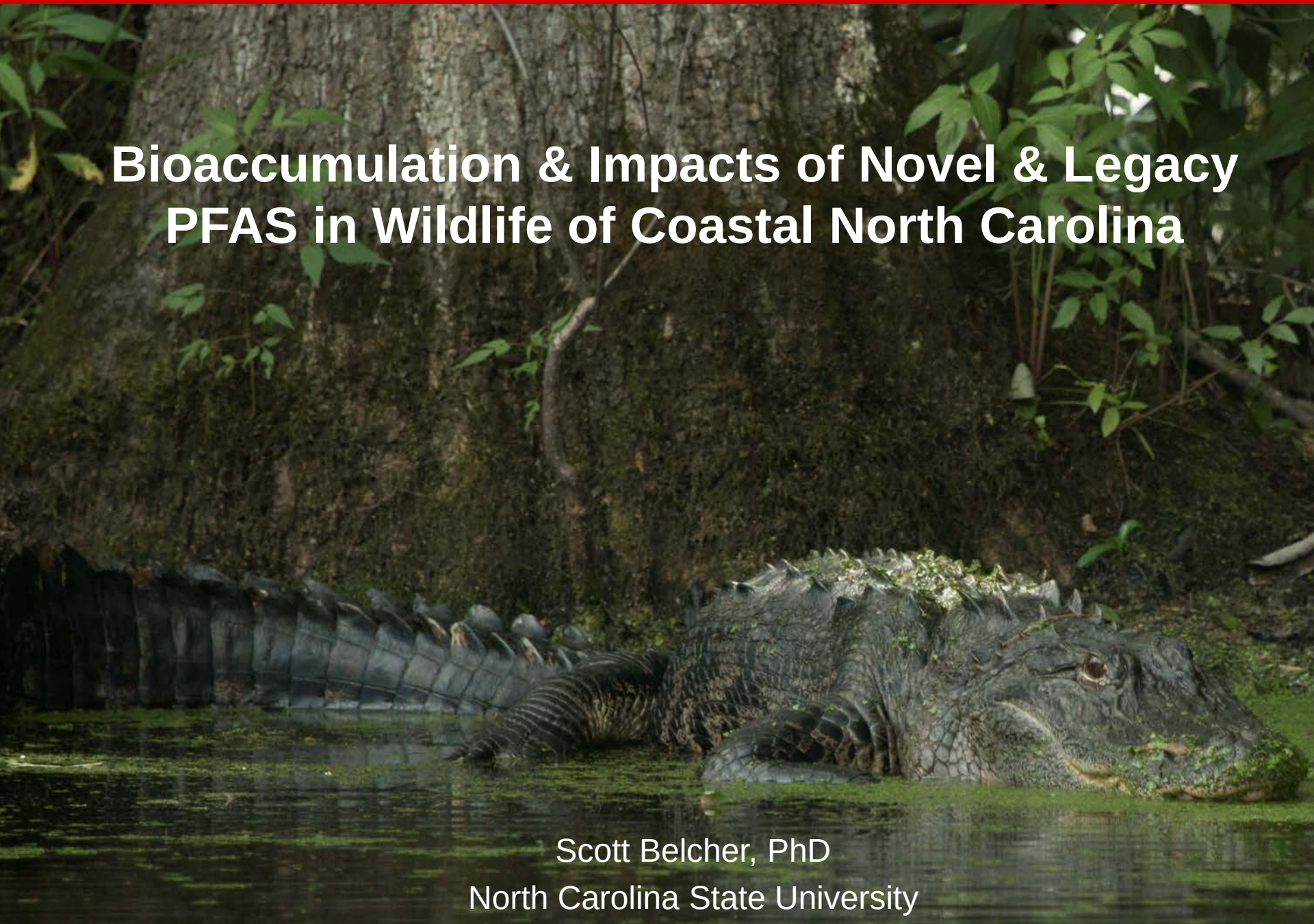

# Bioaccumulation & Impacts of Novel & Legacy PFAS in Wildlife of Coastal North Carolina

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NC STATE UNIVERSITY
Center for Human Health and the Environment

# Acknowledgements:


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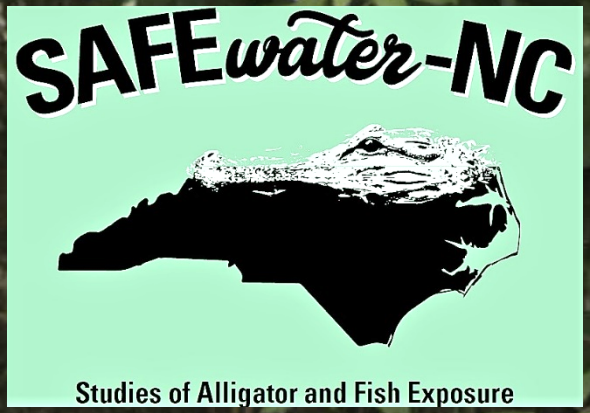



## The BIG Questions:

- Are PFASs present and accumulating in NC wildlife?
  - Which ones and for how long?
  - Do “replacement” PFAS bioaccumulate?
- Are the levels found associated with indicators of adverse wildlife/ecosystems health?
- What can this tell us about effects on humans?

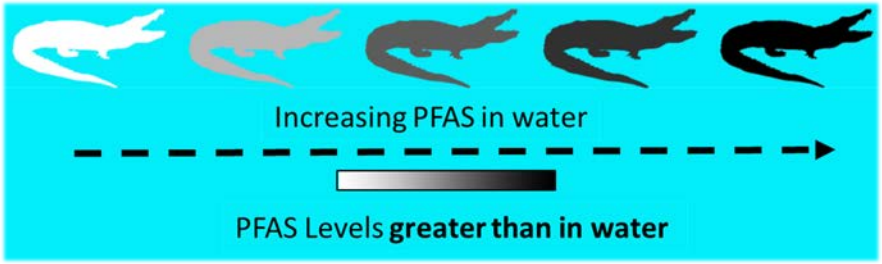
# The Concept of One Health:

*Studies in sentinel species can act as an early warning of environmental problems that are impacting humans*

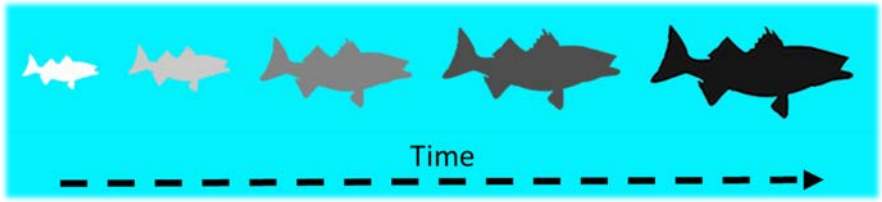
# Sentinel Species

## Bioconcentration, Bioaccumulation, Biomagnification

Bioconcentration/Bioaccumulation

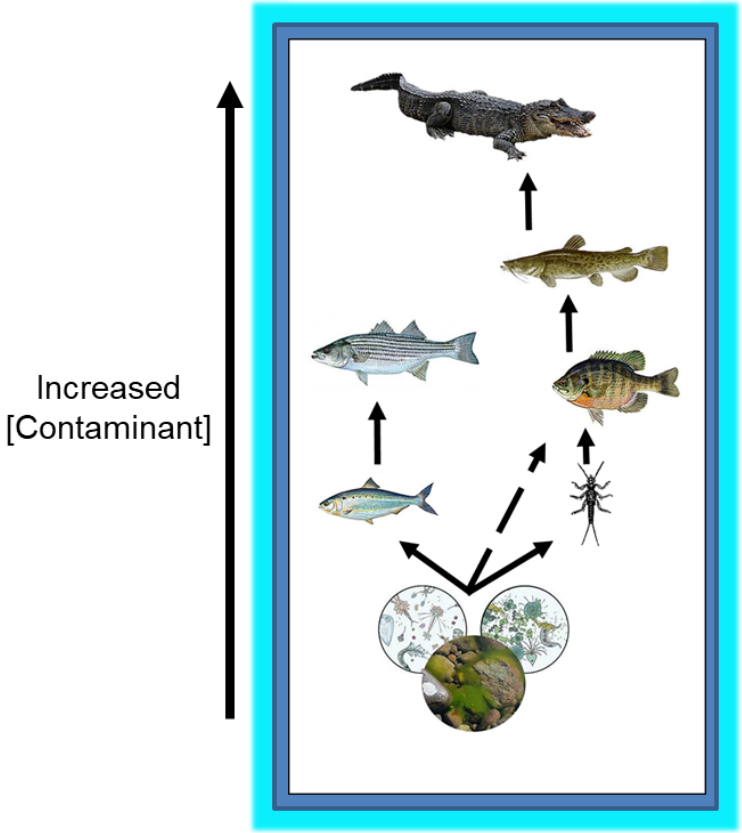



Persistent Organic Pollutants (POPS) or Toxic Metals



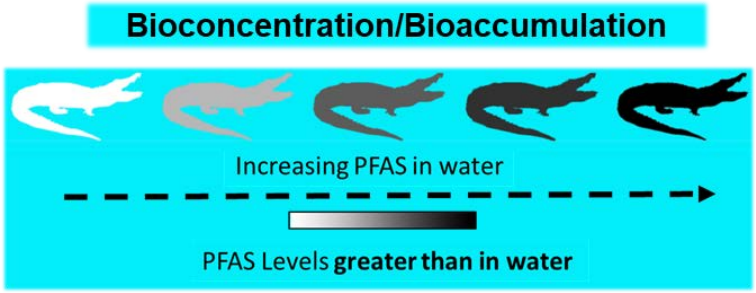

**[Contaminant]**

Biomagnification




Contaminant is eliminated much slower than it is absorbed

# PFAS are bioaccumulating in aquatic vertebrates living in contaminated waters

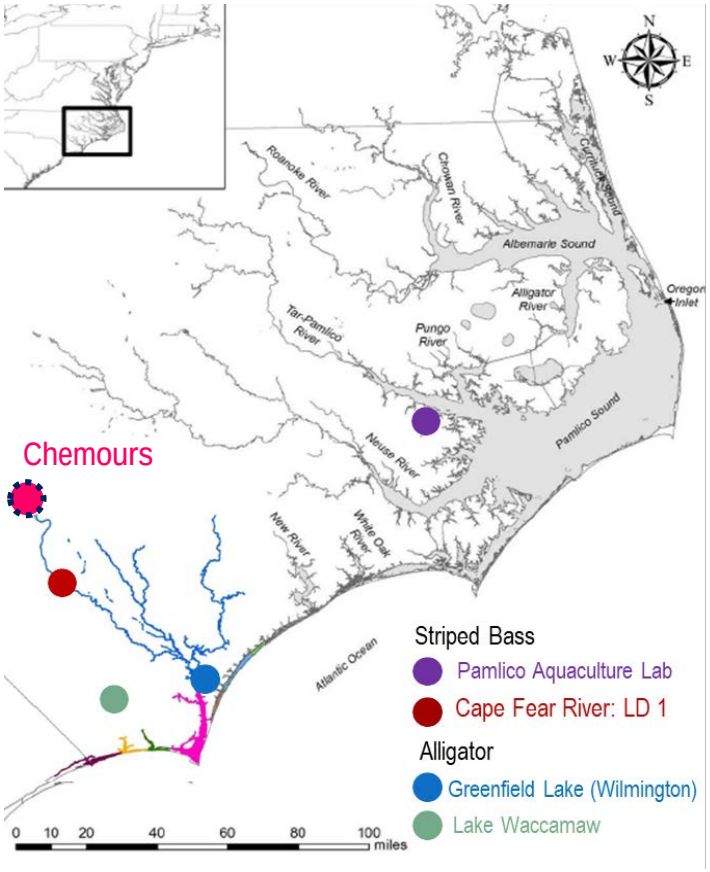



- PFAS in Cape Fear River Water = ppt
- Blood of Fish and Alligator = ppb-ppm

# Alligator: Study Approach

SAFEwater-NC

Studies of Alligator and Fish Exposure

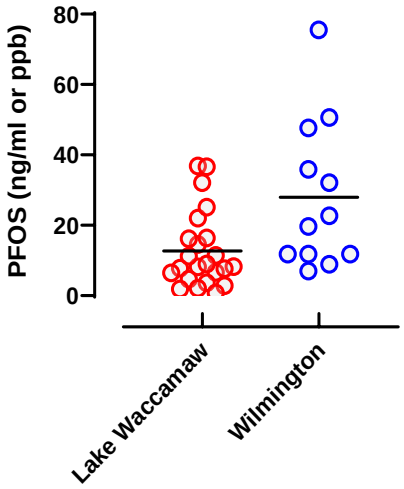



Approach:

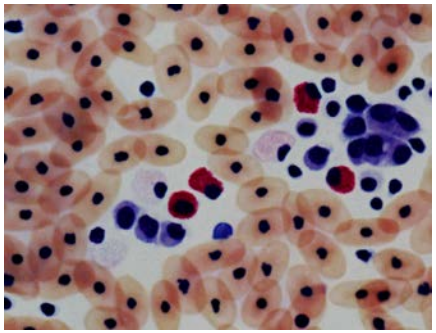
- Active capture adult alligators (6 foot +) and Juvenile
- Sample: Blood/Serum
  - Morphometric measures
  - Determine sex
- Quantitate serum PFAS levels (LC/MS/MS)
- Blood chemistry/hormones/liver enzymes
- Immune function: lysozyme and immune cell counts

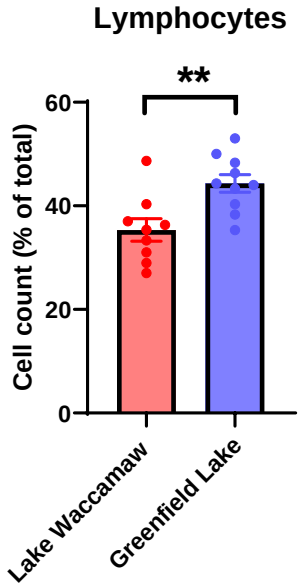
1) Identify a reference population
   - Lake Waccamaw (Lumber River watershed)
2) Compare Cape Fear & Wilmington Area
   - Greenfield Lake

# Preliminary Findings:



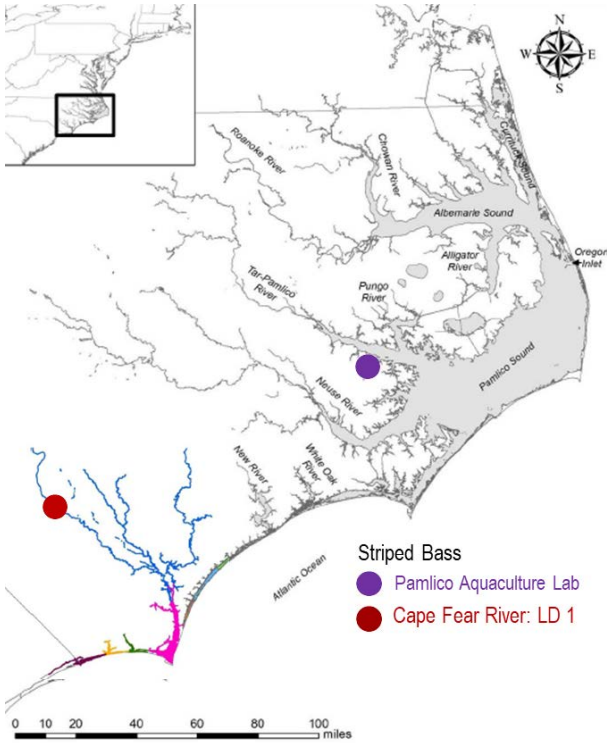

- Increased serum PFAS are found in Cape Fear River alligators (and fish)
- Higher contamination levels - increases in unhealed “lesions” and poor body condition
- Site-specific differences in immune function, liver enzymes, and blood chemistry






Analysis is ongoing……

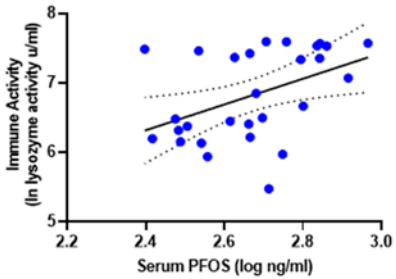
# Striped Bass: *Morone saxatilis*




Commercial and recreational fishery in North Carolina valued at more than **$94 million annually**

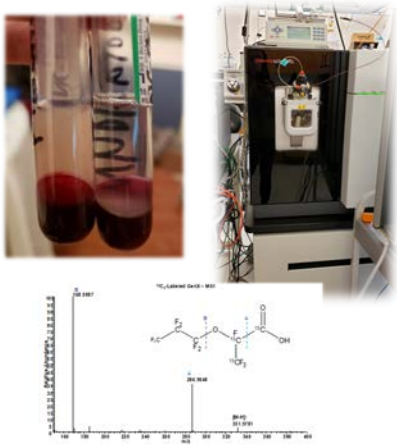
Striped Bass: can live in **both salt and fresh water**

- Cape Fear River Population – <u>Do not migrate</u>
  - riverine/estuarine
    - good model of Cape Fear contaminants
- <u>No natural reproduction in the Cape Fear River</u>
- Tar/Pamlico, Neuse and Cape Fear Rivers:
  - Essentially 100% are hatchery progeny
    - Analyzed fish were between ~2-7 year old
    - Residents of the Cape Fear River from 1-6 years


- Approach:
  - Targeted and untargeted high-resolution mass spectrometry of blood
    - Measure known (≥ 23)
    - Detect unknown PFAS
  - Blood chemistry and health-related biomarkers
    - Liver
    - Kidney
    - Immune system
    - Hormones
  - Regression analysis to detect associations between total and individual PFAS concentrations and health endpoints

# Striped Bass Serum Samples – Preliminary Findings



- **PFAS was detected in every sample**

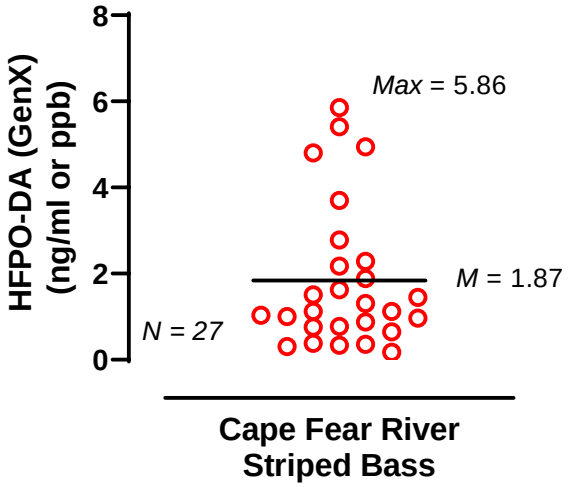
- PFOS, PFNA, PFDA was detected in every Striped Bass
- Nafion bp2 was only detected in Cape Fear samples
- GenX and PFHxS are enriched in Cape Fear samples
- PFBS was detected in PAFL and not Cape Fear Striped Bass

Percentage of samples with PFAS detected >LOD

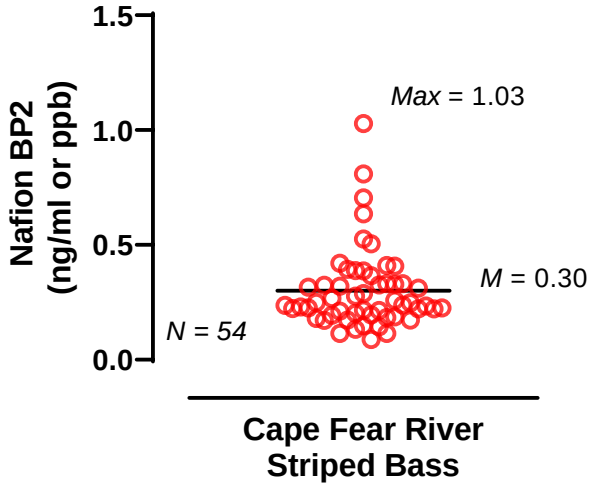
| Striped Bass | PFBA | PFPeA | PFHxA | PFHpA | PFOA | PFNA | PFDA | PMPA | PEPA | GenX | PF02HxA | PFO3OA | PFO4DA | PFO5DoDA | PFBS | PFHxS | PFOS | Naf_bp1 | Naf bp2 | Naf bp2-COOH | Naf_bp4 | NVHOS | 6-2-FTS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAFL -Reference | 0 | 0 | 0 | 0 | 14 | 97 | 97 | 10 | 0 | 10 | 21 | 0 | 0 | 0 | 45 | 3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cape Fear River | 14 | 0 | 0 | 0 | 16 | 100 | 100 | 14 | 0 | 48 | 2 | 0 | 0 | 22 | 0 | 100 | 100 | 0 | 78 | 0 | 0 | 0 | 0 |

# PFAS in Cape Fear Striped Bass Serum

- GenX was detected in half the samples
- Nafion BP 2 was detectable in 78% of samples



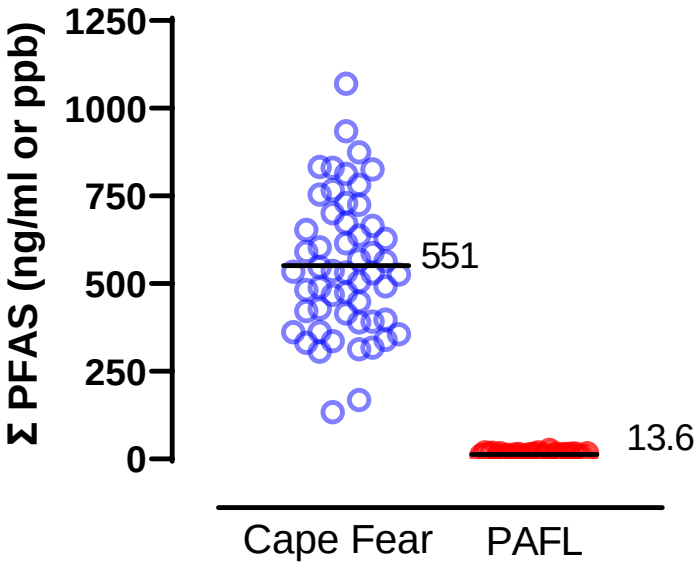

52.6% (30/57 <LOQ 0.24 ng/ml)



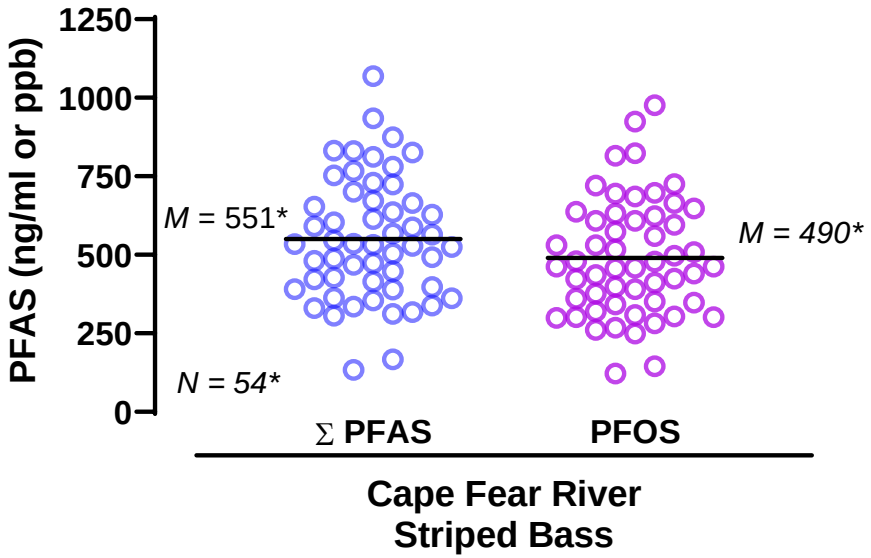

Detected in 78% of samples

# PFAS in Striped Bass Serum

- Total PFAS is >40 higher in Striped Bass from the Cape Fear River

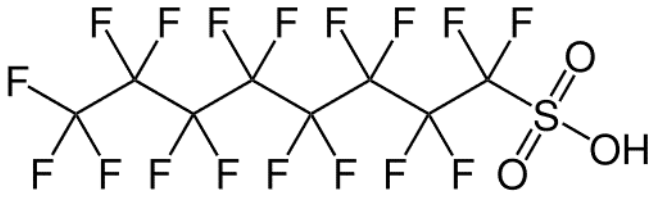


# PFOS accounted for 89% of PFAS present in serum of Striped Bass from the Cape Fear River




**PFOS = 89% of Total PFAS**

* concentration of 4 samples >LOQ

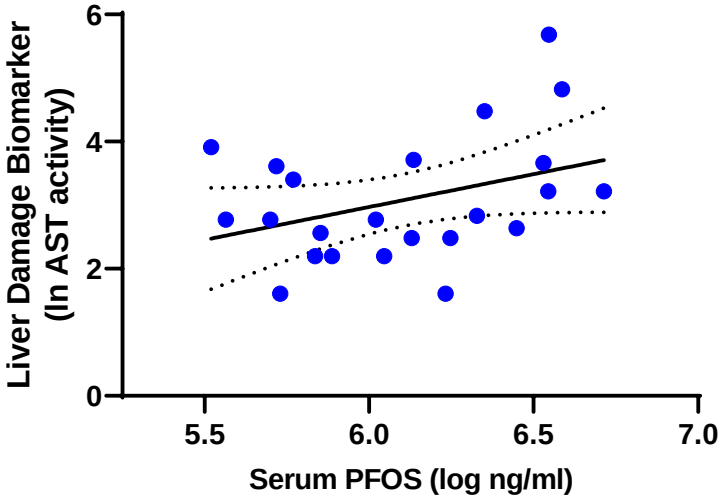


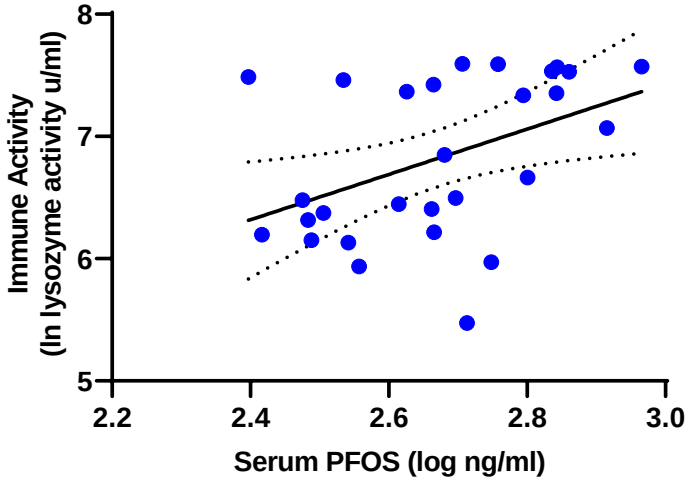
# PFAS exposures in Striped Bass are associated with liver and immune function changes

Comparison of liver enzyme activity and immune enzyme activity

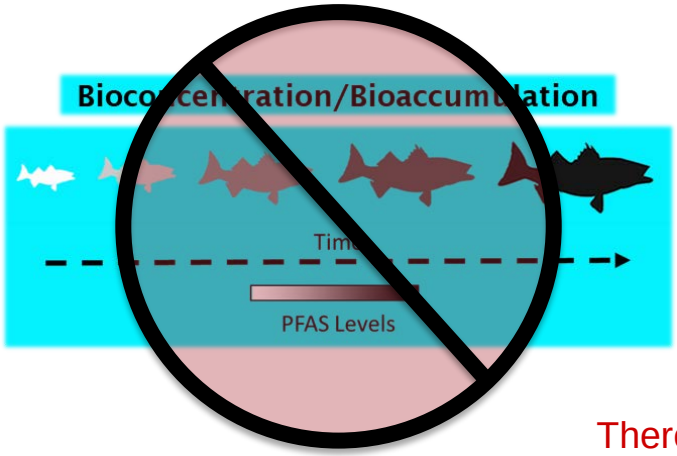
With **regression analysis** found:

Enzyme concentrations are increased with increasing PFAS

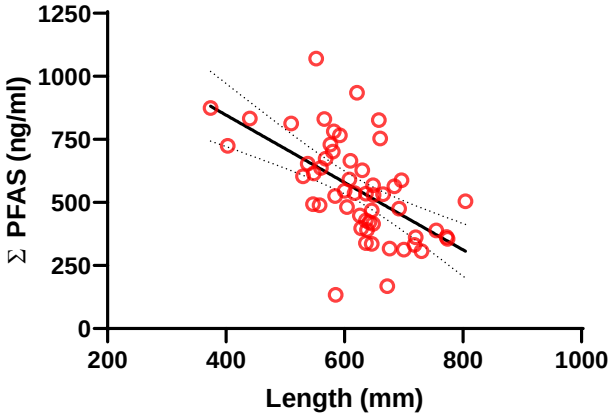


# PFAS in Striped Bass Serum: Bioaccumulation is very complicated




In striped bass PFAS bioaccumulation is not a simple analogy to POPS or toxic metals

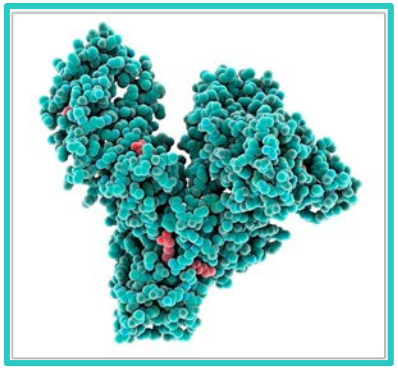



There is a major need to understand fundamental toxicokinetic properties of PFAS

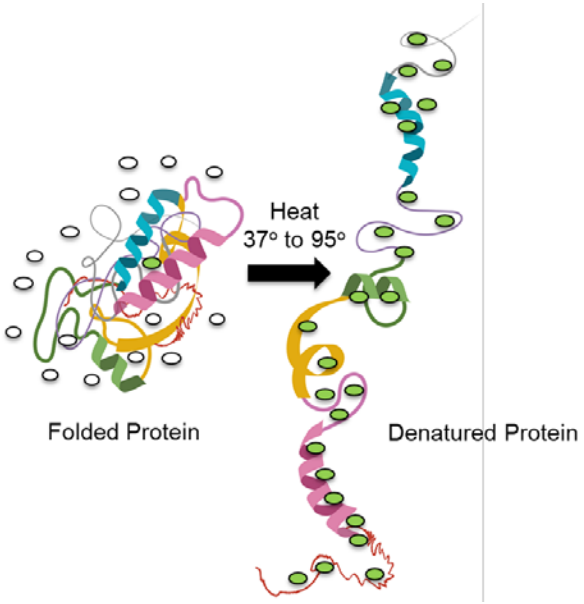
PFAS:

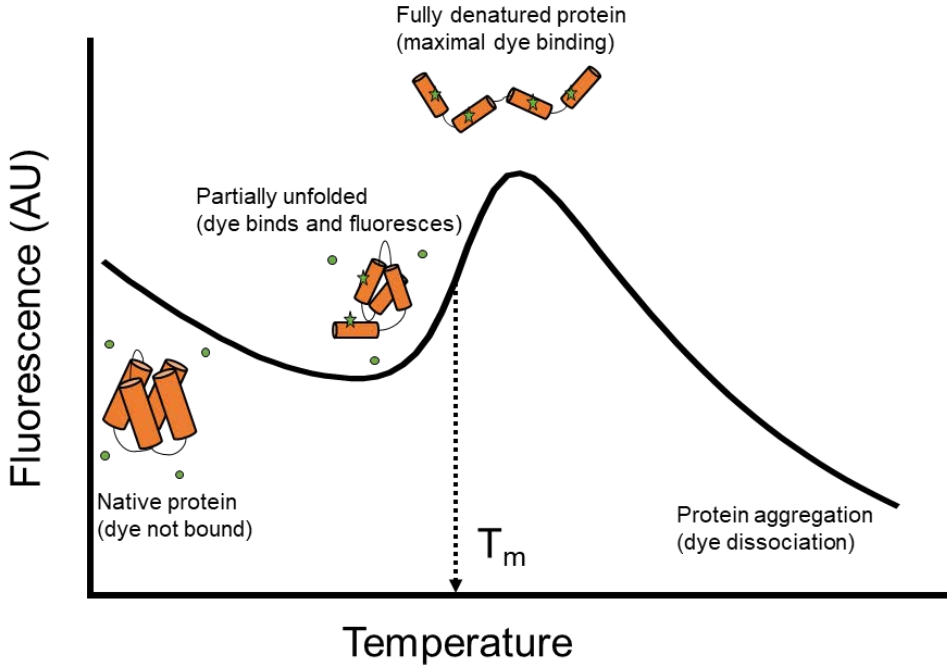
- Can be both polar and hydrophobic
- Can bind proteins (albumin, FABP)
- Accumulate in highly perfused tissues
- Some may bind phospholipids

❖ Affinity of individual PFAS to serum proteins

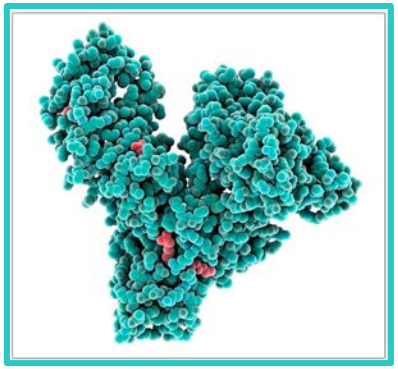


# A Novel HT PFAS Albumin Binding Assay

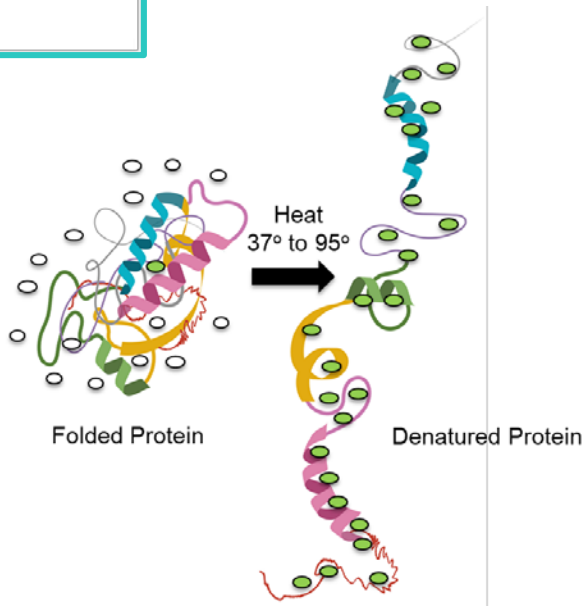


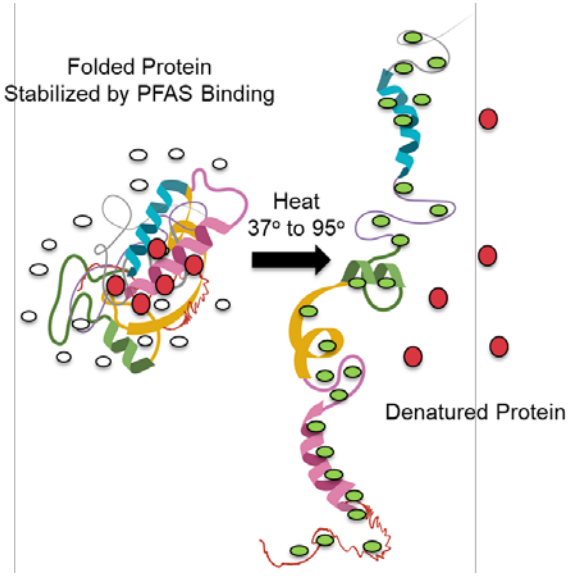




- Hydrophobic dye binding sites sequestered in folded protein
- Denatured protein – dye binding sites exposed
- Measure fluorescence (497nm) increase to calculate Tm



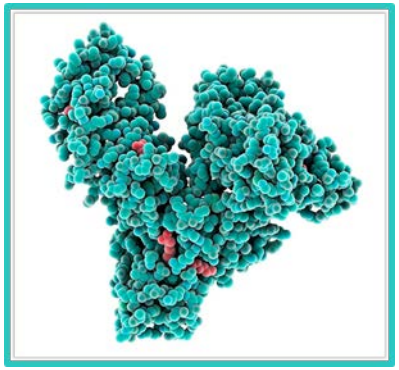
# A Novel HT PFAS Albumin Binding Assay



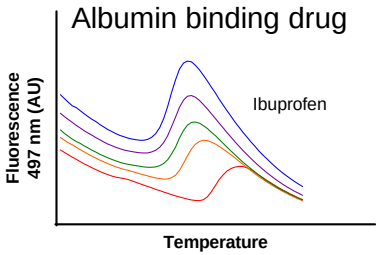

- Hydrophobic dye binding sites sequestered in folded protein
- Denatured protein – hydrophobic dye binding sites exposed
- Measure fluorescence increase to calculate Tm

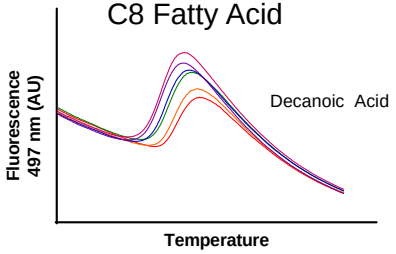
- Bound ligand increases protein stability
- Compare impact of increasing concentrations of ligand/PFAS on Tm (ΔTm)

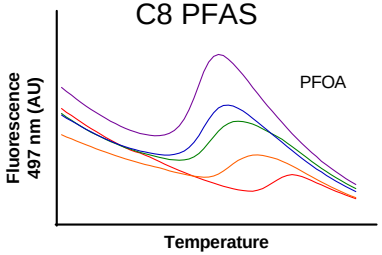


# A Novel HT PFAS Albumin Binding Assay
## - proof of concept -

Concentration Response



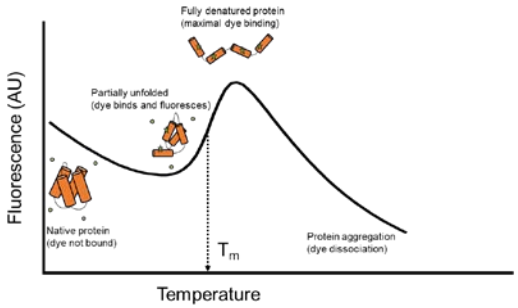



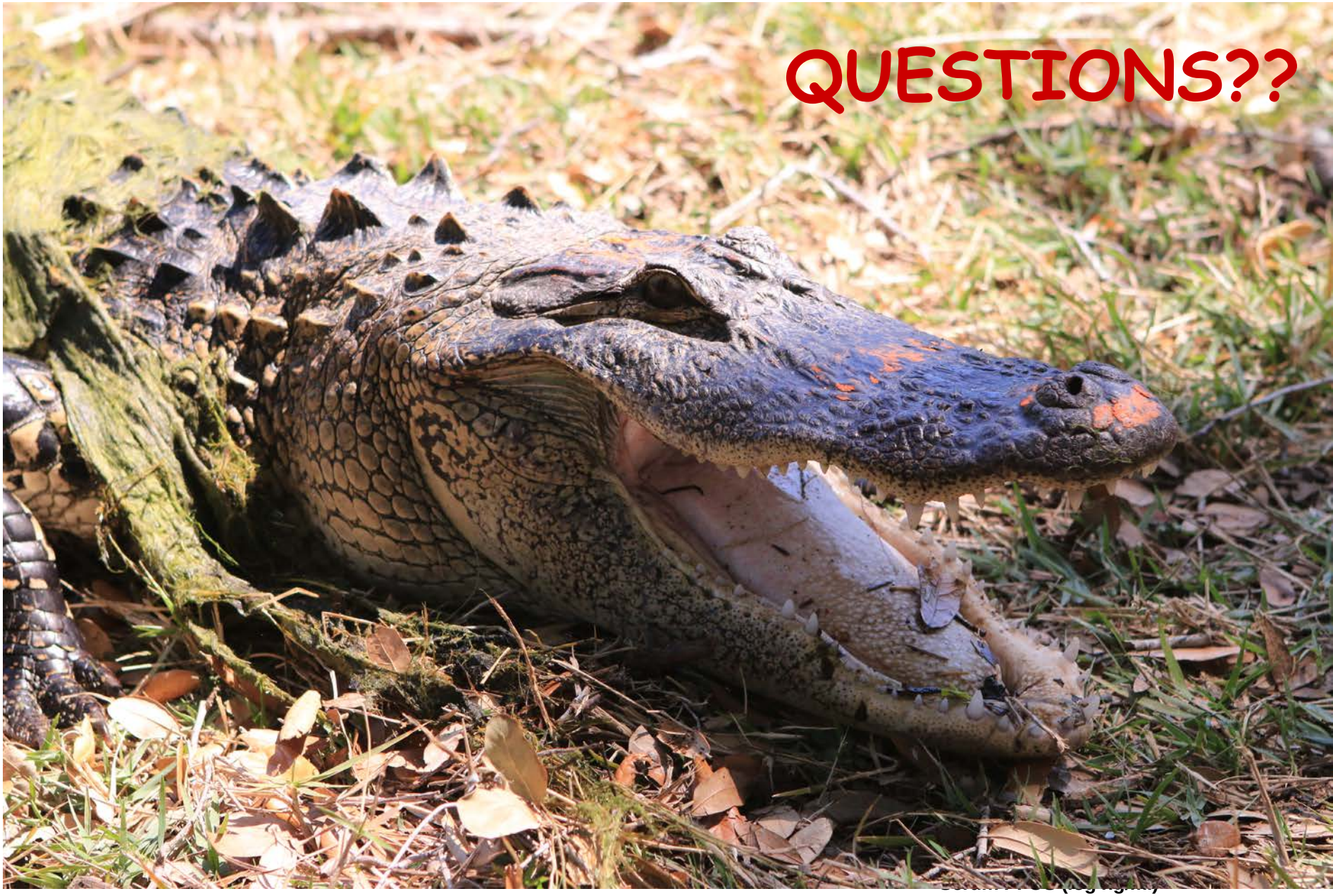

| Compond Name | Abbreviation | Cas No. | Chain Length | ΔTm (◦C) | EC10 [µM] |
|---|---|---|---|---|---|
| Perfluorobutanoic Acid | PFBA | 375-22-4 | 4 | 5.5 | 344 |
| Perfluoropentanoic Acid | PFPeA | 2706-90-3 | 5 | 7.7 | 446 |
| Perfluorohexanoic Acid | PFHxA | 307-24-4 | 6 | 10.8 | 632 |
| Perfluoroheptanoic Acid | PFHpA | 375-85-9 | 7 | 16.4 | 910 |
| Perfluorooctanoic Acid | PFOA | 335-67-1 | 8 | 17.8 | 1183 |

Increasing chain length stabilizes structure

Short chain binds with higher affinity




- Able to calculate Kd and rapidly compare relative binding properties of a variety of PFAS at different proteins


QUESTIONS??

Question 1:

What physiochemical information is necessary to better characterize the fate and transport of individual or groups of PFAS?

Question 2:

What physiochemical information is necessary to better characterize the potential for individual or groups of PFAS to have adverse effects?