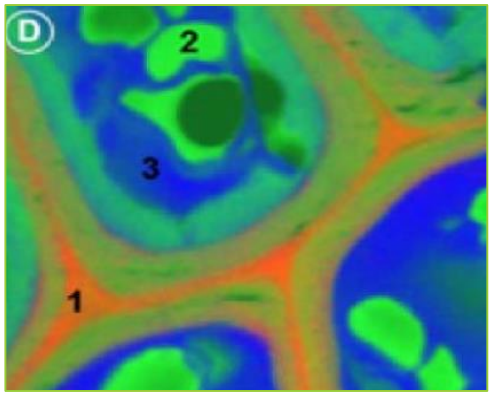
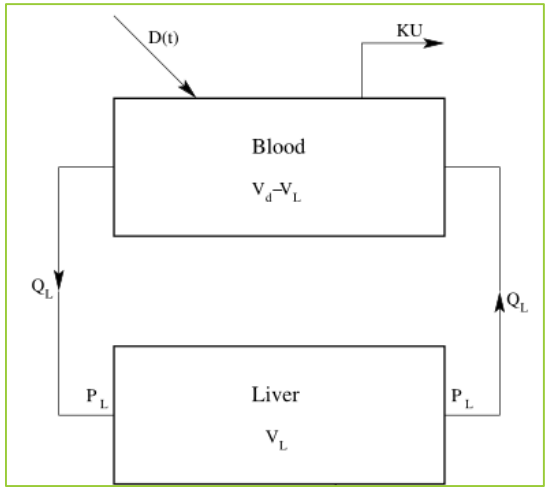


Team #5: Other Applied R&D

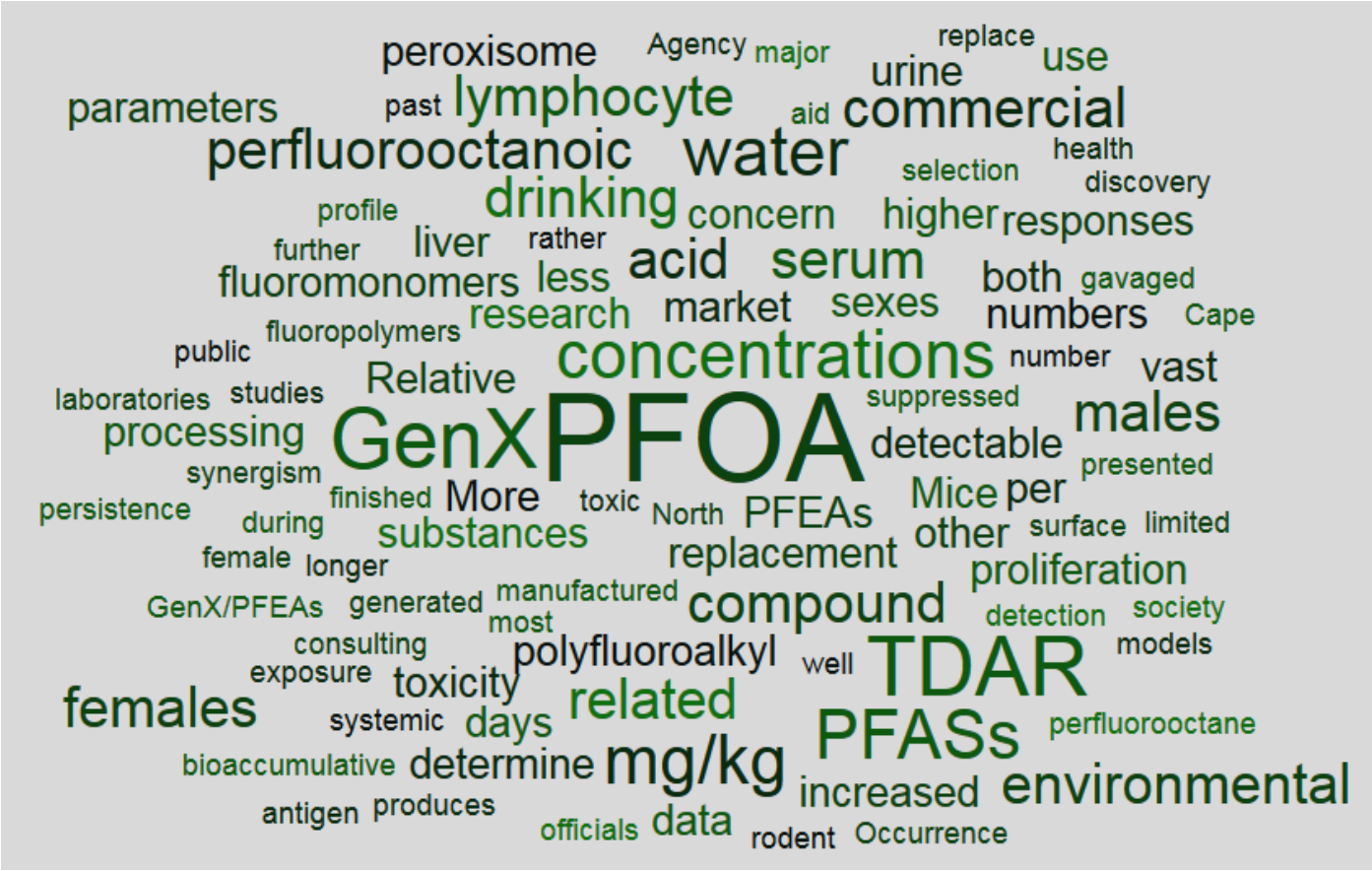
PRESENTED BY: JAMIE DEWITT

TEAM CO-LEADS: JAMIE DEWITT (ECU) AND REBECCA FRY (UNC-CH)

NORTH CAROLINA PFAS Testing Network

NC PFAS Testing (PFAST) Network, a research program funded by the NC Policy Collaboratory

Why do we need this type of PFAS research?



Context

Team 5 is providing diverse contextual information associated with identified PFAS in NC.

Team 5a: The presence of PFAS in landfill leachate

Lead: Dr. Morton Barlaz, NCSU

Collaborator: Dr. Jean-Rene Thelusmond, NCSU

Overall objective:

- Assess the relative importance of MSW landfills and domestic wastewater as sources of PFAS to POTWs and potentially to surface water

Team 5a: The presence of PFAS in landfill leachate

Sub-objectives/research questions:

- Estimate the mass of PFAS that is discharged to Publicly Owned Treatment Works (POTWs) from municipal solid waste landfill leachate
- Estimate the mass of PFAS entering POTWs in NC via municipal wastewater
- Estimate the mass of PFAS leaving POTWs in NC after treatment
- Estimate the release of PFAS from landfills that receive construction and demolition waste

Team 5a: The presence of PFAS in landfill leachate

Research approach:

- *Landfills*
  Sample leachate at point where it is discharged to a wastewater treatment plant; may be a tank, gravity sewer line, or a pumping station
- *Wastewater treatment plants*
  Sample from flow proportioned composite samplers after verification that sampling system does not contain Teflon
- *Construction and demolition (C&D) waste landfills*
  Creative sampling as there are only a few lined C&D

Team 5a: The presence of PFAS in landfill leachate

Research approach:

- *Focus is on mass release*

mass = concentration * volume

- *Concentrations are measured*
- *Volumes are known for landfills and WWTPs but must be estimated using models for C&D landfills that are unlined*

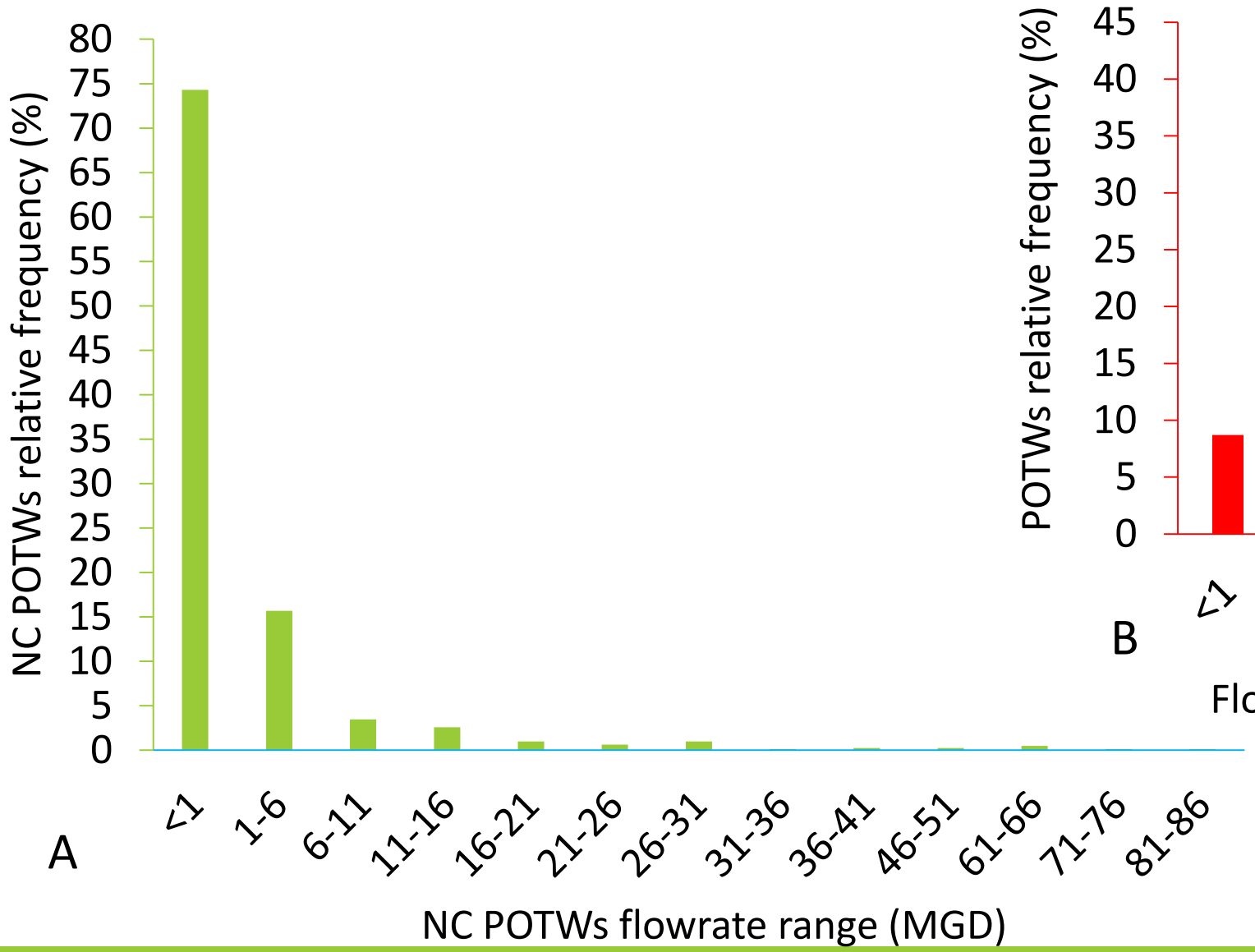
Team 5a: The presence of PFAS in landfill leachate

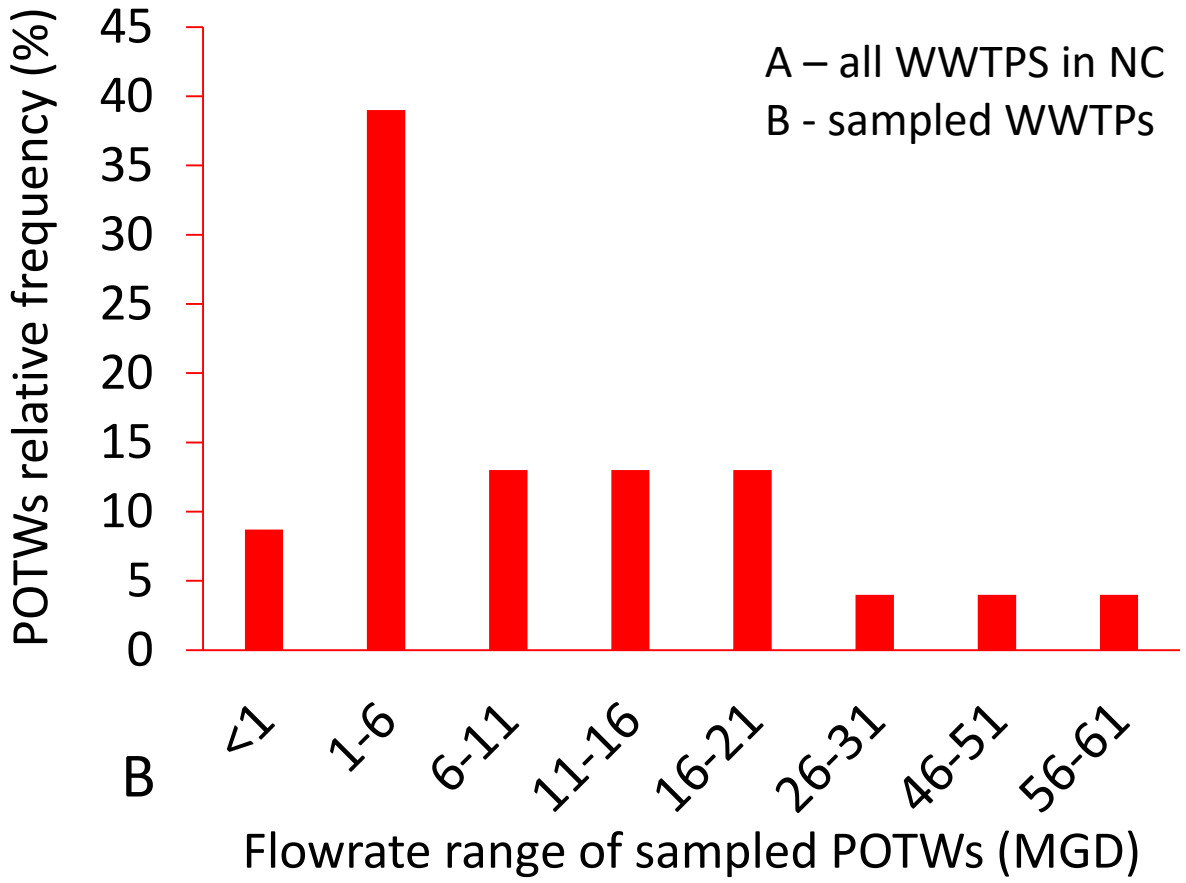
Current status:

- *Team 5a has sampled **23** WWTPs, **11** landfills, and **4** C&D landfills*

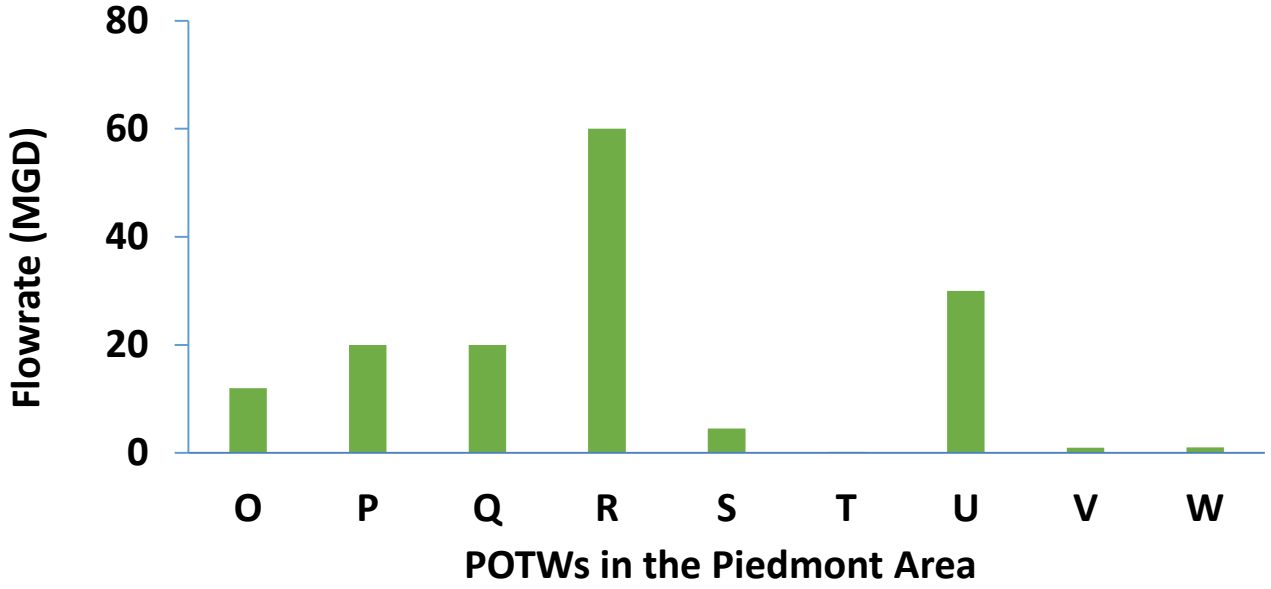
- *Team 5a has sampled **2** WWTPs weekly for four weeks*

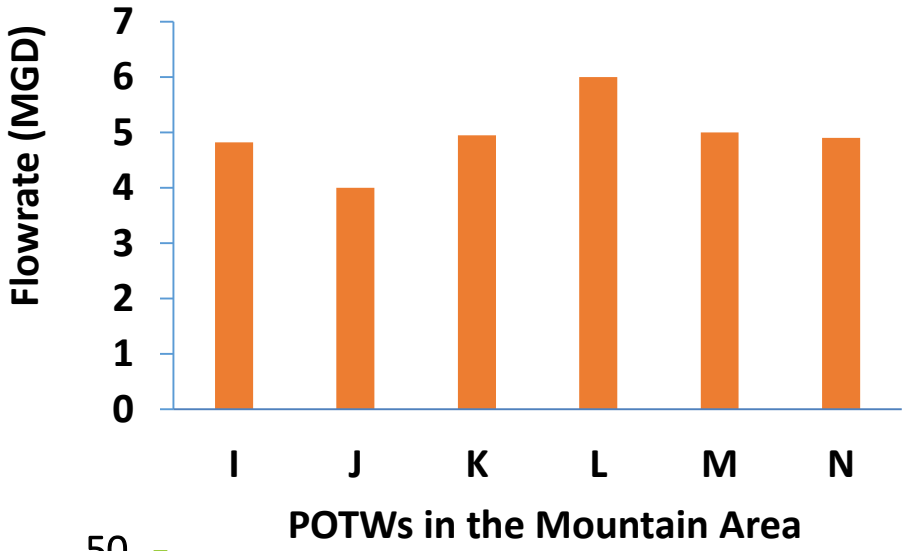
Profile of wastewater treatment plant size in NC

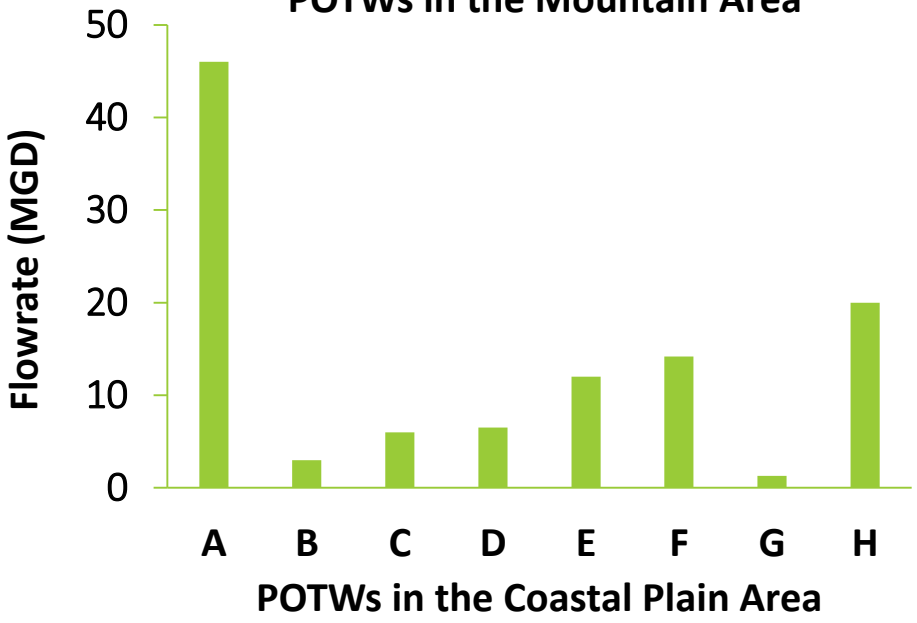




Flowrate of the sampled POTWs in NC by region







Team 5b: The presence and accumulation of PFAS in NC wildlife species

Lead: Dr. Scott Belcher, NCSU

Collaborator: Dr. Theresa Guillette, NCSU

Overall objective:

- Are PFAS present and accumulating in NC wildlife?

Team 5b: The presence and accumulation of PFAS in NC wildlife species

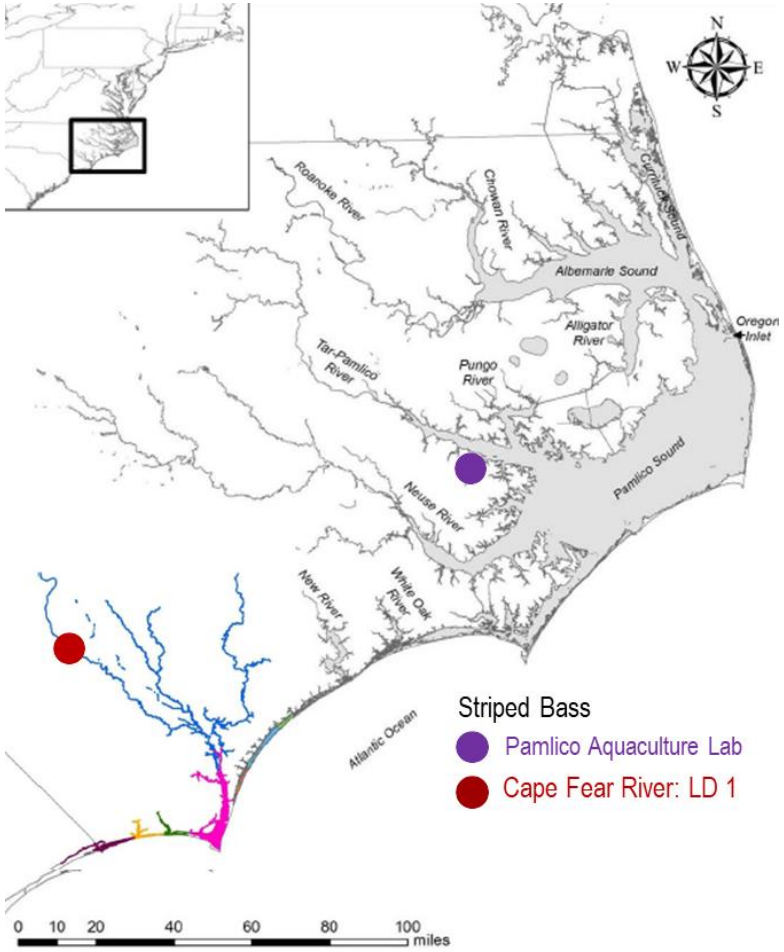
Sub-objectives/research questions:

- Which PFAS are present in NC wildlife and how long do they remain?
- Do replacement PFAS bioaccumulate?
- Are levels of PFAS in NC wildlife associated with indicators of adverse wildlife/ecosystem health? What can these data tell us about human effects?
- Can consuming fish caught from the Cape Fear River increase PFAS in humans?

Team 5b: The presence and accumulation of PFAS in NC wildlife species

Research approach:

- *Striped bass and catfish*
  Collecting from Cape Fear River as well as from populations without known PFAS contamination (“healthy” controls)



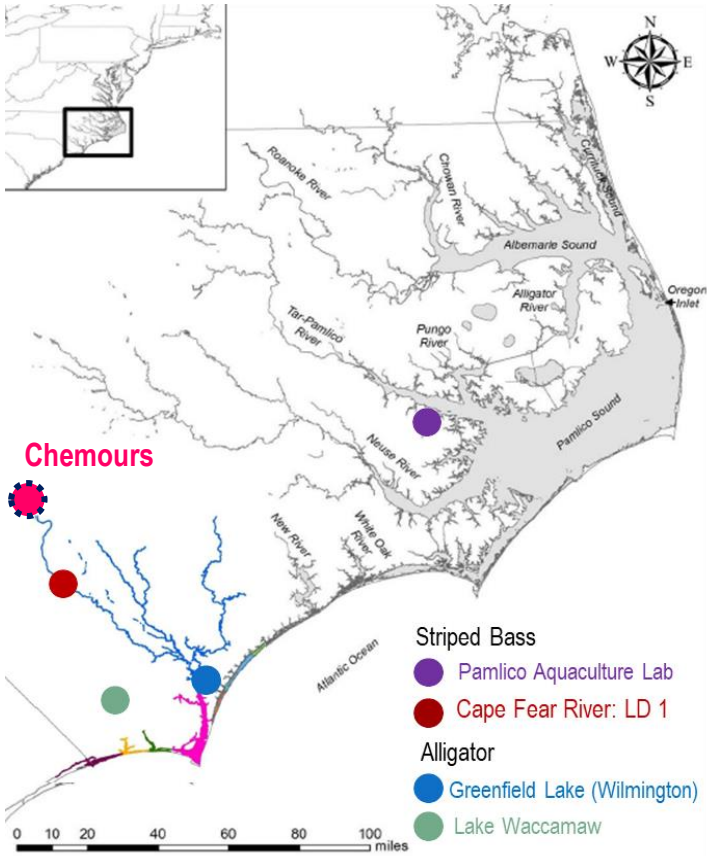
Team 5b: The presence and accumulation of PFAS in NC wildlife species

- *Alligators*

  Active capture of adult (6'+) alligators

  Collection of blood/serum and body measures

  Sex determination

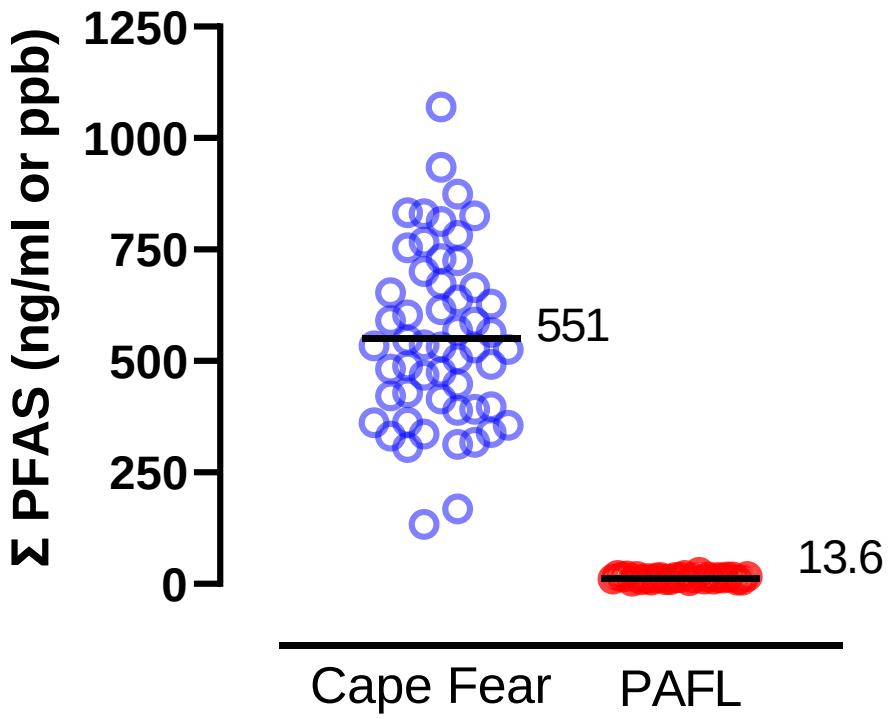


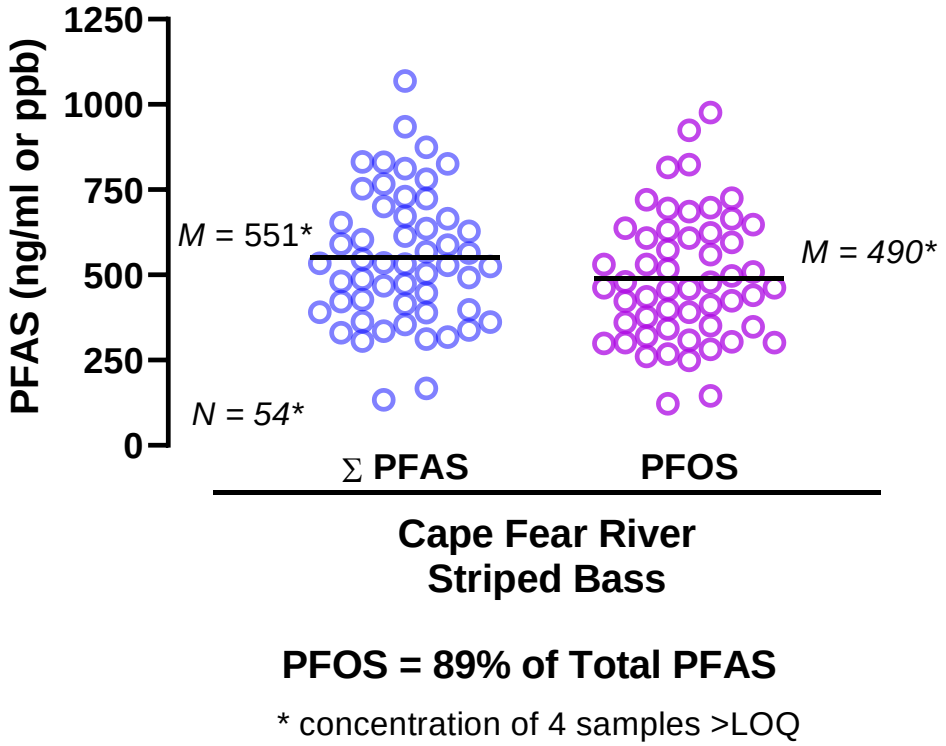
Team 5b: The presence and accumulation of PFAS in NC wildlife species

Current status:

- *Team 5b has collected **63** fish from Cape Fear River Lock and Dam 1*
- *Team 5b has collected **80+** fish from reference sites*

- *Team 5b has sampled **16** alligators from Lake Waccamaw (a reference population)*
- *Team 5b has sampled **~16** alligators from the Cape Fear River and Wilmington areas*

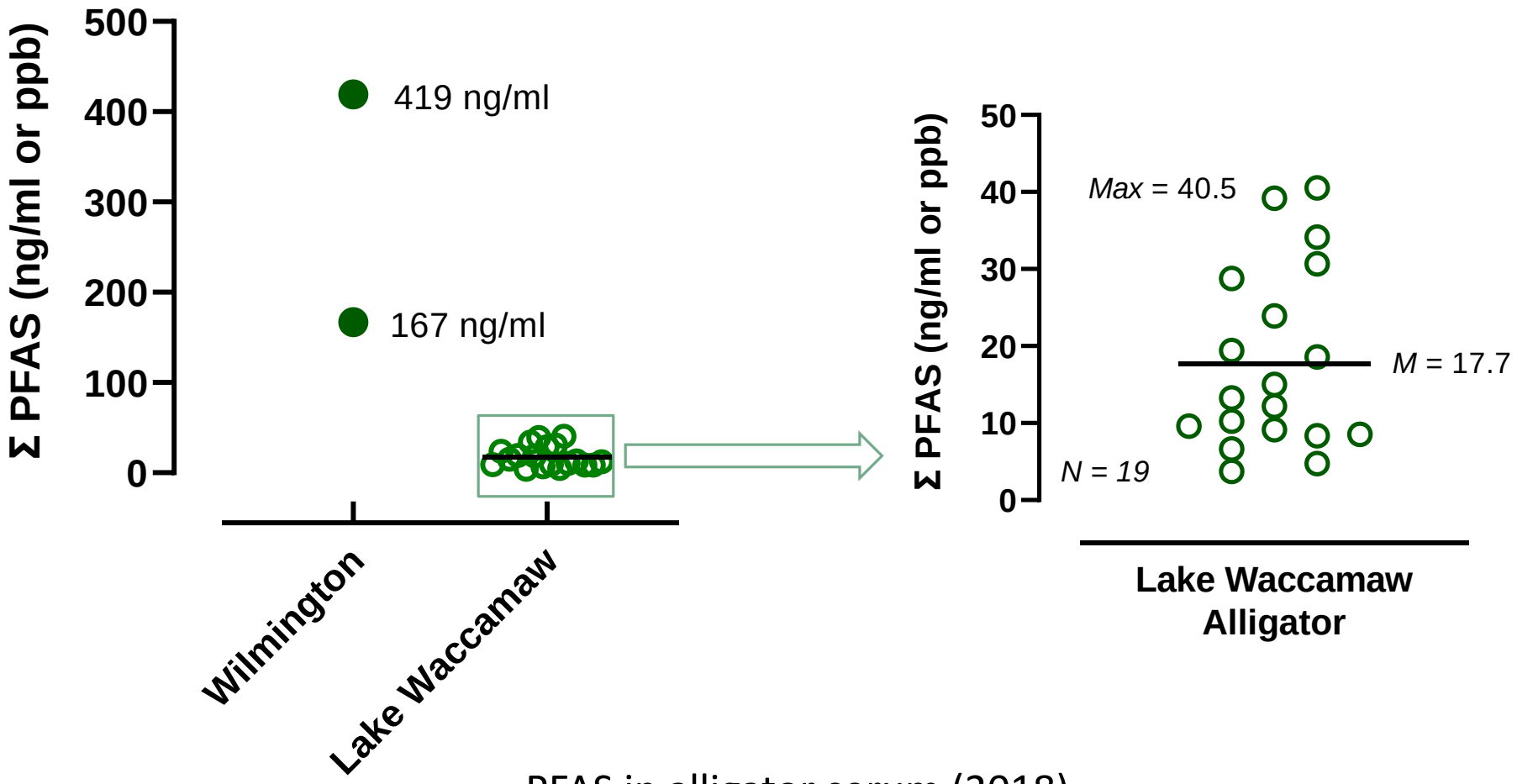




PFOS = 89% of Total PFAS

* concentration of 4 samples >LOQ

- High levels of PFAS were detected in every serum sample
- Reference hatchery fish were contaminated (*M* = 13.6 ng/ml*)*
- Total PFAS is >40 higher in striped bass from the Cape Fear River (*M* = 551 ng/ml)
- PFOS accounted for 89% of PFAS
- PFOS was “phased out” beginning in 2002



PFAS in alligator serum (2018)

Alligator serum LC-MS/MS – total PFAS was >10X higher in alligators from Wilmington

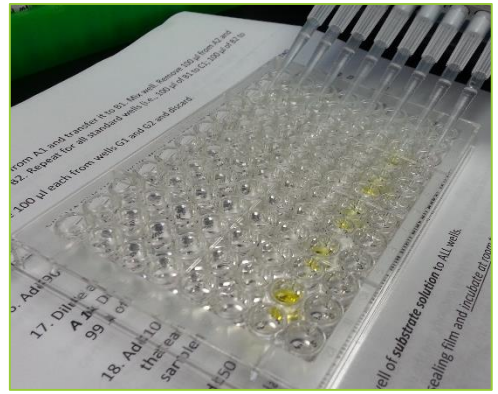
Team 5c: Immunotoxicological effects of PFAS in a rodent model

Lead: Dr. Jamie DeWitt, ECU

Collaborator: Dr. Tracey Woodlief and Samuel Vance, ECU

Overall objective:

- Do emerging PFASs impact the immune system to the same degree as legacy PFASs?



Team 5c: Immunotoxicological effects of PFAS in a rodent model

Sub-objectives/research questions:

- Determine effects of PFAS on major immune cell subpopulations in primary (thymus) and secondary (spleen) lymphoid organs
- Assess functional responsiveness of the adaptive immune system following exposure to PFAS
- Assess functional responsiveness of the innate immune system following exposure to PFAS

Team 5c: Immunotoxicological effects of PFAS in a rodent model

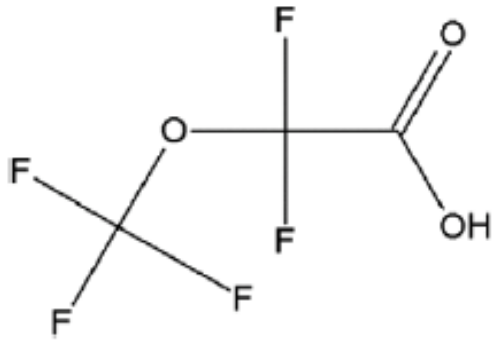
Research approach:

Team 5c: Immunotoxicological effects of PFAS in a rodent model

Current status:

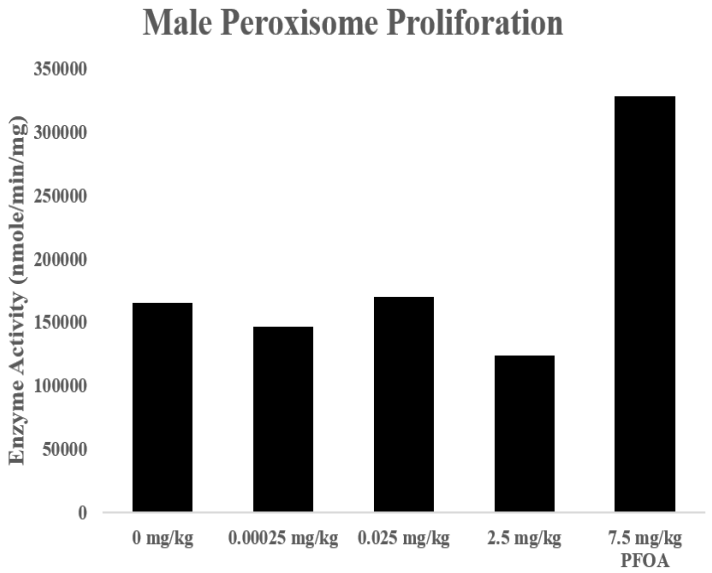
- *Team 5c has conducted studies with **PFMOAA** (3-carbons), **PFMOPrA** (4-carbons), and **PFMOBA** (5-carbons)*

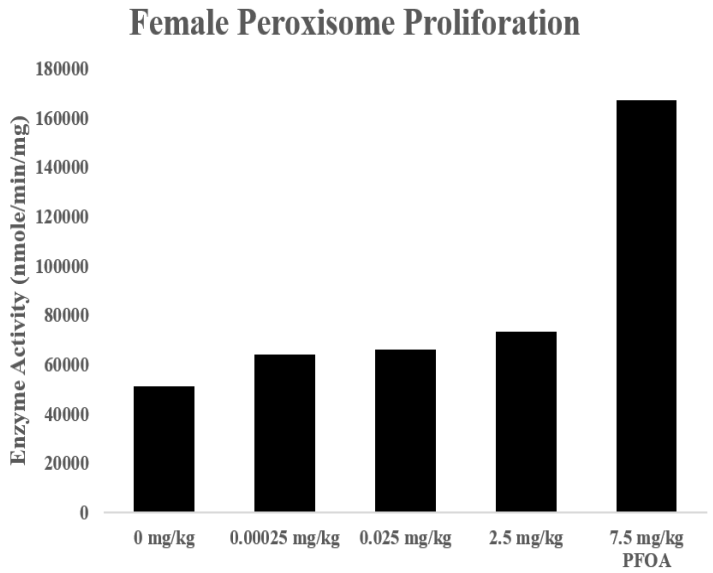
- *Studies with Nafion BP2, PFHxA, and a mixture are planned*

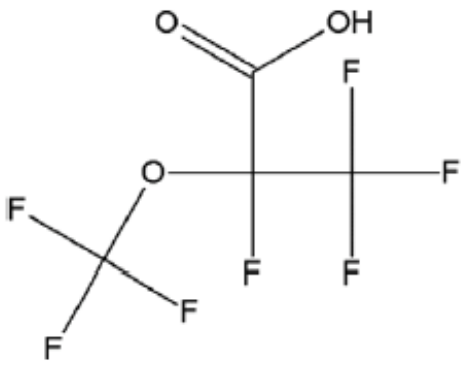


PFMOAA - $C_3HF_5O_3$
perfluoro-2-methoxyacetic acid

Dominant short-chain PFAS detected in Cape Fear River of North Carolina in 2018 at
35,000 ng/L (Hopkins et al., 2018).
Part of Consent Order with NC DEQ/Sound Rivers.



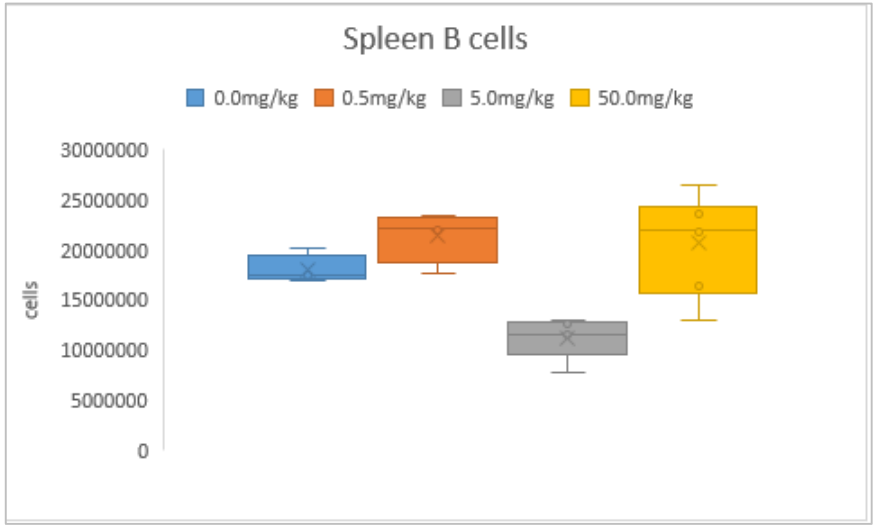




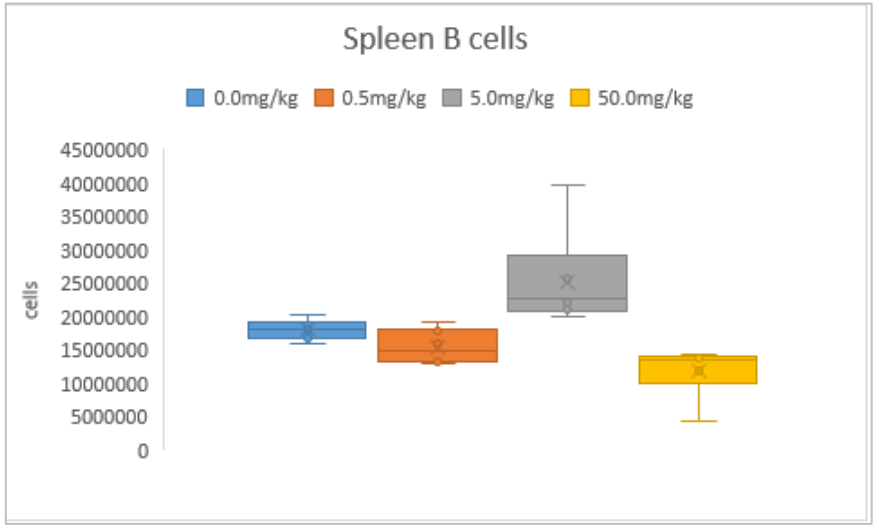
PFMOPrA - $C_4HF_7O_3$

Perfluoro-2-methoxypropanoic acid

Short-chain PFAS detected in Cape Fear River of North Carolina in 2018 (Hopkins et al., 2018).



Males



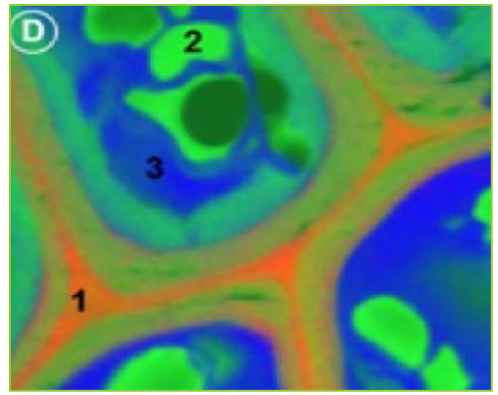
Females

Team 5d: PFAS uptake and accumulation by plants

Lead: Dr. Owen Duckworth, NCSU

Overall objective:

- Assess uptake and accumulation of PFAS by relevant crops and how soil properties and management practices may impact PFAS uptake and distribution within plants



Team 5d: PFAS uptake and accumulation by plants

Sub-objectives/research questions:

- Quantitative linkage between organic matter content of soil and uptake of PFAS into crops prevalent in eastern NC
- Improved recommendations about soil management practices that can reduce uptake of PFAS from soil by plants
- Molecular scale information about where PFAS go in plant tissues

Team 5d: PFAS uptake and accumulation by plants

Research approach:

- *Soil amendments*
  Compost addition to soil to determine if this reduces plant uptake of PFAS from soil

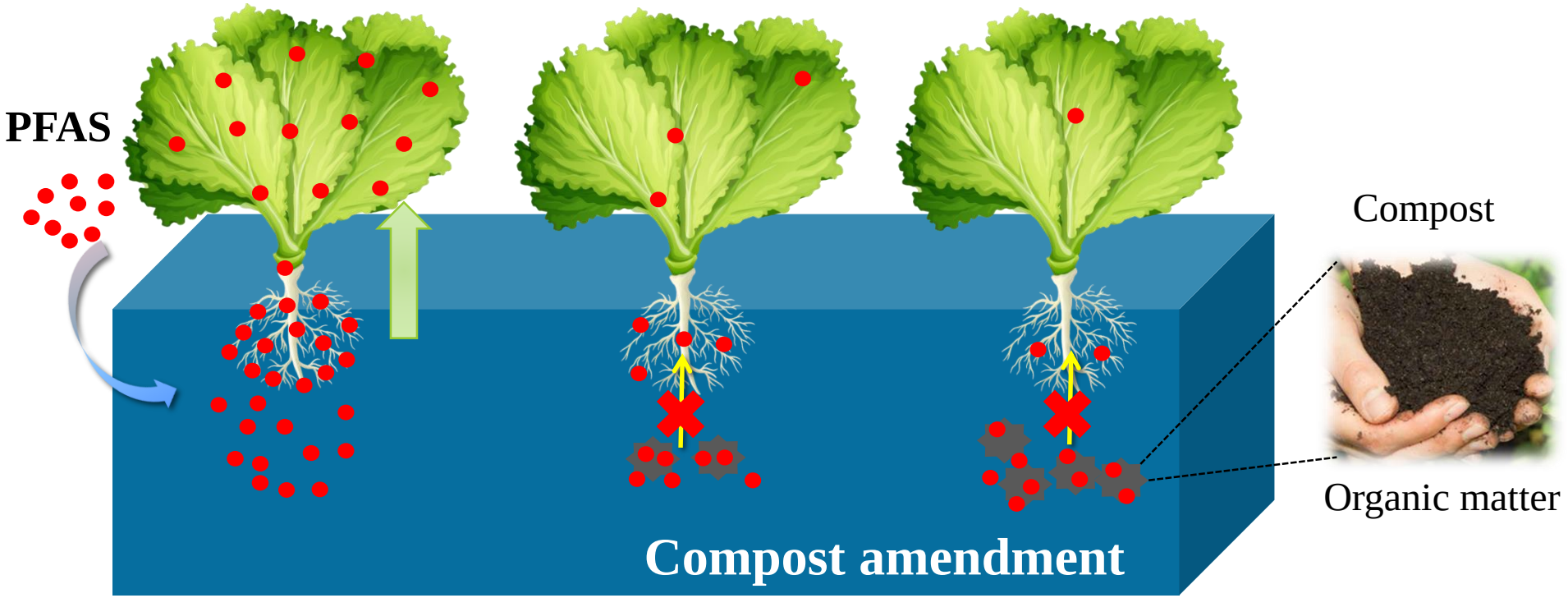
- *Evaluation of PFAS in plants*
  Measurement of PFAS in whole plant and in specific parts of plants to see where PFAS go

Team 5d: PFAS uptake and accumulation by plants

Current status:

- *Team 5d has conducted experiments with compost-amended soil spiked with PFAS and is waiting for plants to reach harvestable size*

Effects of compost addition on reducing the plant uptake of PFAS from soil



Hypothesis: Increasing the compost content could increase the sorption of PFAS chemicals thus reduce plant uptake.

Lettuce uptake experiment (to be harvested)

Two spiking concentration: 10 and 100 μg/kg

0% compost **5% compost** **10% compost** **20% compost**

Expecting results:

- ✓ Application of compost could increase the lettuce biomass.
- ✓ Application of compost could reduce the lettuce accumulation of PFAS chemicals.

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?

Lead: Dr. Rebecca Fry, UNC Chapel Hill

Overall objective:

- Determine effects of PFAS on placental health and function

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?

Sub-objectives/research questions:

- What are levels of PFAS in:
  Drinking water
  Placenta
  Cord blood
  Maternal serum in pregnant women in NC

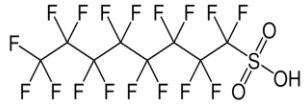
- What is effect of PFAS on placental health and function?

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?

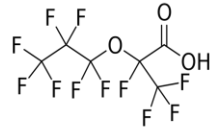
Research approach:

- *Well water sampling*
  Collection of drinking water from pregnant women who obtain water from wells
- *Cord blood and maternal serum sampling*
  Collection of blood for analysis of PFAS
- *Placenta sampling and work with placental cell lines*
  Collection of placenta for evaluation cell-level changes and responses to PFAS in collected placenta cells and placenta cell lines

Perfluorooctanoic acid (PFOA)



Perfluorooctanoic sulfate (PFOS)



2,3,3,3-tetrafluoro-2-(hepta fluoropropoxy) propanoate (GenX)

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?

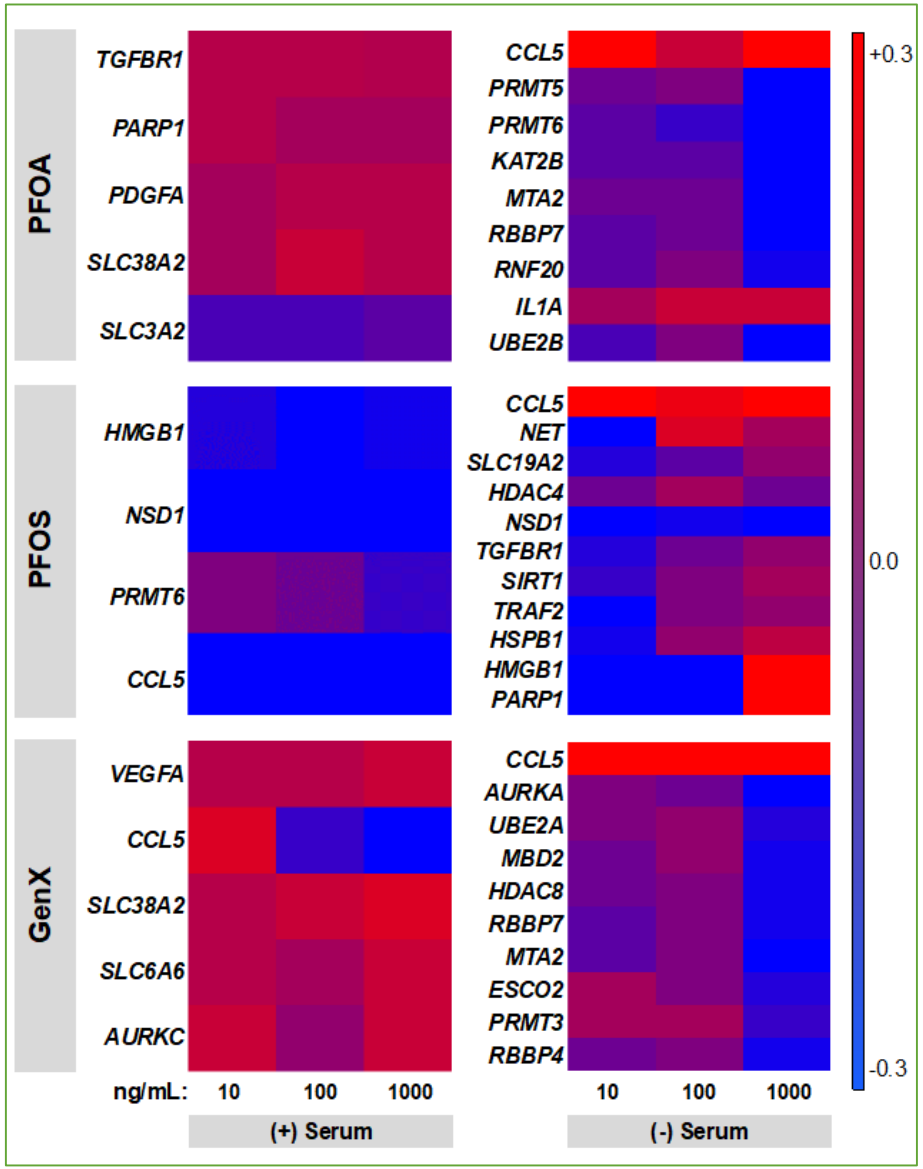
Current status:

- *Team 5e has recruited human subjects and collected urine and serum*
- *Team 5e has collected and continues to collect drinking water, cord blood, and placenta samples*
- *Team 5e has started measuring PFAS in water, serum, placenta, and cord blood*
- *Team 5e has run and continues to run cell-level experiments*

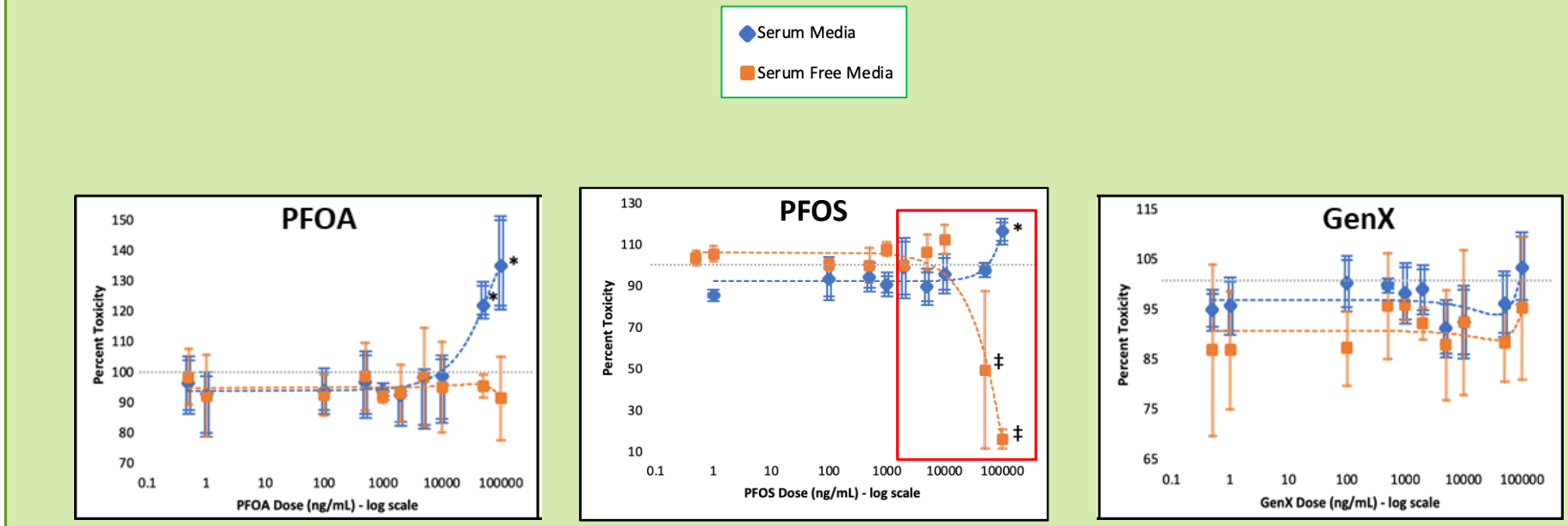
- Monitored for 14 PFAS in 12 well water samples
- Found 5 PFAS detected in several well water samples

	PFPeA	PFHxA	PFHpA	PFHxS	PFOA
Limit of detection (LOD) (ng/ml)	0.02	0.02	0.02	0.1	0.02
% of samples with detectable levels	**16.7**	**25.0**	**33.3**	**8.3**	**25.0**
Median	0.015	0.015	0.012	0.055	0.015
Range	< LOD -0.015	< LOD -0.016	< LOD -0.013	< LOD -0.055	< LOD -0.027

- Changes in gene expression associated with inflammation (CCL5) when placental cells were treated with PFAS
- Additional changes in gene expression in placenta cell line treated with PFAS



Changes in PFAS toxicity on a placental cell-line in two types of cell culture media

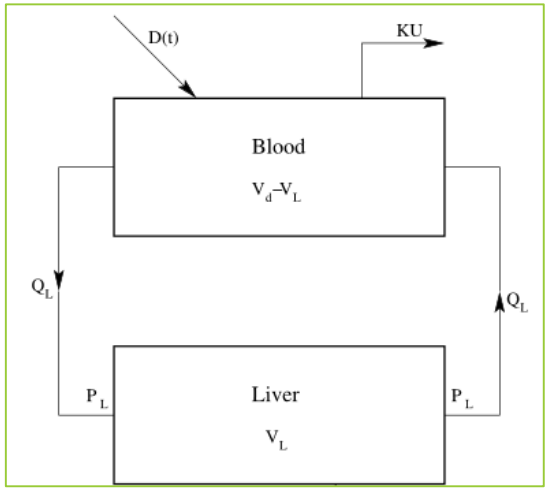


Team 5f: Predictive models for PFAS movement in the environment and in living organisms

Lead: Dr. Nick Luke, NC A&T

Overall objective:

- Can we develop models to predict where PFAS go in living organisms and in the environment?



Team 5f: Predictive models for PFAS movement in the environment and in living organisms

Sub-objectives/research questions:

- Quantitate analysis of immunotoxicity and systemic toxicity data generated PFAST Network collaborators, including half-life estimates to generate a physiologically based pharmacokinetic (PBPK) model

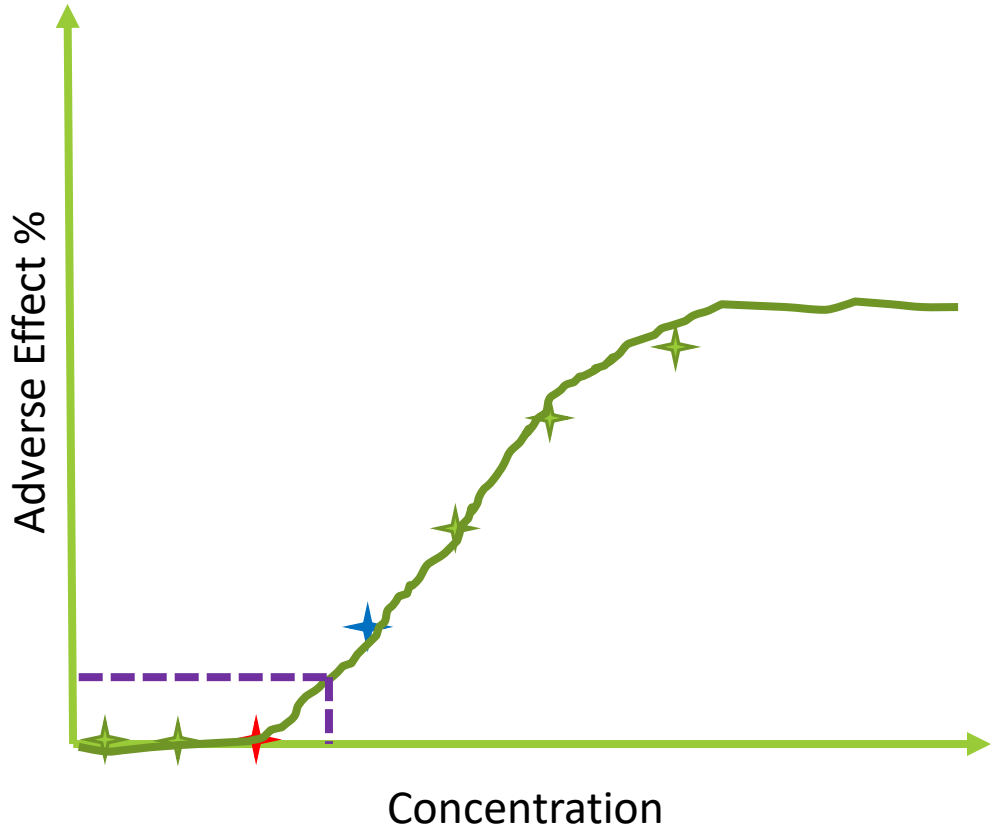
- Quantitate analysis of environmental concentrations of PFAS generated by PFAST Network collaborators to develop models to predict fate and transport of PFAS in the environment

Team 5f: Predictive models for PFAS movement in the environment and in living organisms

Sub-objectives/research questions:

- *Benchmark dose modeling*

  Experiments conducted to find at what level of exposure adverse effects begin to occur
  - NOAEL (No Adverse Effects Level) the highest concentration at which no adverse effects are observed
  - LOAEL (Lowest Adverse Effects Level) the lowest concentration at which no adverse effects are observed
  - BMD (Benchmark Dose) a quantitative model is formed to fit the adverse effects data, and a reference dose is identified using the model. The reference dose may be between the NOAEL and LOAEL.

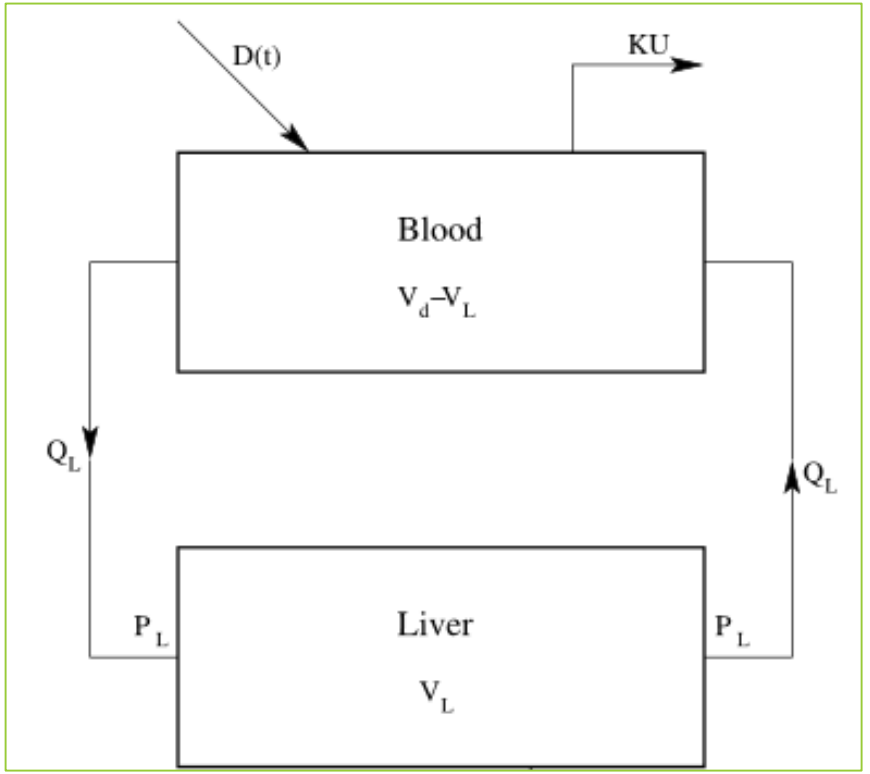


Team 5f: Predictive models for PFAS movement in the environment and in living organisms

Research approach:

- *Physiologically based pharmacokinetic modeling (PBPK)*

  The body is separated into a series of compartments
  - A mathematical model is created to represent how a chemical is distributed throughout the body, excreted from the body, and metabolized in the body.
  - The model is calibrated using experimental data.
  - The calibrated model can be used to examine how long a chemical will remain within the body, how much of the chemical builds up within the body.



Schematic for a 2 compartment PBPK model






Team #5: Other Applied R&D

THANK YOU! WE WELCOME YOUR QUESTIONS

NORTH CAROLINA PFAS Testing Network

NC PFAS Testing (PFAST) Network, a research program funded by the NC Policy Collaboratory

Photo references

From left to right on title slide:

- http://efc.web.unc.edu/2013/11/05/solid-waste-finance/.
- http://www.starnewsonline.com/sports/20180816/scientists-look-for-genx-in-lake-waccamaw-alligators.
- https://www.slideshare.net/syednayyeralvi/immunotoxicity.
- Karunakaran C. et al., 2015, Introduction of soft X-Ray spectromicroscopy as an advanced technique for plant biopolymers research, PLoS ONE 10, e0122959.
- https://www.shutterstock.com/search/placenta.
- Luke N. et al., 2010, Development of a quantitative model of pregnane X receptor (PXR) mediated xenobiotic metabolizing enzyme induction, Bulletin of Mathematical Biology, 72:1799-1819.