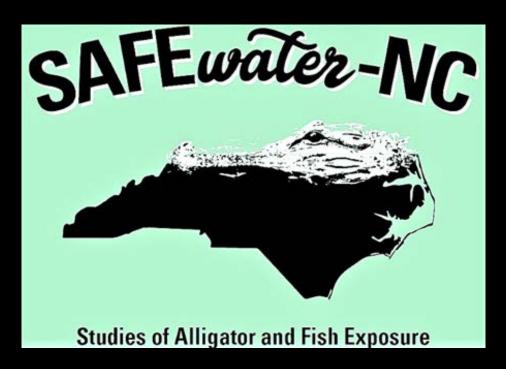
Insight into toxicity of PFAS through analysis of aquatic wildlife: the SAFEwaterNC Study

Cape Fear River Striped Bass Update



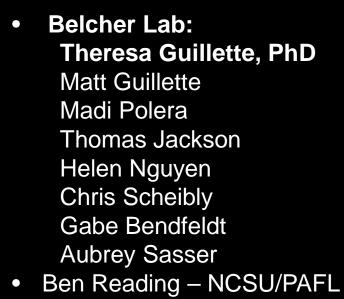
Scott Belcher, PhD
North Carolina State University



Acknowledgements:













Kyle Rachels

Clint Morgenson



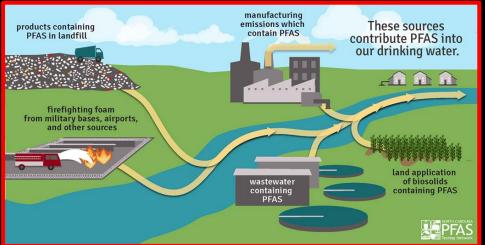
NORTH CAROLINA







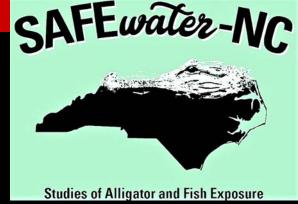
PFAS Contamination in the U.S WYCO On Military Sites On Drinking Water On Drinking Water On Other Known Sites On Other Known Si



PFAS contamination is pervasive

- PFAS are detectable through out the Cape Fear watershed
- Contamination concern is focused downstream of known manufacturing site
- Other sources of PFAS
- Contamination of drinking water
 - GenX
 - Nafion by products
 - Others, known & unknown







Study goals:

Characterize levels of PFASs in blood/serum and water (LC/MS/MS)

- Alligator
- Striped Bass
- Identify reference populations for comparisons
- Muscle/Tissue of other fish that are eaten

Evaluate: 1) Exposure

- 2) Bioaccumulation paired water samples
- 3) Impacts on health (biomarkers)

Communicate findings to stakeholders – Community Engagement



Striped Bass Pamlico Aquaculture Lab Cape Fear River: LD 1

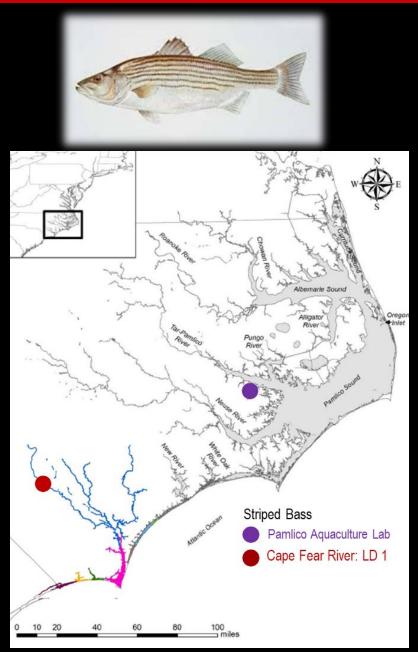
Striped Bass: Morone saxatilis

Commercial and recreational fishery in North Carolina

valued at more than \$94 million annually (2016)

Striped Bass: can live in both salt and fresh water

- Cape Fear River Population
 - Do not migrate
 - No natural reproduction in the Cape Fear River
 - 100% are hatchery progeny (genetics)
 - Good model of Cape Fear contaminants
- Aquaculture facility as a reference population for exposure



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Elevated levels of per- and polyfluoroalkyl substances in Cape Fear River Striped Bass (*Morone saxatilis*) are associated with biomarkers of altered immune and liver function



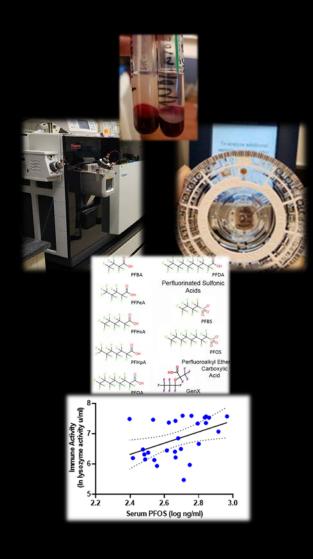
T.C. Guillette^{a,b}, James McCord^e, Matthew Guillette^a, M.E. Polera^a, Kyle T. Rachels^f, Clint Morgeson^f, Nadine Kotlarz^{b,c}, Detlef R.U. Knappe^{b,c}, Benjamin J. Reading^d, Mark Strynar^e, Scott M. Belcher^{a,b,*}

- May 2018 Striped Bass from Cape Fear River by electrofishing
 LD1 & 3
- Blood/Serum Collected
- Analyzed fish were between ~2-7 year old
 - Residents of the Cape Fear River from 1-6 years
- Exposure compared to aquaculture-reared fish (background)

Fish Serum Collection Standard Operating Procedure



- Caudal tail venipuncture
- Collect 2-3 ml serum and plasma tubes
- Serum allowed to clot for 30 minutes
- Stored on ice, return to lab
- Centrifuged and aliquoted into cryovials
- Store frozen at -80C



Analytical Approach

- Untargeted high-resolution LC-mass spectrometry Orbitrap Fusion
 - Measure known: 23 PFAS calibration standards
 - Detect unknown PFAS
- Blood chemistry and health-related biomarkers (14)
 - Liver
 - Kidney
 - Immune system lysozyme
 - Hormones
- Regression analysis to detect associations between PFAS concentrations and health endpoints

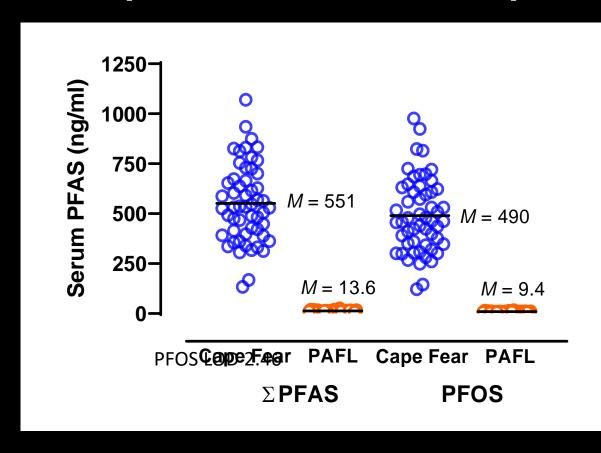
Table 2PFAS detection frequency and concentration in Striped Bass serum.

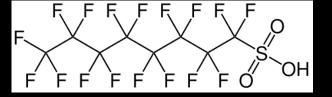
	LOD	CFR PAFL % > LOD		Concentration (ng/mL) mean (range)	
_	(ng/mL)	(n=58)	(n=29)	Cape Fear	PAFL
PFNA	0.34	100	96.9	4.49 (0.81-11.6)	0.48 (0.34-0.82)
PFDA	1.68	100	96.9	68.0 (10.2-146)	2.5 (1.68-4.6)
PFOS	2.47	100	100	490 (122-977)	9.41 (4.62-16.5)
PFHxS	0.09	98.3	3.4	0.78 (0.15-2.23)	0.59
Nafion byproduct 2	0.25	77.6	0	0.30 (0.25-1.03)	All < LOD
GenX	0.24	48.3	10.3	1.91 (0.31-5.85)	1.64 (0.24-2.3)
PFBS	0.01	24.1	44.8	0.15 (0.01-1.35)	0.01 (0.01-0.2)
PFO5DoDA	0.01	22.4	0	0.49 (0.01-1.35)	All < LOD
PFOA	0.16	15.1	13.8	0.57 (0.16-4.29)	0.16 (0.16-1.14)
PMPA	0.12	13.8	10.3	0.12 (0.12-0.19)	0.12 (0.12-0.14)
PFBA	0.11	13.8	0	0.11 (0.11-0.18)	AII < LOD

LOD, limit of detection; CFR, Cape Fear River; PAFL, Pamlico aquaculture field laboratory

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







PFAS are also present in Striped Bass Tissue (muscle/filet)

PFAS detected in muscle

Table 2	
PFAS detection frequency and concent	ration in Striped Bass serum.

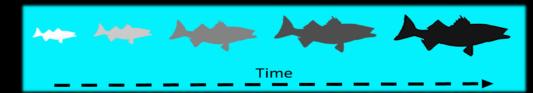
	LOD	CFR % > LOD	PAFL % > LOD		Concentration (ng/mL) mean (range)	
	(ng/mL)	(n=58)	(n=29)	Cape Fear	PAFL	
PFNA	0.34	100	96.9	4.49 (0.81-11.6)	0.48 (0.34-0.82)	
PFDA	1.68	100	96.9	68.0 (10.2-146)	2.5 (1.68-4.6)	
PFOS	2.47	100	100	490 (122-977)	9.41 (4.62-16.5)	
PFHxS	0.09	98.3	3.4	0.78 (0.15-2.23)	0.59	
Nafion byproduct 2	0.25	77.6	0	0.30 (0.25-1.03)	All < LOD	
GenX	0.24	48.3	10.3	1.91 (0.31-5.85)	1.64 (0.24-2.3)	
PFBS	0.01	24.1	44.8	0.15 (0.01-1.35)	0.01 (0.01-0.2)	
PFO5DoDA	0.01	22.4	0	0.49 (0.01-1.35)	All < LOD	
PFOA	0.16	15.1	13.8	0.57 (0.16-4.29)	0.16 (0.16-1.14)	
PMPA	0.12	13.8	10.3	0.12 (0.12-0.19)	0.12 (0.12-0.14)	
PFBA	0.11	13.8	0	0.11 (0.11-0.18)	All < LOD	
LOD, limit of detection; CFR, Cape Fear River; PAFL, Pamlico aquaculture field laboratory						

	LOD (ng/g)	CFR % > LOD	Concentration (ng/g) mean (range)
	(ng/g)	(n=5)	Cape Fear
PFNA	0.3	100	0.55 (0.17-1.44)
PFOS	0.1	100	7.02 (2.80-13.3)
NVHOS	0.01	100	1.14 (0.59-3.05)
PFBA	0.01	100	0.35 (0.11-0.84)
PFHxS	0.09	100	4.35 (0.3-18.9)
PFDA	0.1	80	20.1 (15.9-27.8)
PFHpA	0.02	80	37.8 (25.1-61.6)
HFPO-DA (GenX)	0.04	60	1.63 (0.3-5.09)
PMPA	0.01	20	0.06
Nafion byproduct 2	0.02	20	0.43
PFBS	0.01	20	18.7
PFO3DoDA	0.01	20	9.74
PFO5DoDA	0.01	20	0.21
PFO2HxA	0.05	0	ND
PFO4DA	0.01	0	ND

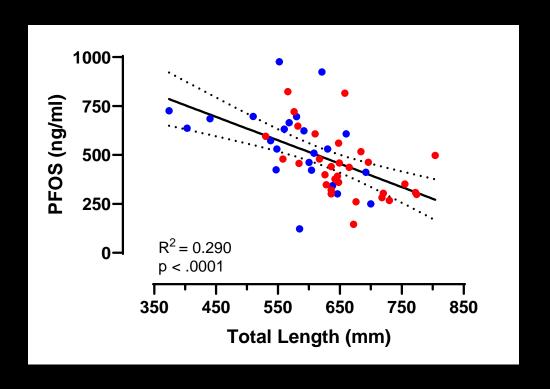
N = 5 May 2019, Lock and Dam #1 (unpublished)

PFAS do not increase with body weight/length/age

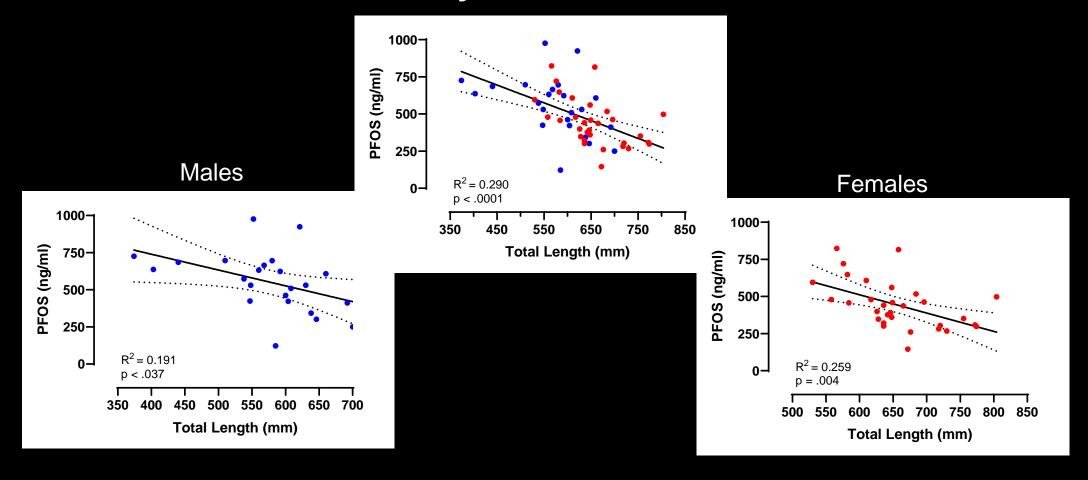
Persistent Organic Pollutants (POPS) or Toxic Metals



Contaminant is eliminated much slower than it is absorbed



PFAS Decrease with Body Size in Both Males and Females



Are PFAS partitioning to germ cells and impacting reproduction?



- High levels of PFAS present in Striped Bass from the Cape Fear
- Most serum PFAS is **PFOS**
- PFAS exposure is associated with adverse effects on <u>liver</u> and <u>immune</u> function of Striped Bass
- Both exposure and effects mirror known toxic impacts of PFAS
- Ongoing analysis: has determined tissue PFAS distribution in Catfish, American Shad, Sunfish and Striped bass
- Analyzing PFAS in Striped Bass serum 2019: CFR vs Roanoke
- Planned for Spring 2020:
 - Resampling from all NC watershed
 - Experiments to investigate PFAS impacts on reproduction
- Paired analysis humans consumption and PFAS in fish caught

