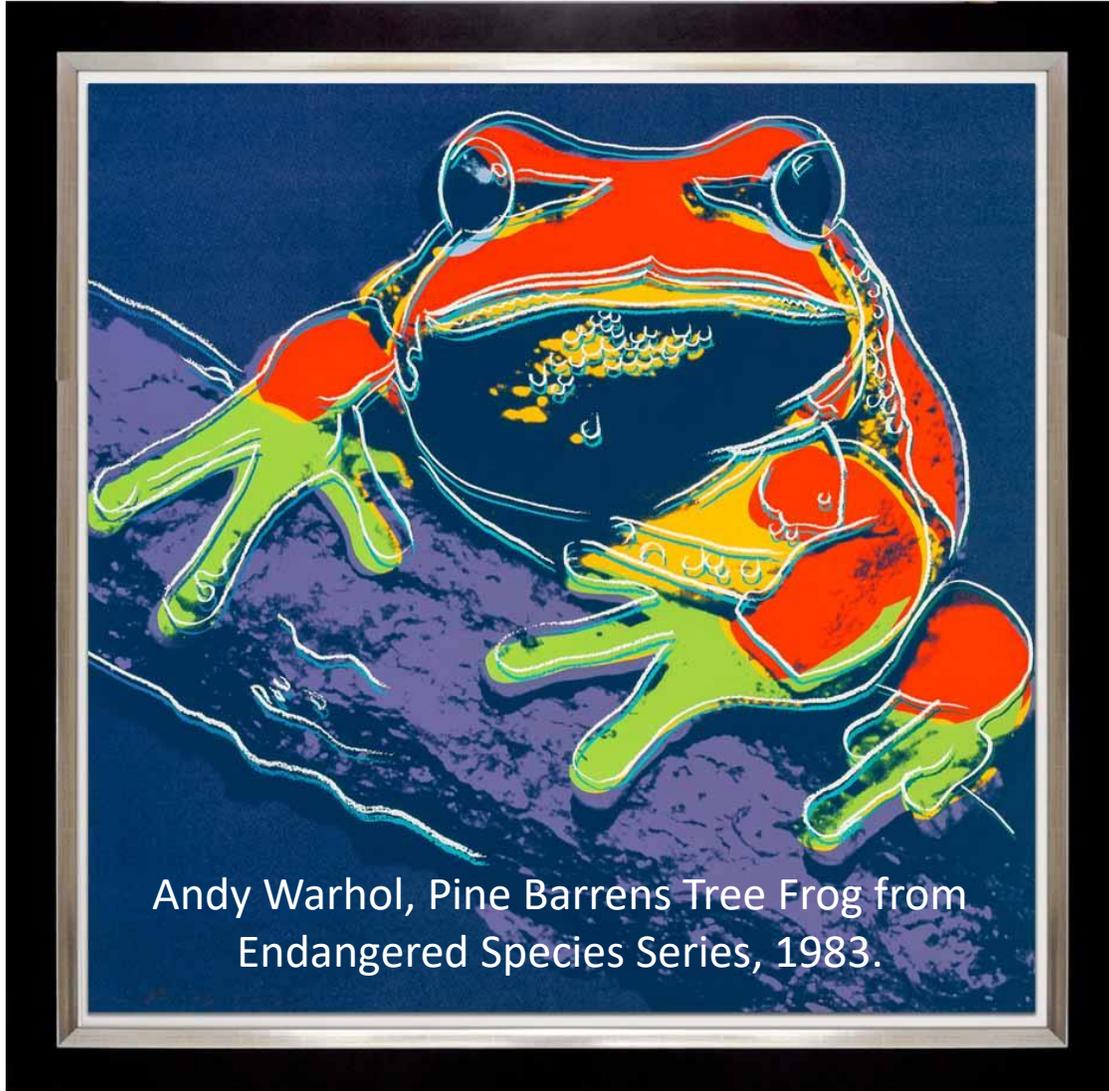


Point and non-point sources of endocrine disrupting compounds and their potential effects on fish and frogs in the New Jersey Pinelands

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NJ Pinelands Commission Meeting
November 10, 2022





Andy Warhol, Pine Barrens Tree Frog from
Endangered Species Series, 1983.

Project Funding

- ✓ New Jersey Pinelands Commission
- ✓ William Penn Foundation through the Academy of Natural Sciences at Drexel University Delaware Watershed Research Fund
- ✓ USGS Cooperative Water Program
- ✓ USGS Amphibian Research & Monitoring Initiative
- ✓ USGS Environmental Health Program



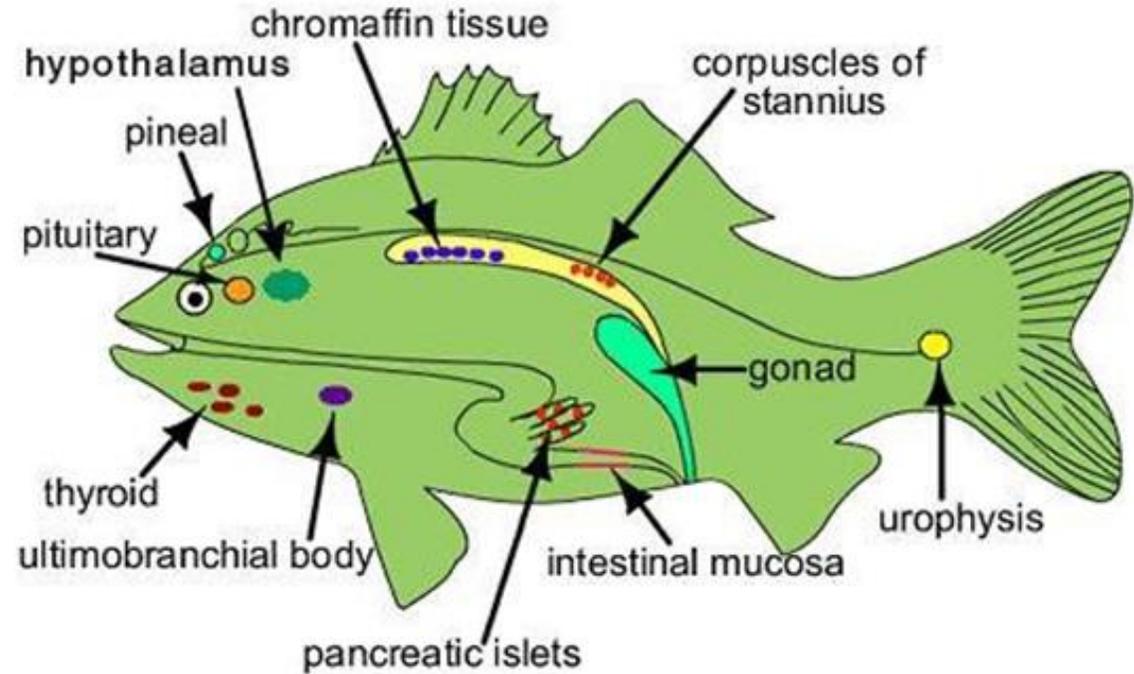
Outline

- ✓ Brief overview of EDCs
- ✓ Questions
- ✓ Study design
- ✓ Surface water sampling
- ✓ Chemical mixtures in surface water
- ✓ Fish and frog sampling
- ✓ Health effects
- ✓ Why does it all mean?

The endocrine system is...

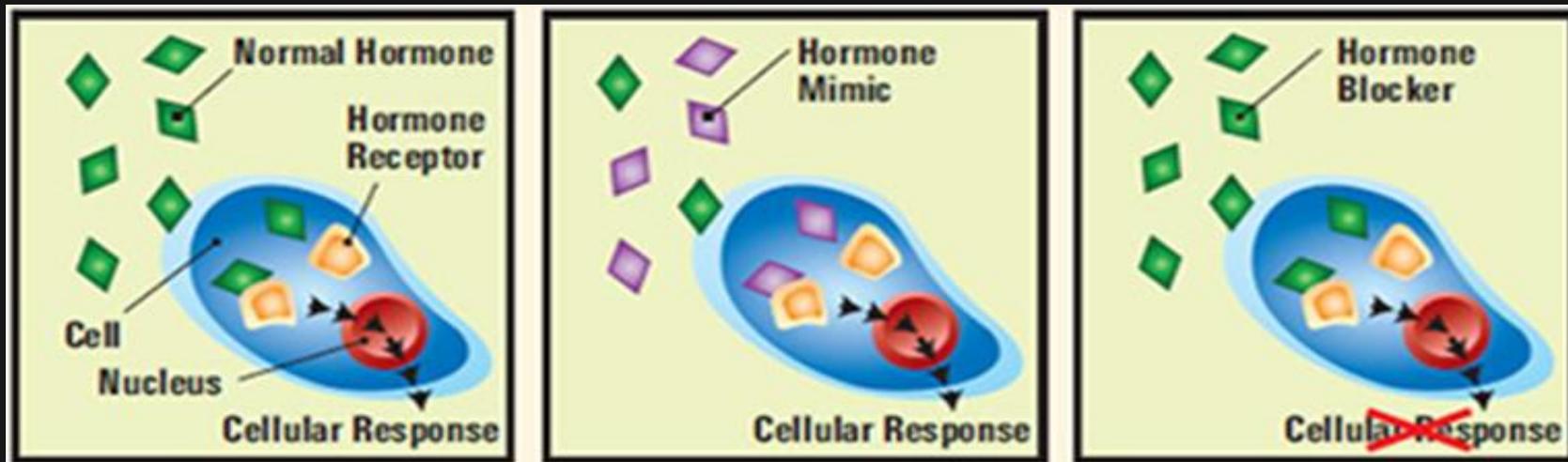
a collection of tissues in animals that produce hormones to regulate essential life processes

e.g., reproduction, development, etc.



What is an endocrine disrupting chemical?

Compounds within an **ecosystem** with the potential to interfere with the **endocrine function** of natural populations of fish and wildlife, resulting in deleterious effects on **development, metabolism, reproduction, immune response** or other biological outcomes.

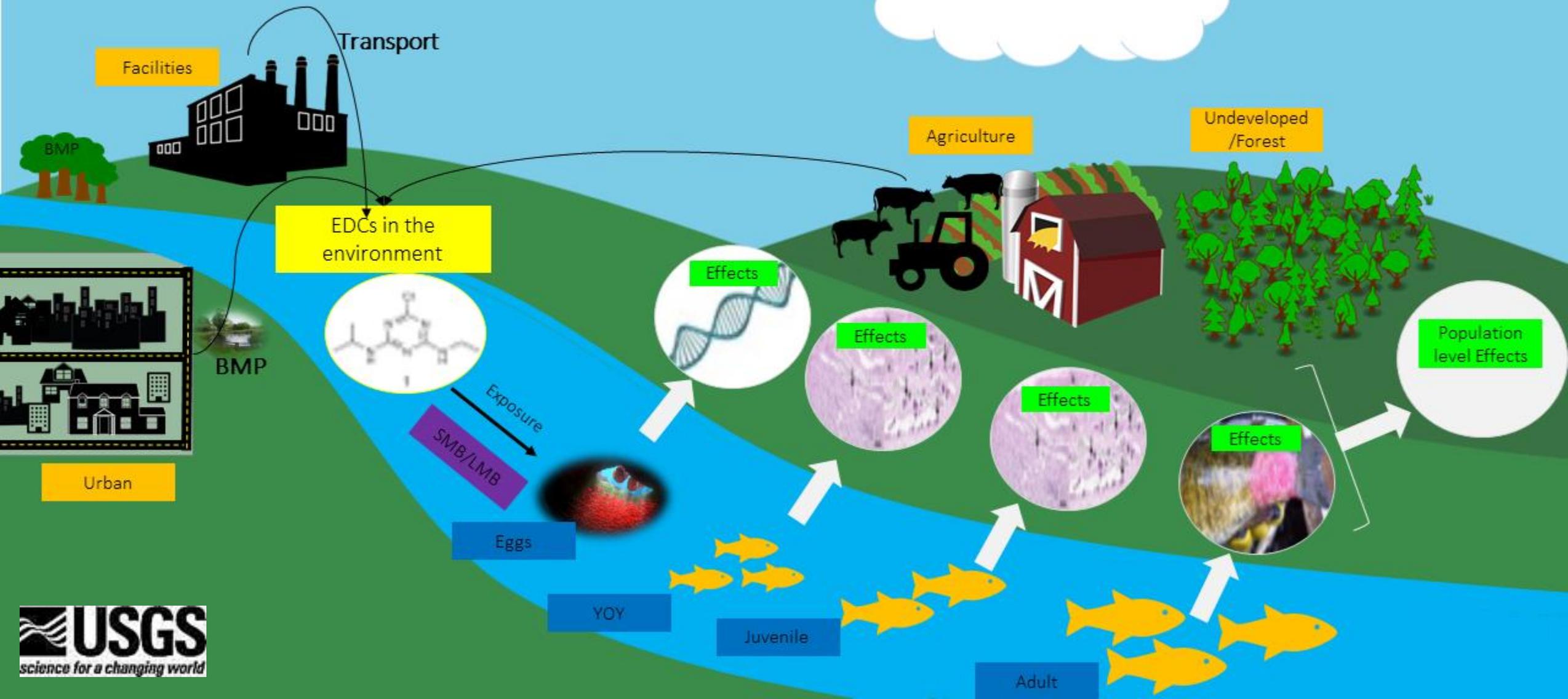
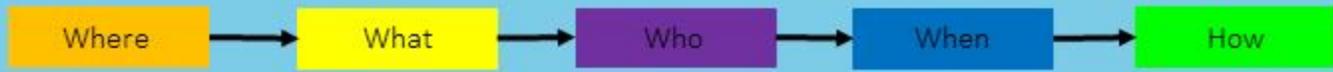




Sources and examples of EDCs

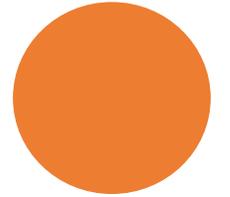
- Hormones (natural or synthetic)
- Pharmaceuticals/antibiotics
- Pesticides
- Surfactants
- Plastics
- Flame retardants
- Personal care products



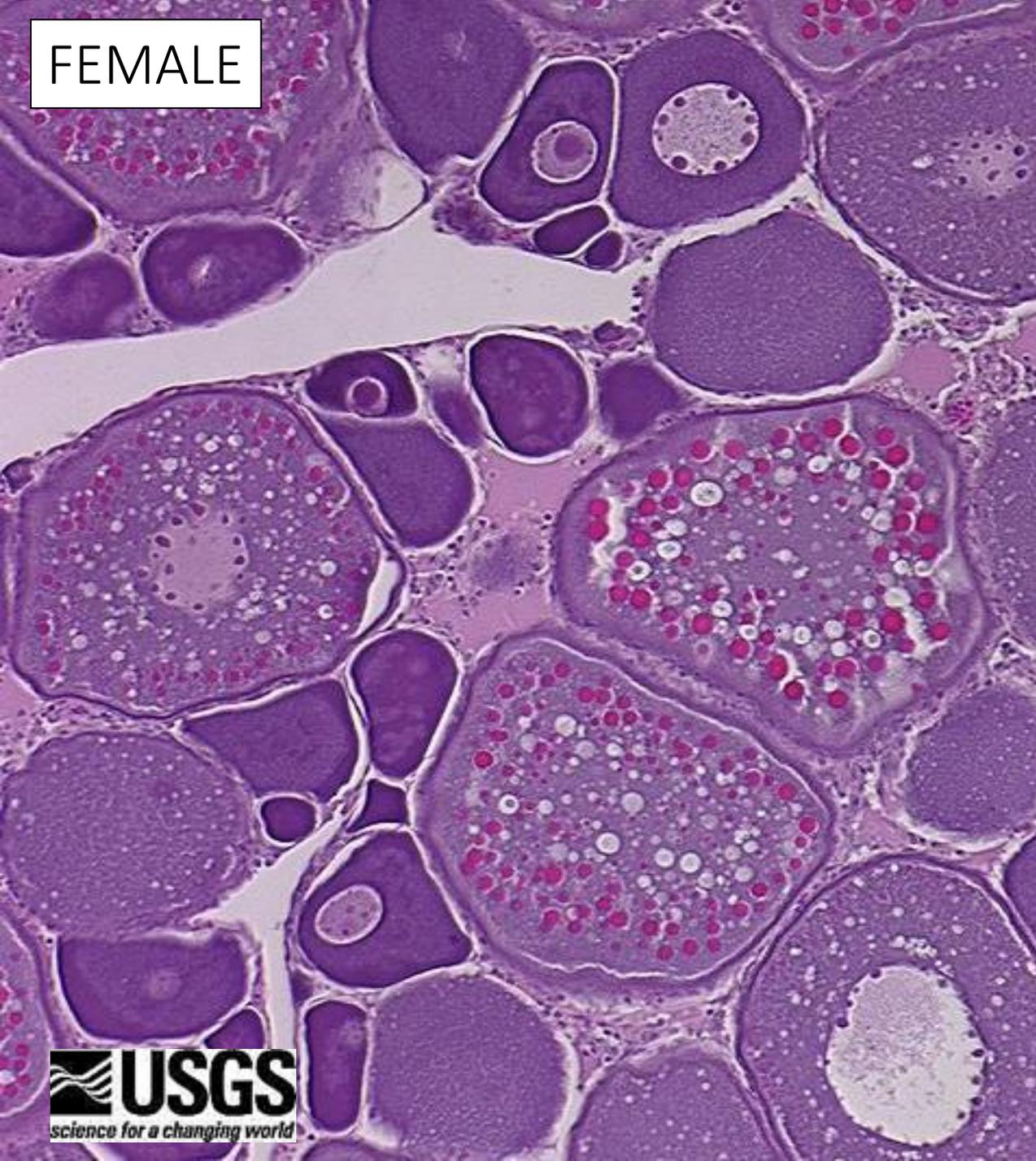


Potential effects

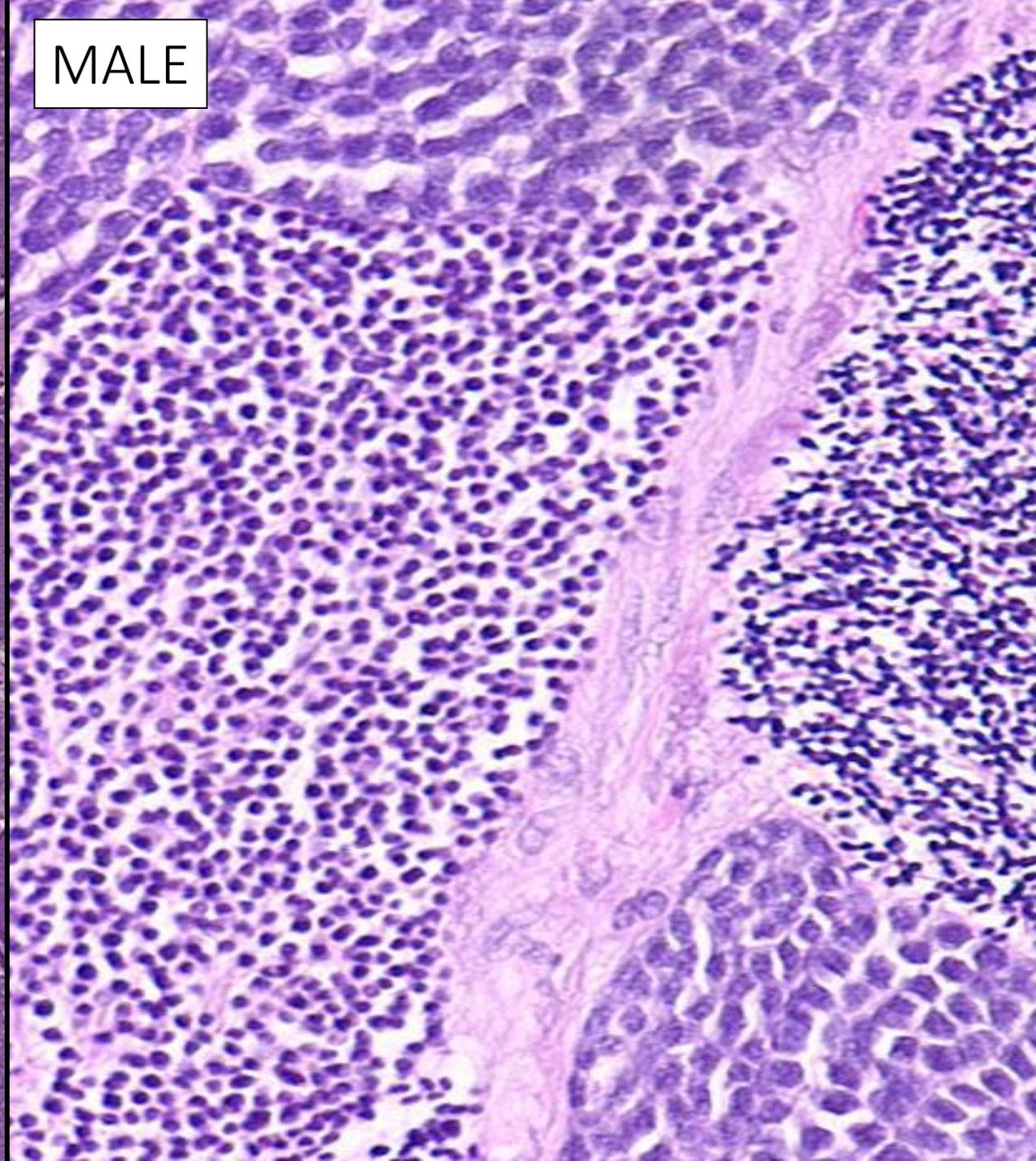
- Female based sex ratio
- Intersex
- Immune suppression (parasites)



FEMALE



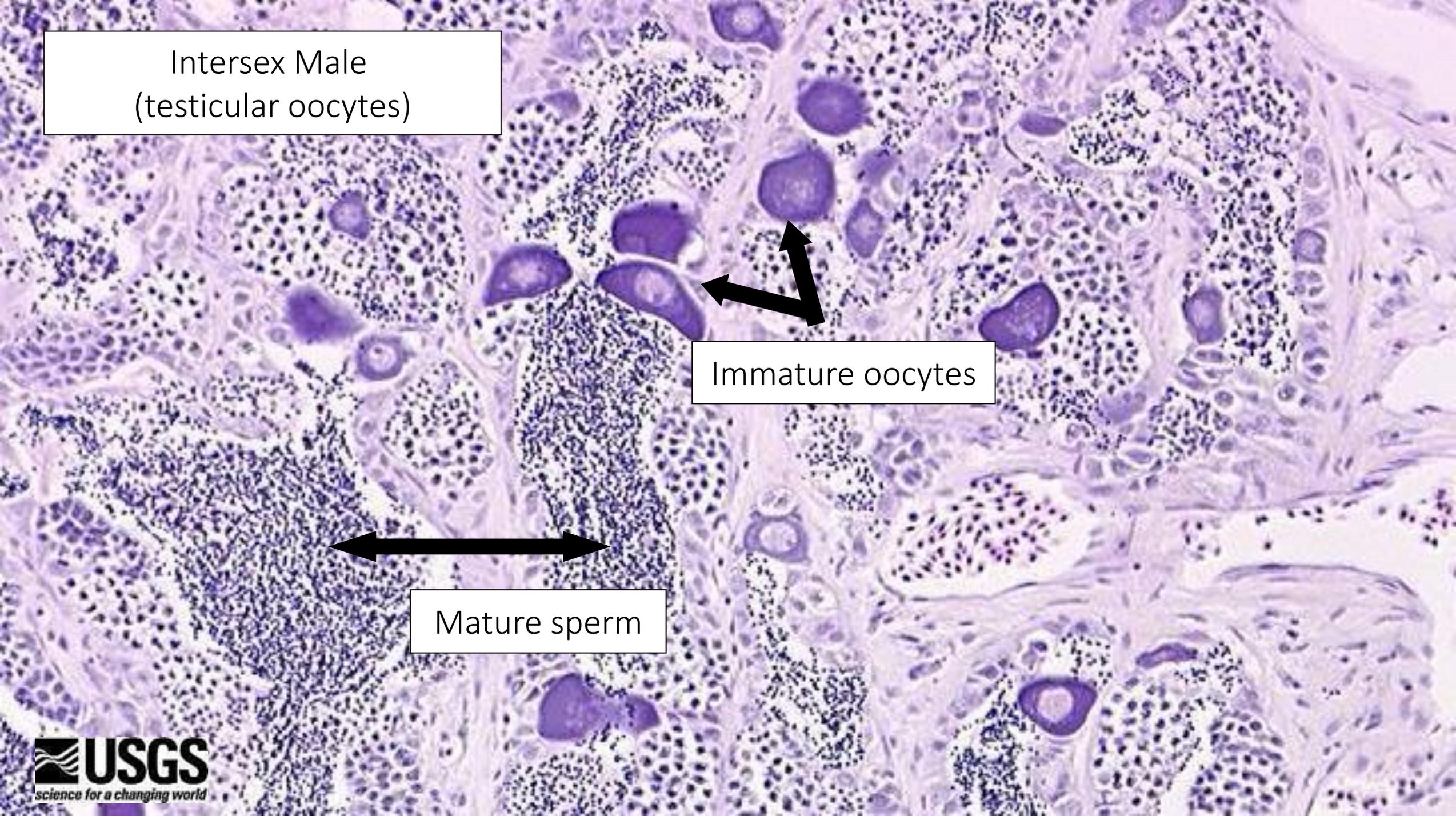
MALE



Intersex Male
(testicular oocytes)

Immature oocytes

Mature sperm



Study Questions

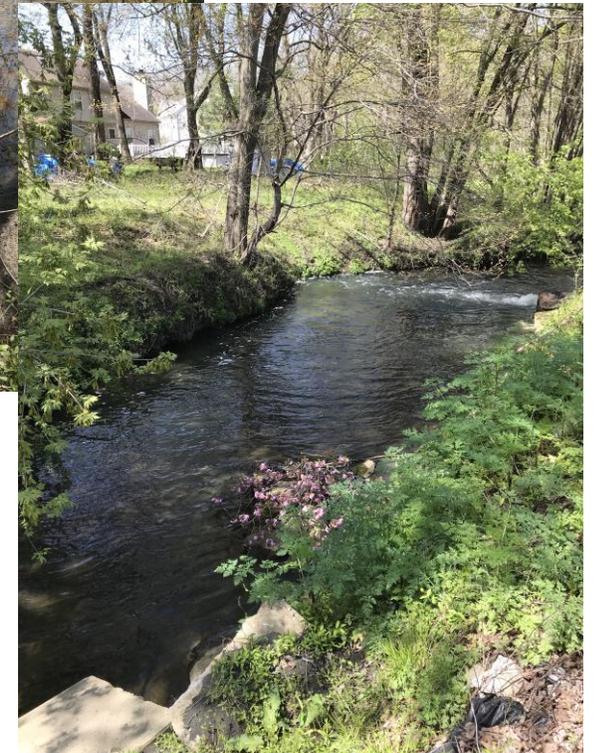
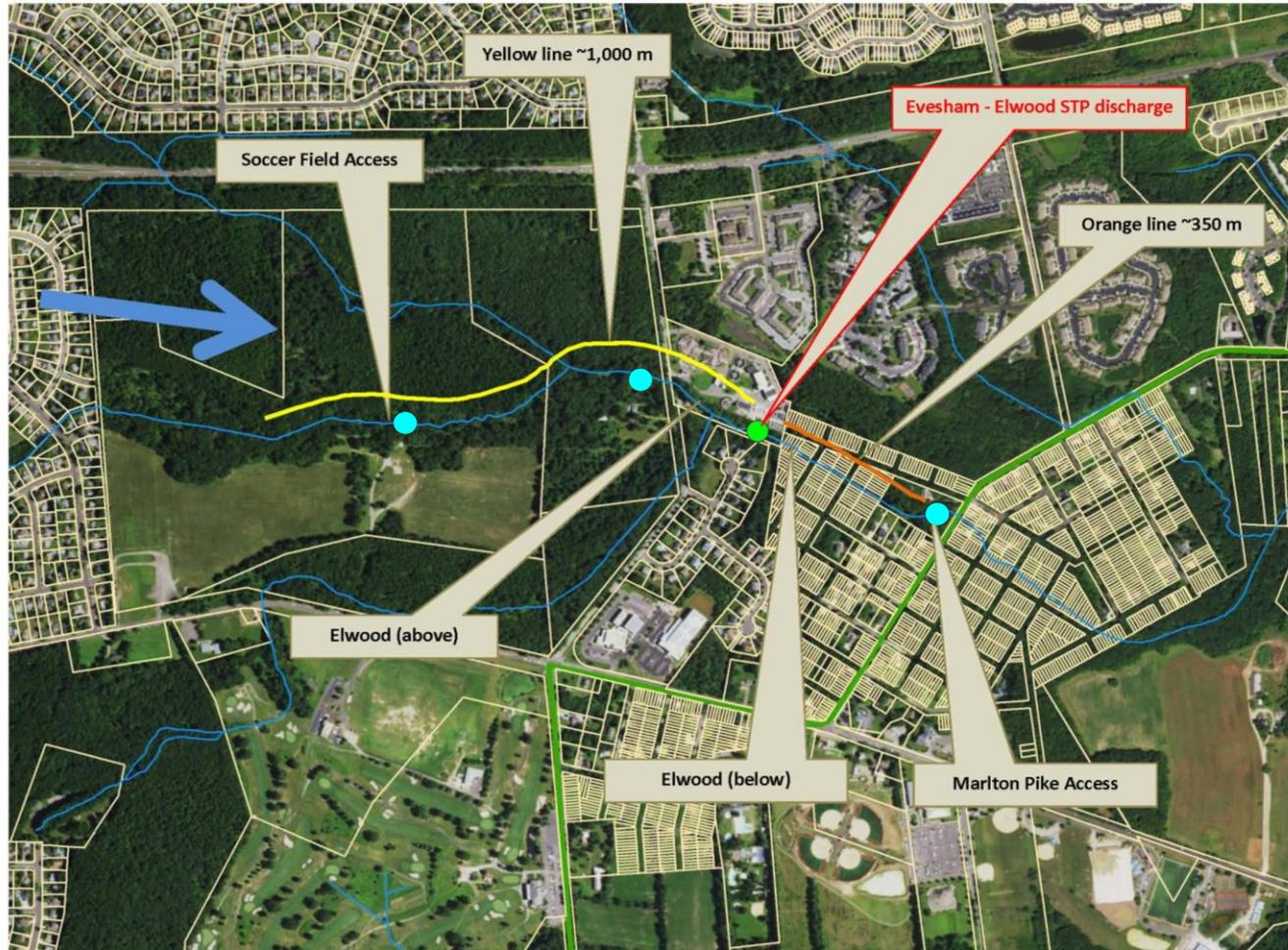
- Are EDCs present in the surface water of streams above and below municipal sewage treatment plants and in the surface water of stormwater basins and ponds located in altered landscapes?
- Do biological manifestations of endocrine disruption occur in native and non-native fish and frog species at these sites?
- Do differences in the proposed biological and chemical measures of endocrine disruption exist between point and non-point sources in the Pinelands?
- How do the results for these impacted sites compare to the results for minimally impacted reference streams and ponds?

Study Design

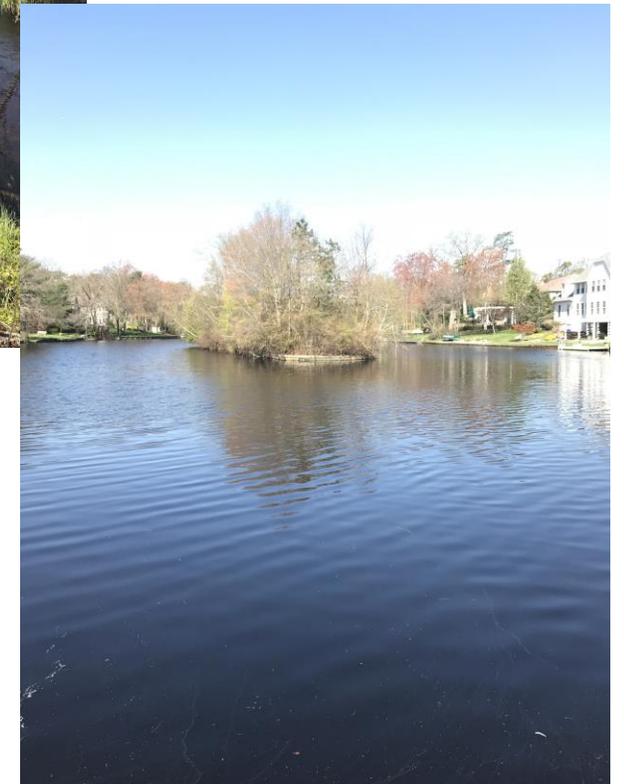
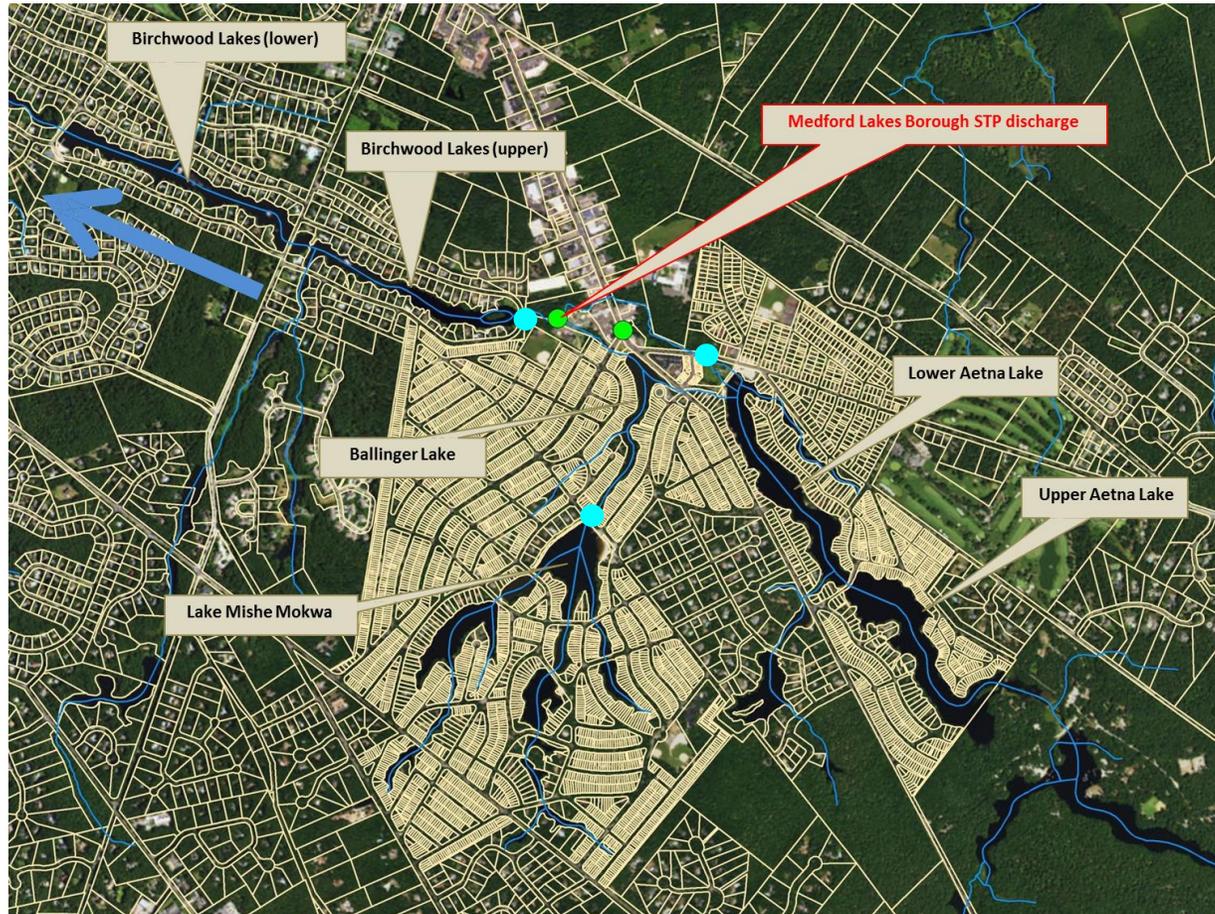
- **On-Stream** - point source impacted
 - 1 stream complex
 - 1 lake complex
 - 2 reference lakes
- **Off-stream** - nonpoint source impacted
 - 10 degraded ponds/stormwater basins
 - 3 reference ponds



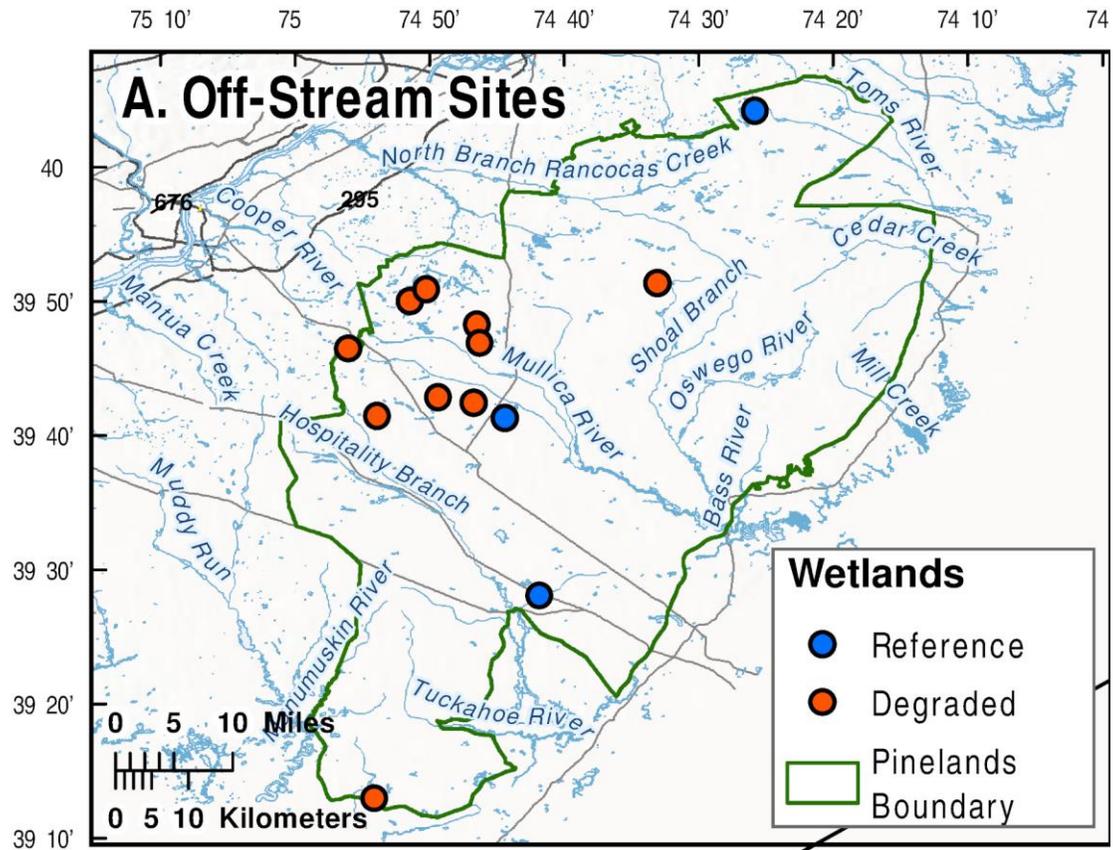
On-stream sites



On-lake sites



Off-stream sites



Land-use information

Site Type	n	Upland Agriculture	Development	Altered Land
Off-Stream Sites (Wetlands)				
Reference	3	0%	<4.5%	<4.5%
Degraded	10	0–30%	0.7–43%	12–64%
On-Stream (Streams/Lakes)				
Reference	2	0–0.4%	0.3–2.8%	<3.5%
Above WWTP	4	0–1.6%	40–75%	41–75%
Below WWTP	2	0.2–1.1%	48–73%	41–75%

Water Sampling

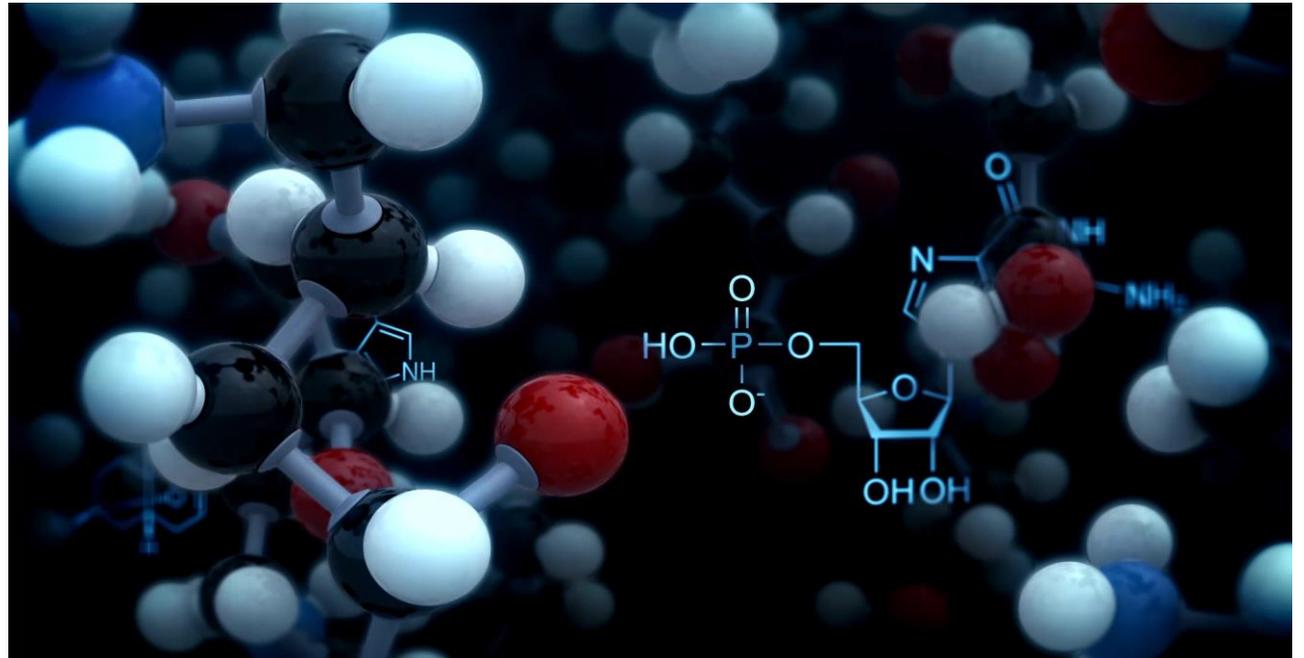
- On-stream: 32 total samples (8 sites x 4 sampling events)
- Off-stream: 52 total samples (13 sites X 4 sampling events)



Photo credit: New Jersey Pinelands Commission

Chemical Analysis:

- Basic water quality parameters
- Naturally occurring phytoestrogens & mycotoxins
- Hormones & hormone conjugates
- Trace metals
- Compounds indicative of human activity



Water chemistry

- 23 trace elements
- Estrogenicity - bioindicator of estrogenic activity
- 6 phytoestrogens and 8 mycotoxins
- 42 hormones and hormone conjugates
- 70 waste indicator compounds including: surfactants, fragrances, antioxidants, disinfectants, food additives, plastics, industrial solvents, PAHs, fecal and plant sterols, phosphate flame retardants and pesticides



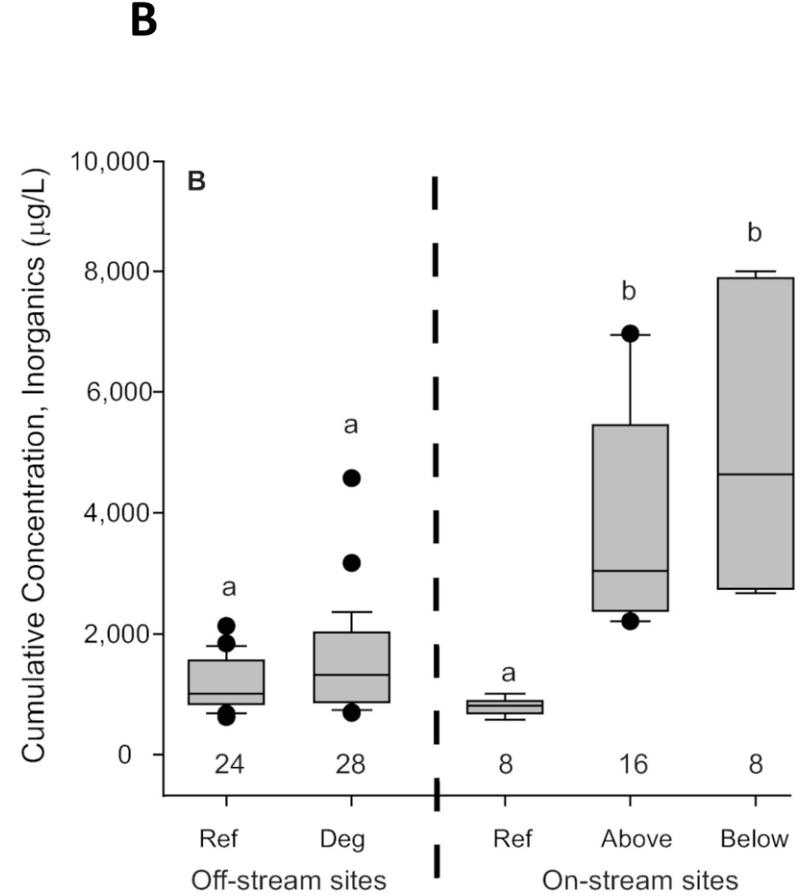
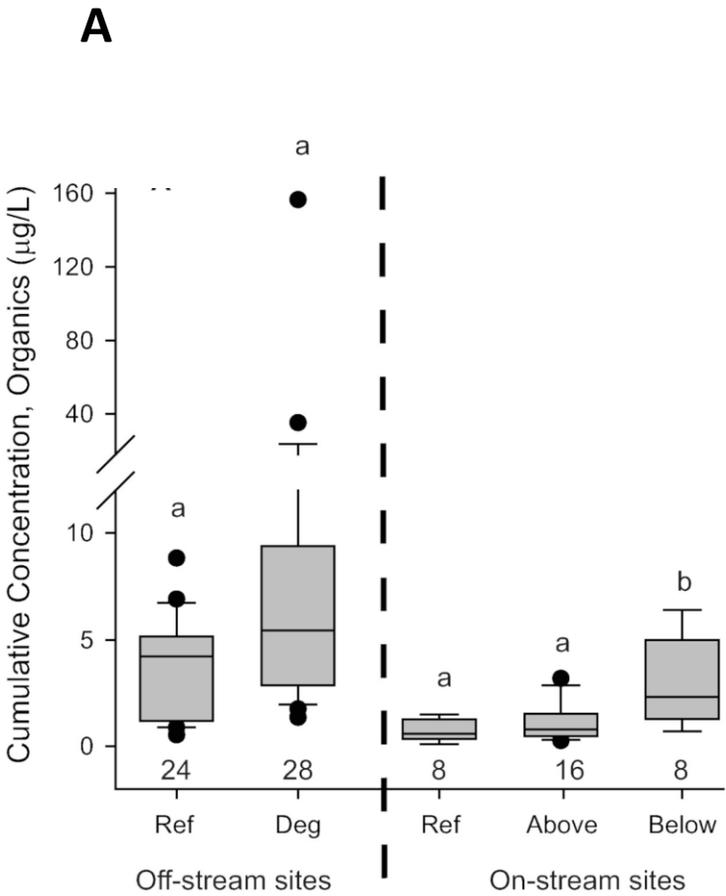
Inorganic Results – All sites

Contaminant Class	Number of compounds observed	Concentration Range (ug/L)	Compounds observed
Toxic Heavy Metals	4	0.25-8.69	Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb)
Metalloestrogens	12	21-814	Aluminum (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Antimony (Sb), Selenium (Se), Vanadium (V)
Cumulative inorganics	22	250-7,700	

Organic Results – All sites

Contaminant Class	Number of compounds observed	Concentration Range (ug/L)	Compounds observed
Steroids	10	0-15	17-alpha-Estradiol, 17b-Estradiol 3-sulfate, 17-beta-Estradiol, 3-beta-Coprostanol, 4-Androstene-3,17-dione, Cholesterol, Equilin, Estriol 17-sulfate, Estrone, Progesterone
Phytochemicals	12	0-122	3-Methyl-1H-indole, beta-Sitosterol, beta-Stigmastanol, Caffeine, Camphor, Daidzein, Equol, Formonentin, Genestein, Indole, Methyl salicylate, p-Cresol
Pesticides	5	0-0.68	1,4-Dichlorobenzene, Atrazine, Metolachlor, Pentachlorophenol, Prometon
Personal Care Products	11	0-15.8	4-Cumylphenol, 4-Nonylphenol (all isomers), Acetophenone, AHTN, Benzophenone, D-Limonene, HHCB, Isophorone, N,N-Diethyl-m-toluamide, Triclosan, Triethyl citrate
PAHs	10	0-27	2,6-Dimethylnaphthalene, 2-Methylnaphthalene, 9,10-Anthraquinone, Anthracene, Benzo-a-pyrene, Carbazole, Fluoranthene, Phenanthrene, Phenol, Pyrene
Mycotoxins	3	0-0.073	Beauvericin, Deoxynivalenol, Zearalenone
Industrial chemicals	9	0-2.19	5-Methyl-1H-benzotriazole, Chloroethylphosphate, Diethyl phthalate, FYROL FR 2, TBEP, Tetrachloroethene, Tribromomethane, Tributyl phosphate, Triphenyl phosphate
Cumulative organics	60	0.09-156	

Cumulative concentrations of organic and inorganic contaminants

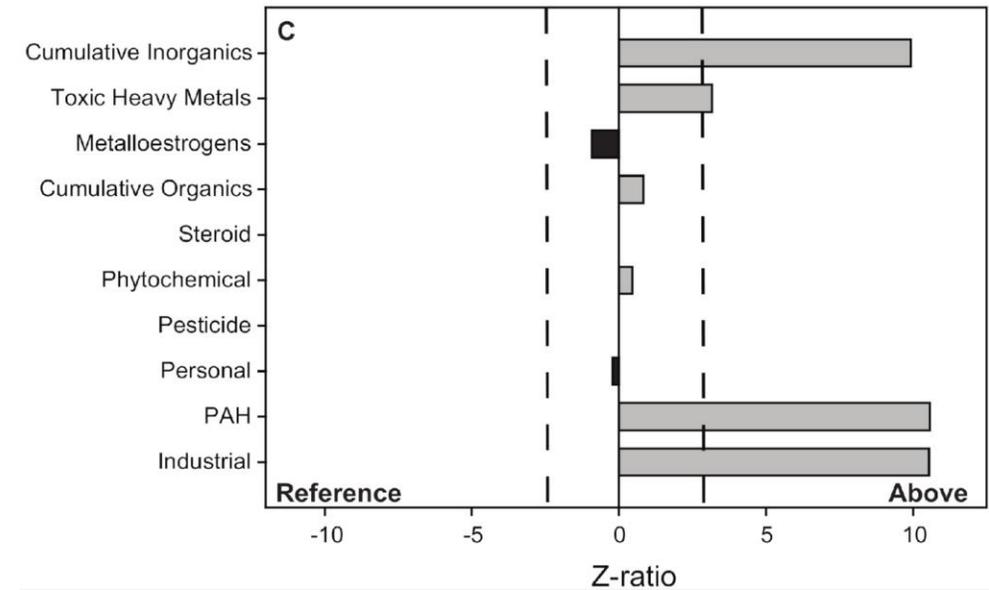
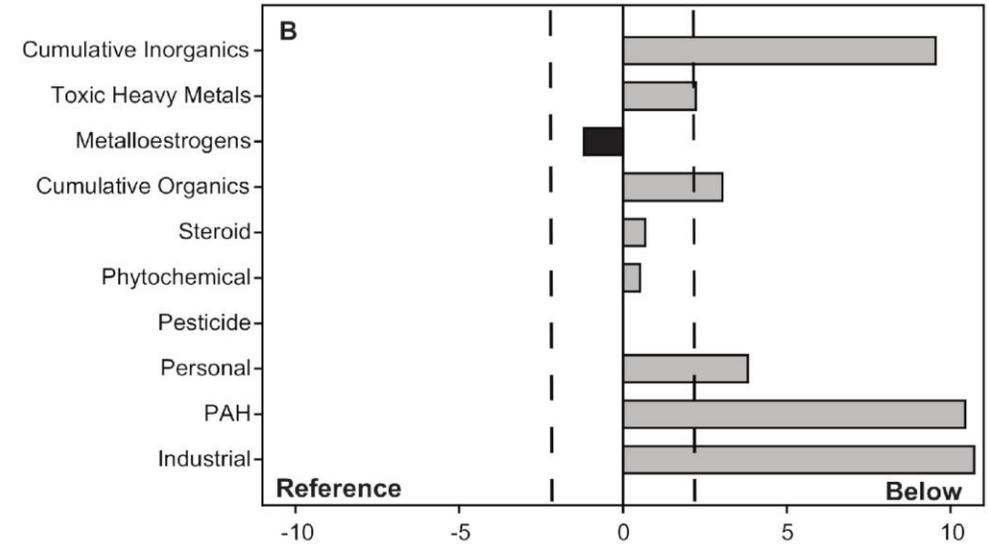
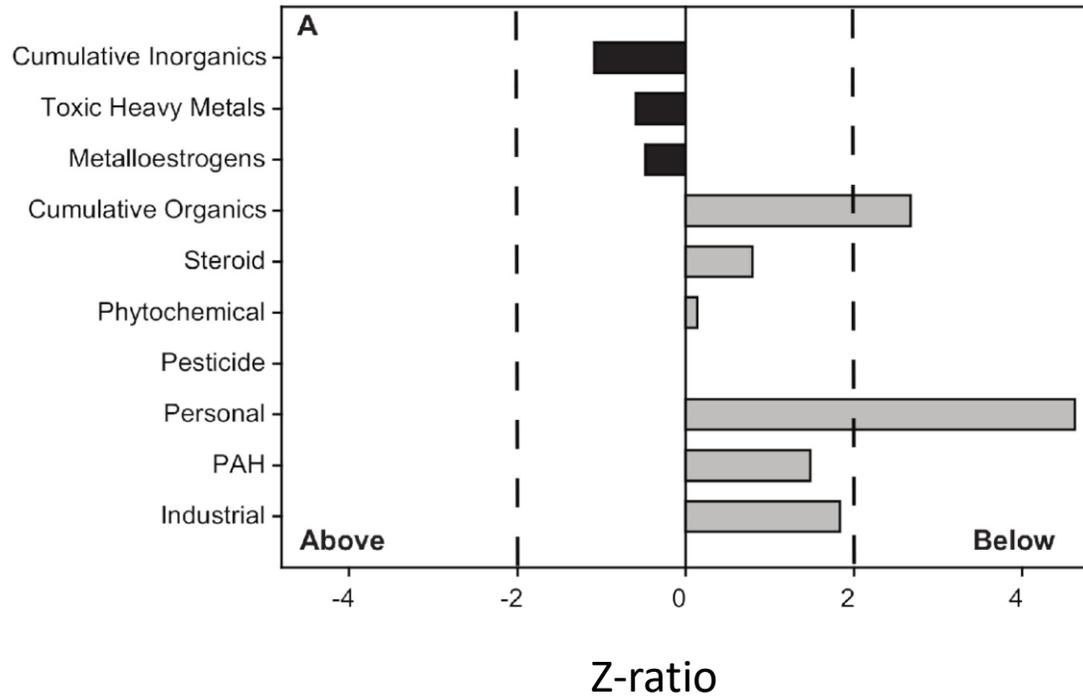


A Pinelands Preserve impoundment; Photo credit: John Bunnell, New Jersey Pinelands Commission



A Pinelands stream; Photo credit: Paul Leekan, New Jersey Pinelands Commission

On-Stream sites

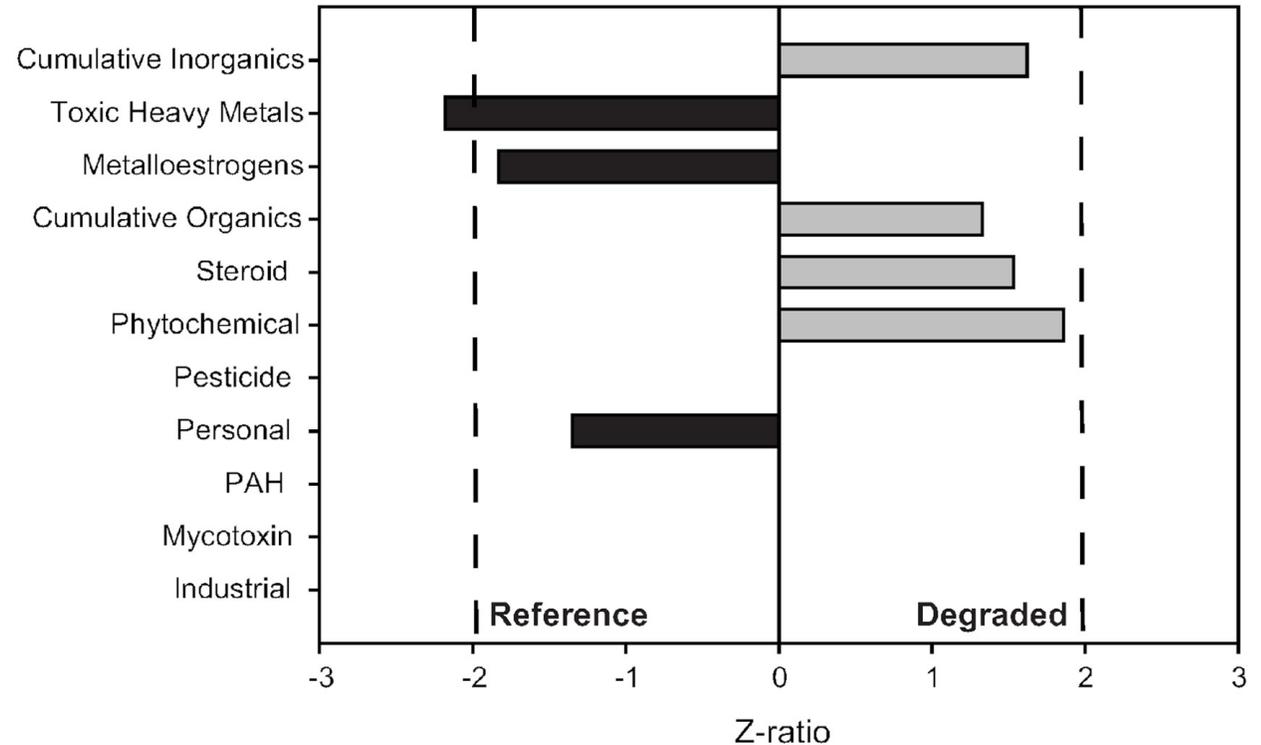


Contaminant grouping concentration (µg/L)	On-stream (streams/lakes)		
	Reference lakes (n=2)	Above WWTP (n=4)	Below WWTP (n=2)
Cumulative inorganics	490 (254–686)	2,710 (1,880–6,640)	4,310 (2,350–7,680)
Toxic heavy metals	0.626 (0.459–0.981)	1.40 (0.490–2.61)	1.42 (0.413–2.19)
Metalloestrogens	112 (48.9–140)	87.3 (58.6–270)	89.9 (30.1–245)
Cumulative organics	0.58 (0.09–1.49)	0.80 (0.26–3.19)	2.31 (0.69–6.41)
Steroids	0.20 (0–0.90)	0.25 (0–1.8)	0.50 (0.0005–0.80)
Phytochemicals	0.255 (0–0.481)	0.310 (0–1.92)	0.377 (0–0.764)
Pesticides	0 (0–0.01)	0 (0–0.68)	0 (0–0.64)
Personal care products	0.10 (0.02–0.80)	0.09 (0–0.38)	0.59 (0.17–2.96)
PAHs	0 (0–0.09)	0.03 (0–0.55)	0.11 (0.02–0.34)
Mycotoxins	ND	ND	0 (0–0.048)
Industrial chemicals	0 (0–0.2)	0.05 (0–0.54)	0.21 (0.06–2.19)

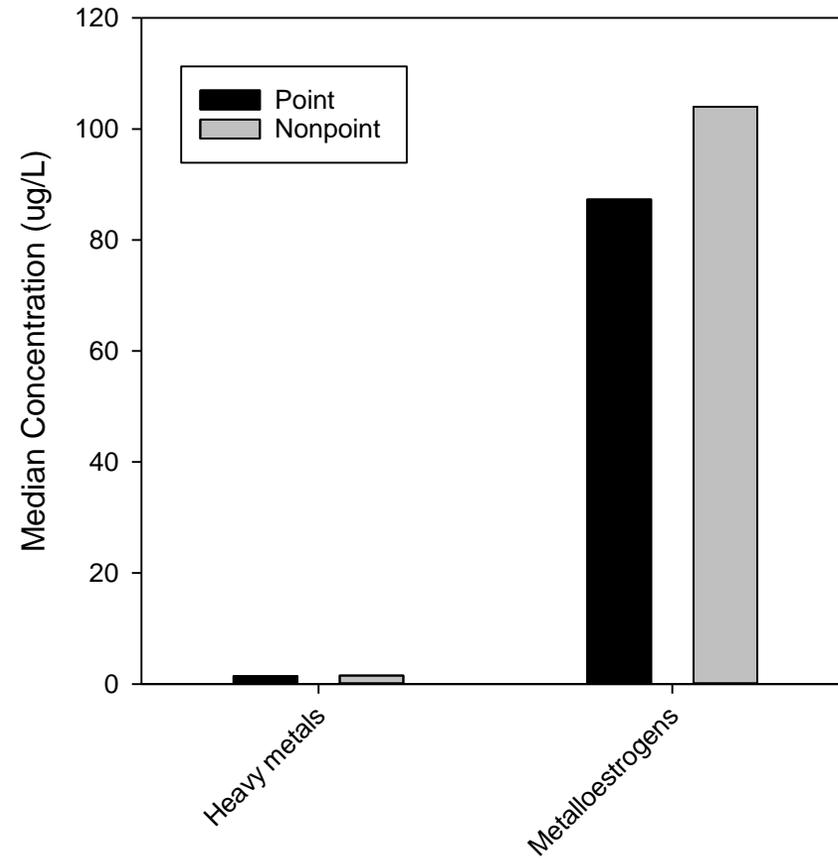
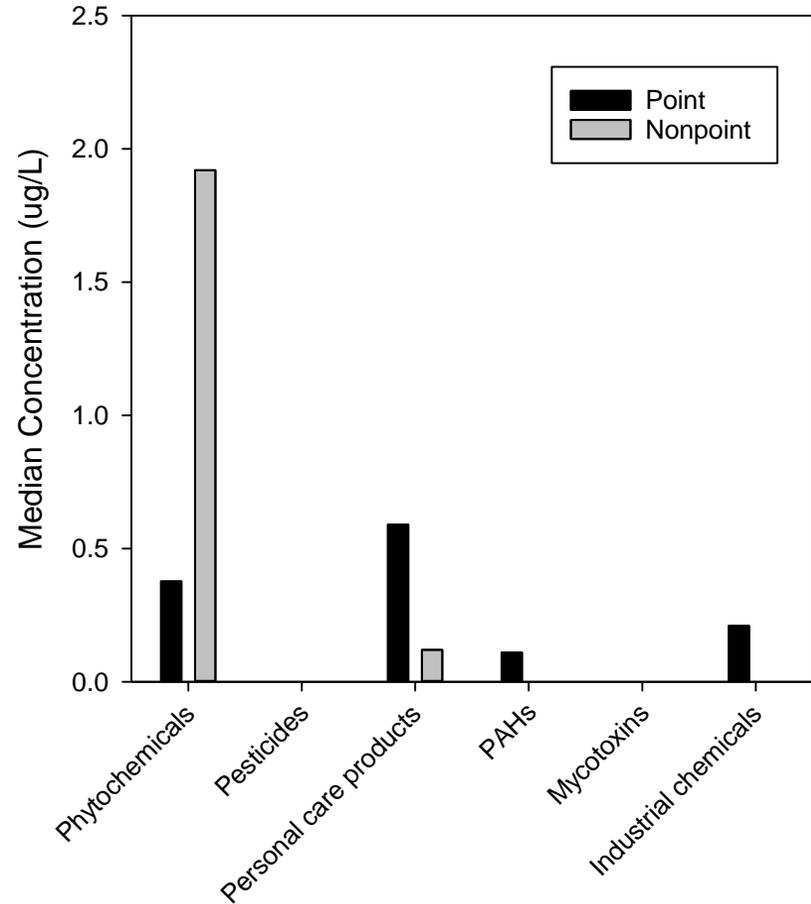


Contaminant grouping concentration (µg/L)	Reference (n=3)	Degraded (n=10)
Cumulative inorganics	654 (297–1,510)	966 (364–4,250)
Toxic heavy metals	2.37 (0.818–8.39)	1.54 (0.248–5.48)
Metalloestrogens	197 (70.5–814)	104 (21.0–538)
Cumulative organics	4.04 (0.53–8.82)	4.80 (0.880–156)
Steroids	0.865 (0–4.20)	2.00 (0–15.0)
Phytochemicals	0.930 (0–3.86)	1.92 (0–122)
Pesticides	0 (0–0.020)	0 (0–0.600)
Personal care products	0.170 (0.060–1.95)	0.120 (0–15.8)
PAHs	0 (0–2.17)	0 (0–27.0)
Mycotoxins	0 (0–0.002)	0 (0–0.073)
Industrial chemicals	0 (0–1.40)	0 (0–0.430)

Off-Stream Sites



Point vs Nonpoint Sources



Landuse and contaminants

- **Landscape alteration driver of several contaminant groupings in study area**
- PAH, industrial, and cumulative inorganics increase with development
- Cumulative organics, steroids, phytochemicals, and metalloestrogens increased with agricultural land-use.

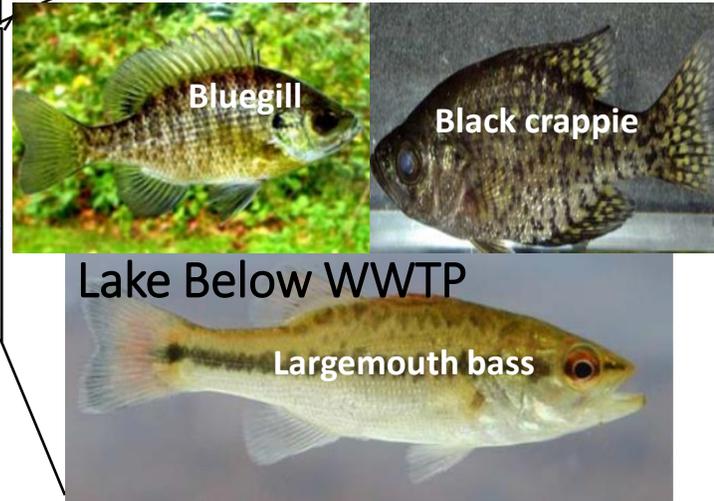
	Upland Agriculture	Development
Cumulative Organics	0.30	--
Cumulative Inorganics	--	0.79
Pesticides	--	--
Steroids	0.24	--
Industrial Chemicals	--	0.34
PAHs	--	0.39
Mycotoxins	--	--
Personal Care Products	--	--
Phytochemicals	0.29	--
Heavy Metals	--	--
Metalloestrogens	--	-0.43

Frog and Fish Sampling

Streams Above & Below WWTP



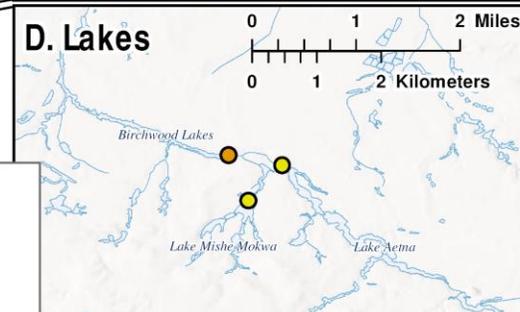
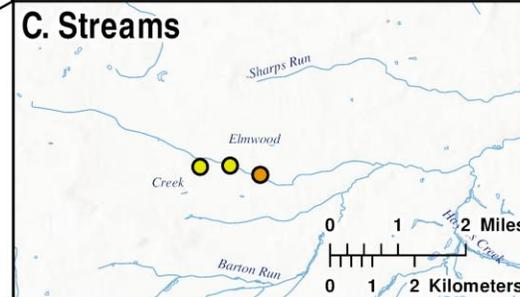
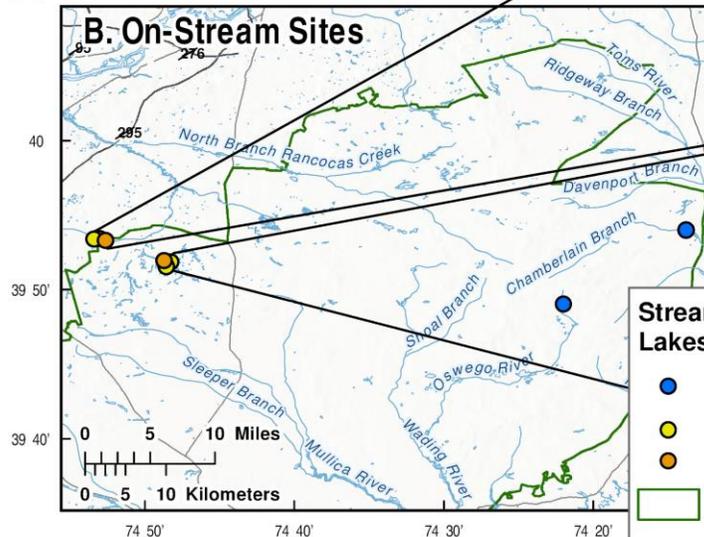
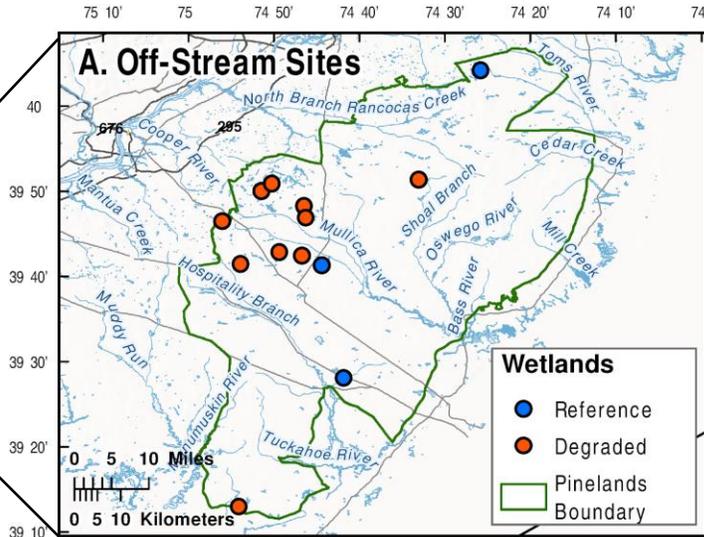
Lakes Above & Below WWTP



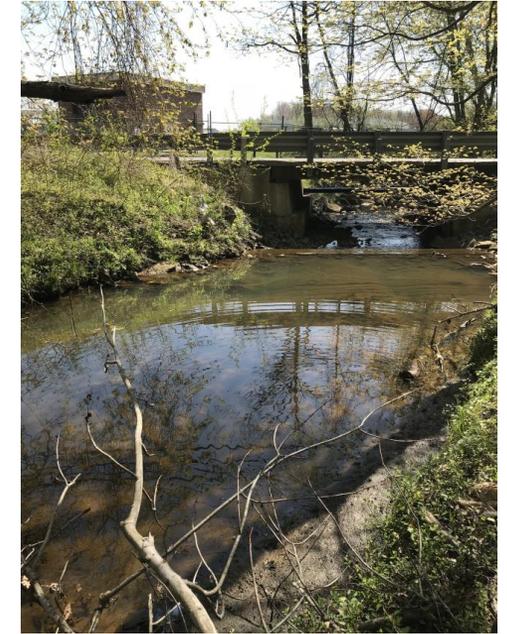
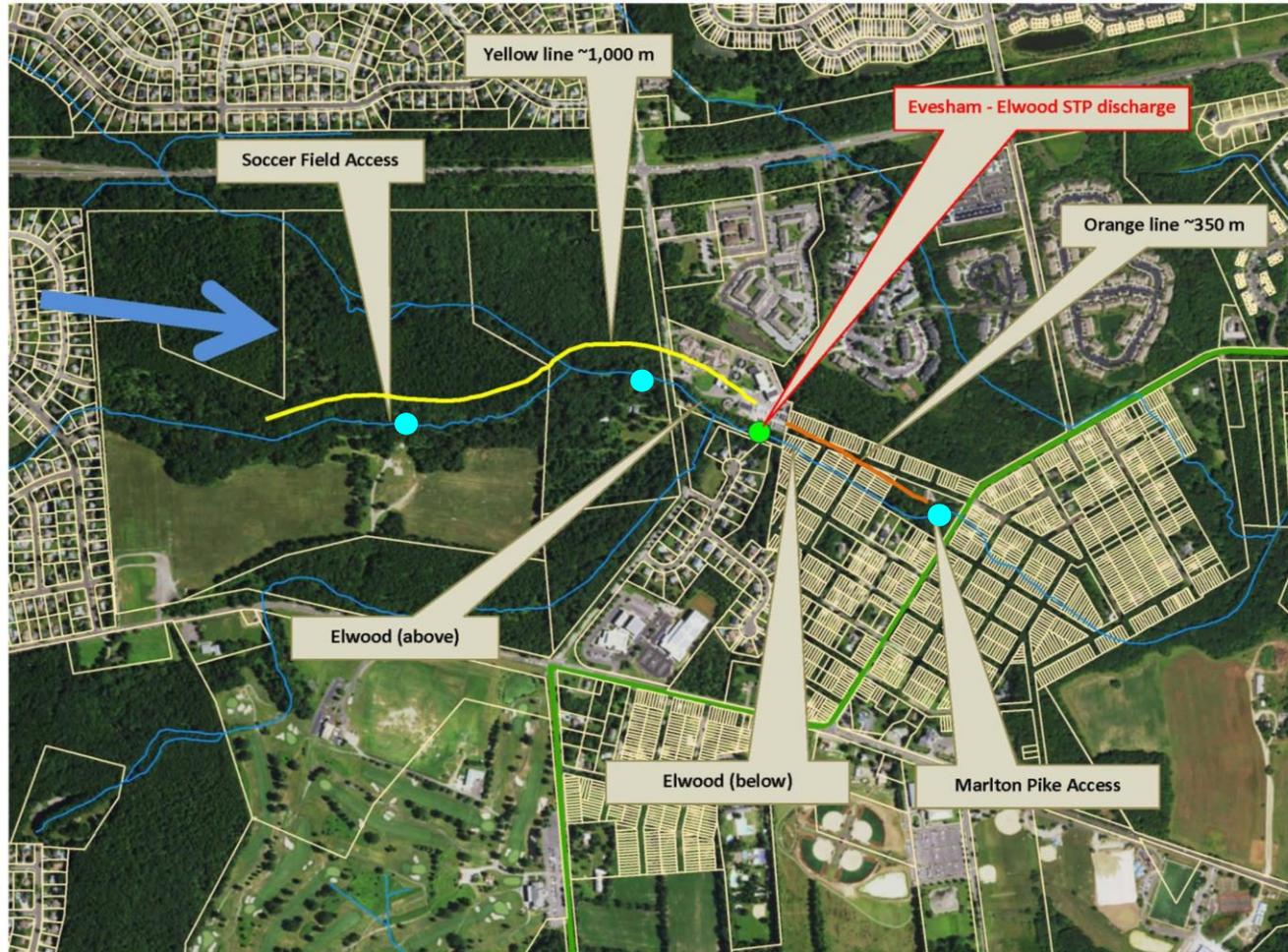
Wetlands



Reference Lakes



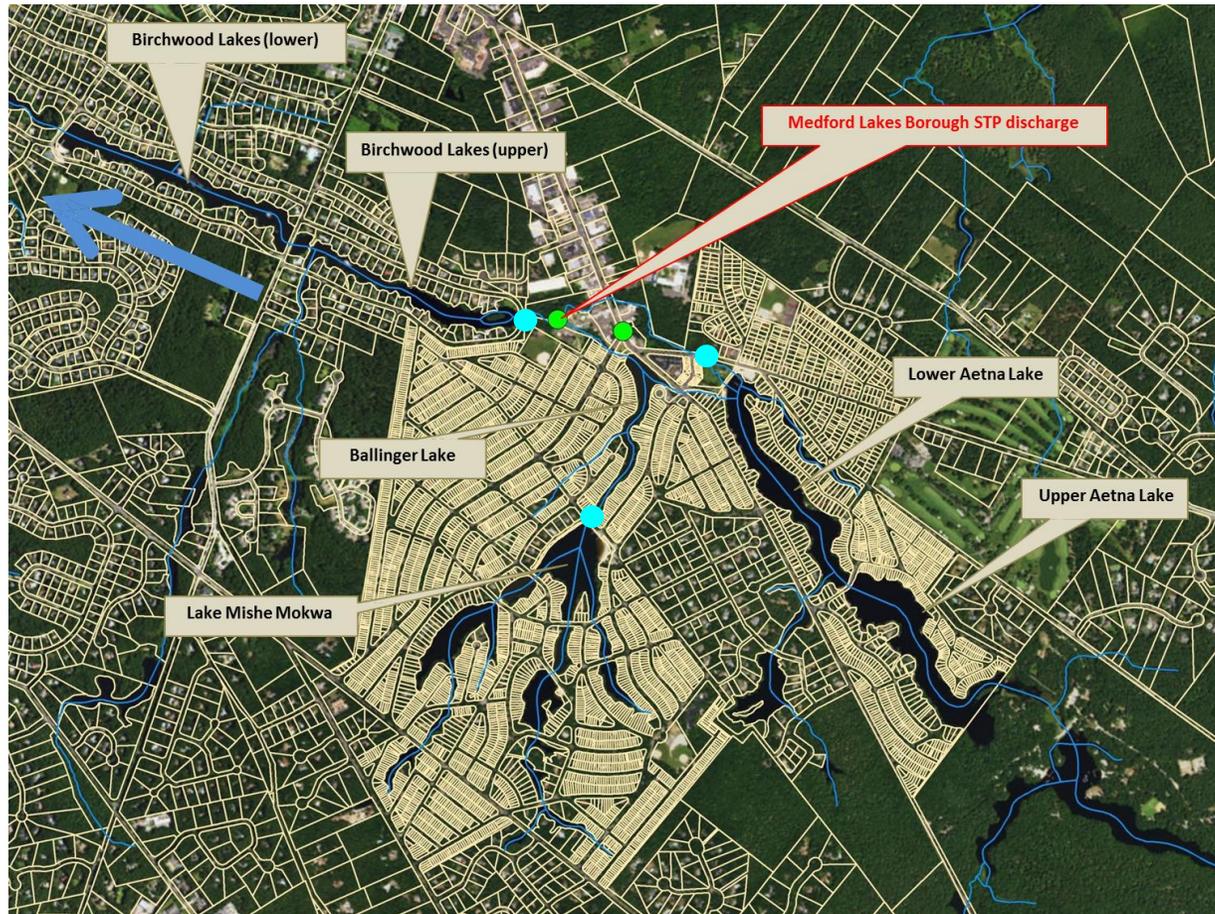
On-stream sites



Fish: tessellated darters, redbreast sunfish

Endpoints: intersex, external abnormalities, immune function (parasite loads)

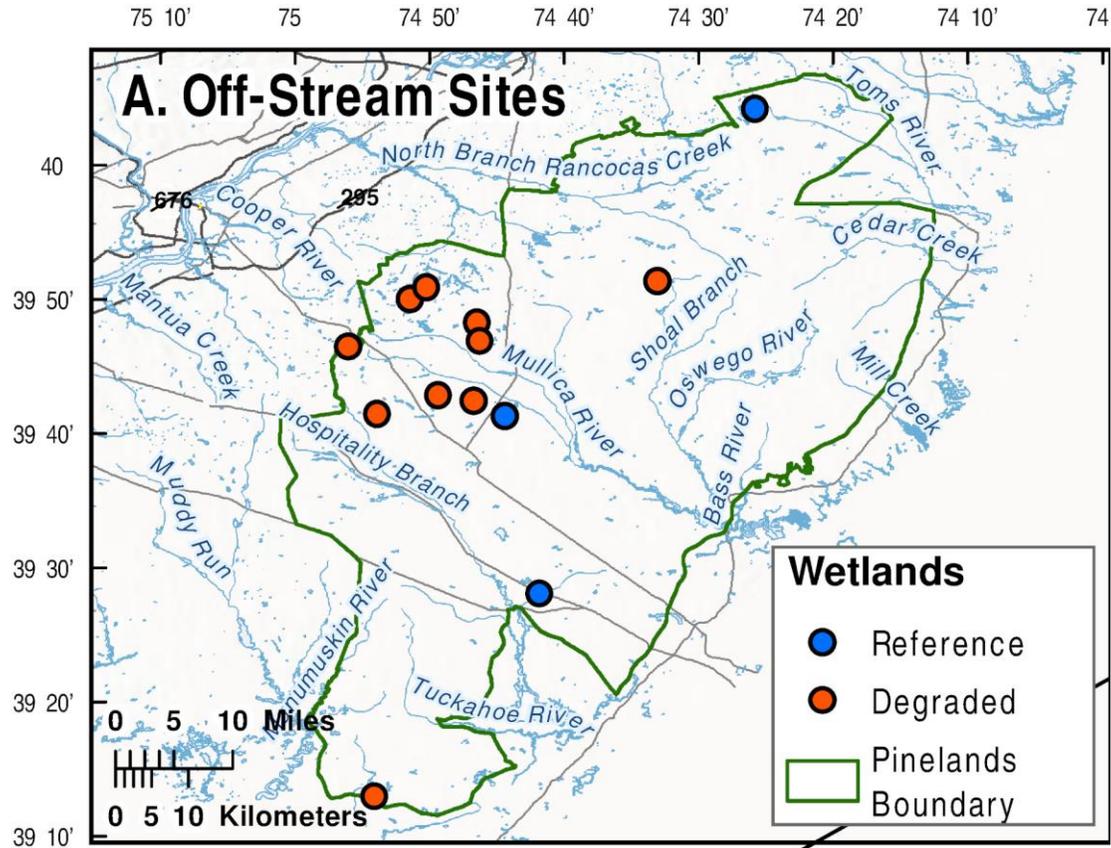
On-lake sites



Fish: largemouth bass, black crappie, bluegill

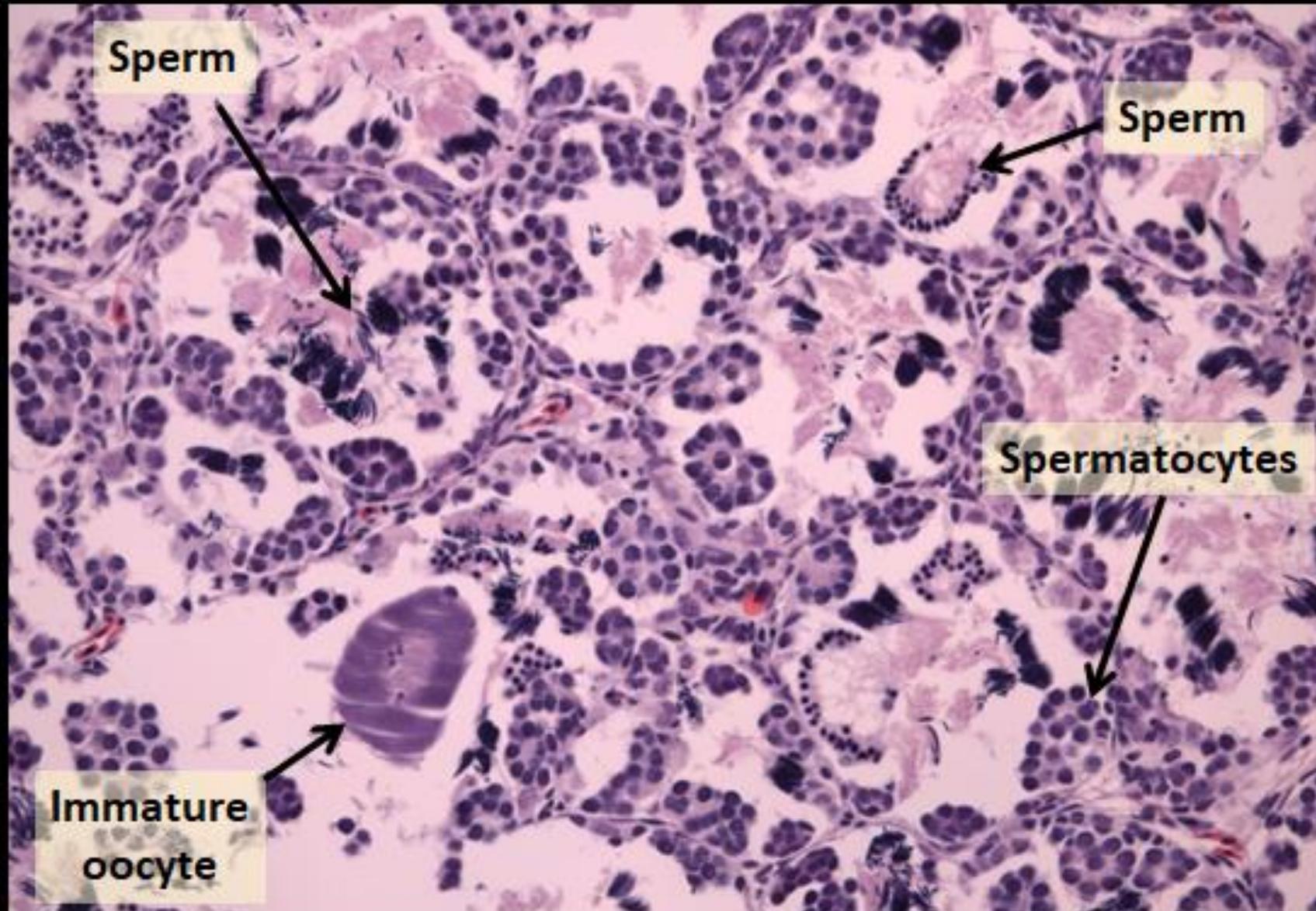
Endpoints: intersex, plasma vitellogenin (bass only), external abnormalities, immune function (parasite loads)

Off-stream sites

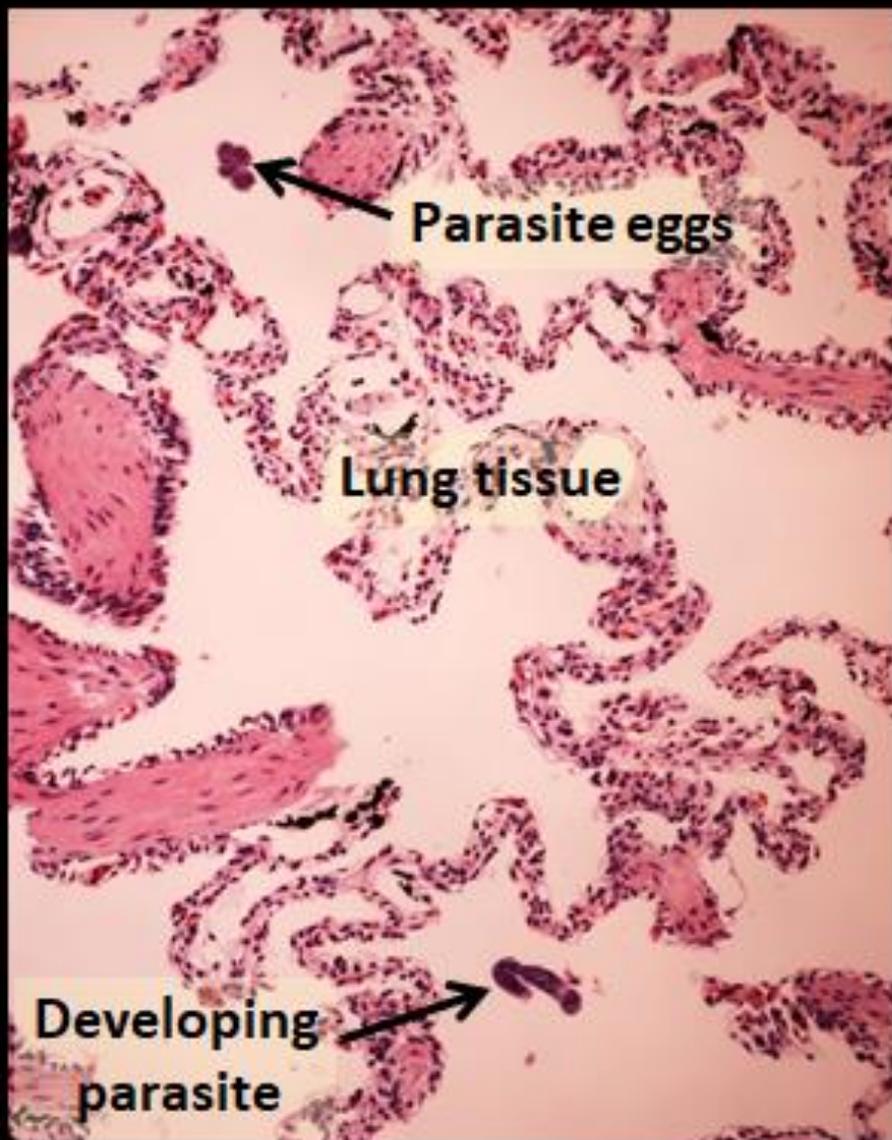


Green frog: adult and metamorphs
Endpoints: sex ratio, intersex,
immune function (parasite loads)

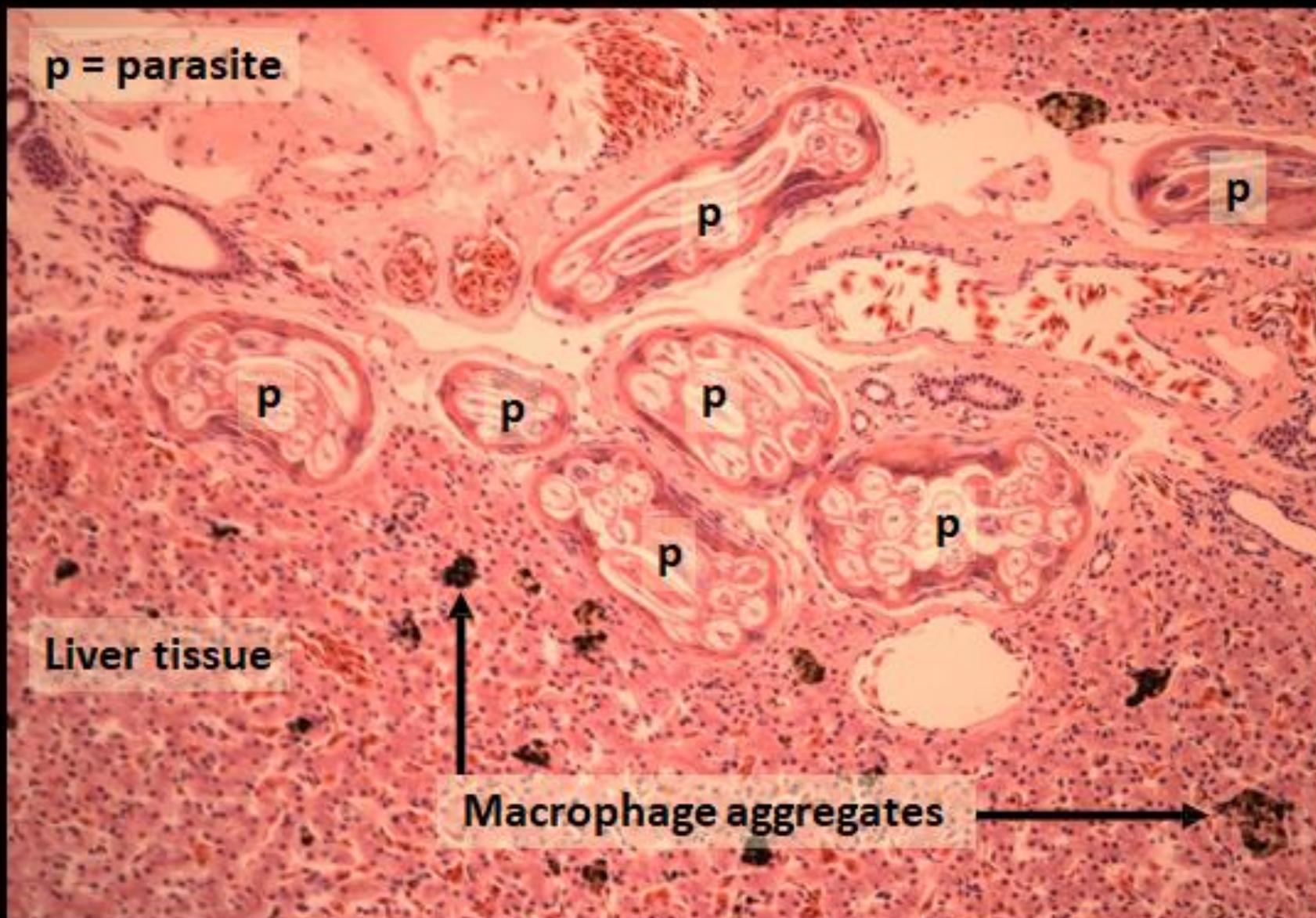
INTERSEX



PARASITES IN THE LUNGS



LIVER OBSERVATIONS



Sunfish

Native Banded Sunfish

- Fish collected from 2 reference lakes had parasites in gill, liver, muscle and kidneys
- Sex ratio = 19% male
- Intersex prevalence = 20%

Redbreast Sunfish

- Fish collected below WWTP had higher condition factor and eye parasites compared to fish above
- No other differences
- Intersex ranged from 7% above WWTP to 25% below



Banded sunfish

Photo credit: John Bunnell, New Jersey Pinelands Commission.



Redbreast sunfish

Tessellated Darter

- Longer and heavier above compared to below
- No other differences
- Intersex = 0%
- Parasite occurrence and diversity was low compared to other fish species
- May not be as sensitive to infection despite changes in water quality



Photo credit: John Bunnell, New Jersey Pinelands Commission.

Bluegill

Introduced Species: Bluegill (*Lepomis macrochirus*)



- introduced gamefish
- circular nest
- male guards nest
- up to 10 inches long



- Heavier and longer above compare to below WWTP outfall
- No other differences observed
- Intersex:
 - Above outfall = 7%
 - Below outfall = 0%

Photo credit: John Bunnell, New Jersey Pinelands Commission.

Black Crappie

Introduced Species: Black Crappie (*Pomoxis nigromaculatus*)

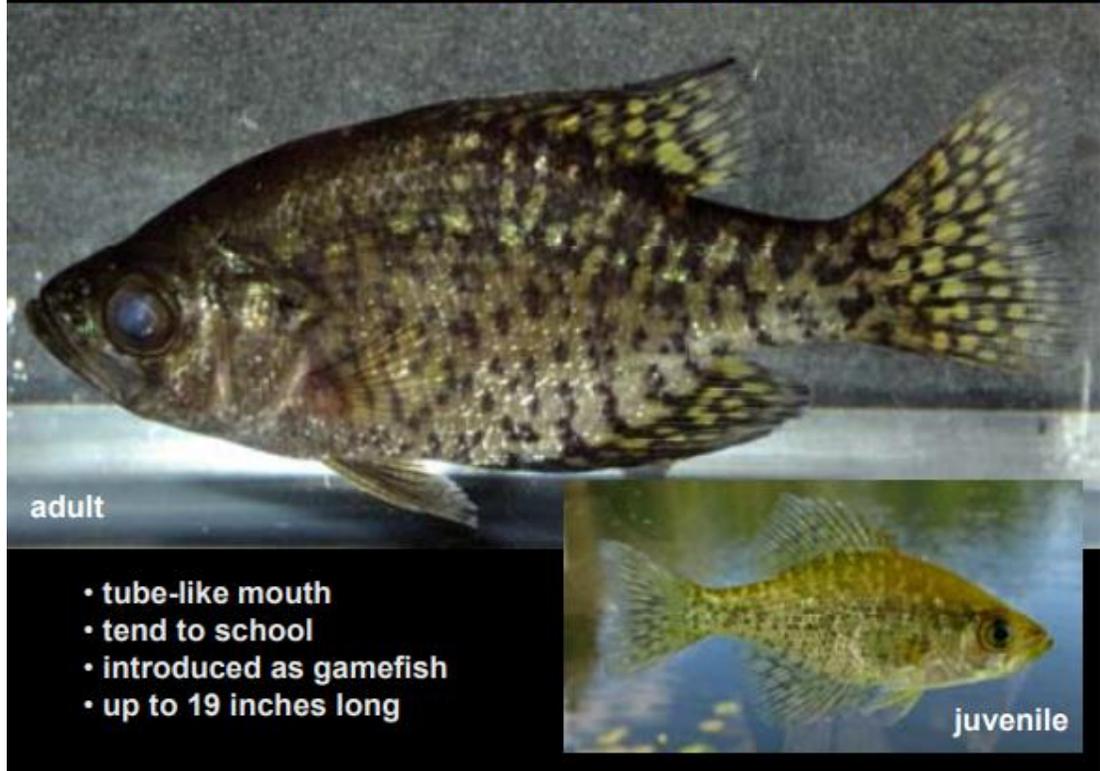


Photo credit: John Bunnell, New Jersey Pinelands Commission.

- Heavier and longer above compared to below outfall
- Higher prevalence of liver and spleen parasites above compared to below outfall
- No differences in sex ratio or intersex
- Intersex
 - Above outfall = 43%
 - Below outfall = 50%

Largemouth Bass

- Only collected below the outfall
- Parasites observed in liver, kidneys, spleen, and gills
- Several had external parasites or white spots
- Sex ratio = 31% males
- Intersex = 80%



Photo credit: John Bunnell, New Jersey Pinelands Commission.

Centrarchids (Sunfish)

	Heavy Metals	Phytochemicals	Pesticides	Personal care products	Industrial compounds	Total Organics	PAHs	Total Inorganics	Steroids	Mycotoxins	Metalloestrogens
Percent male	0.32	0.09	-0.07	-0.04	0.25	0.00	0.08	0.51	-0.02	0.20	-0.15
Intersex	0.03	0.08	0.20	0.44	0.17	0.54	0.11	-0.04	0.55	0.10	-0.18
Eye parasites	-0.48	-0.29	0.21	0.14	0.68	0.27	0.41	0.68	0.14	0.74	-0.55
Gill parasites	-0.24	0.00	0.62	0.05	0.65	-0.15	0.59	0.38	-0.42	0.10	0.25
Liver parasites	0.77	0.48	0.17	0.26	0.18	0.22	0.07	0.24	0.14	-0.21	0.14
Spleen parasites	0.47	0.32	0.19	0.50	0.34	0.36	0.25	0.23	0.27	0.10	0.11
Anterior kidney parasites	0.47	0.20	0.16	0.10	0.19	-0.02	0.08	0.21	-0.07	-0.10	0.20
Posterior kidney parasites	0.45	0.16	0.16	0.20	0.24	0.06	0.09	0.23	-0.01	0.00	0.16
Gut parasites	-0.82	-0.60	-0.07	-0.47	0.04	-0.22	-0.05	0.13	-0.16	0.33	-0.44
Heart parasites	0.25	0.09	0.10	-0.03	0.22	-0.04	0.12	0.30	-0.06	0.00	0.04
Muscle parasites	-0.78	-0.57	-0.15	-0.53	-0.14	-0.36	-0.18	-0.03	-0.34	0.22	-0.33
External parasites	-0.06	0.32	0.30	0.06	0.26	-0.06	0.52	0.06	-0.13	-0.19	0.39
Eye exophthalmia	-0.53	-0.07	0.25	0.02	0.73	0.15	0.67	0.73	0.02	0.58	-0.36



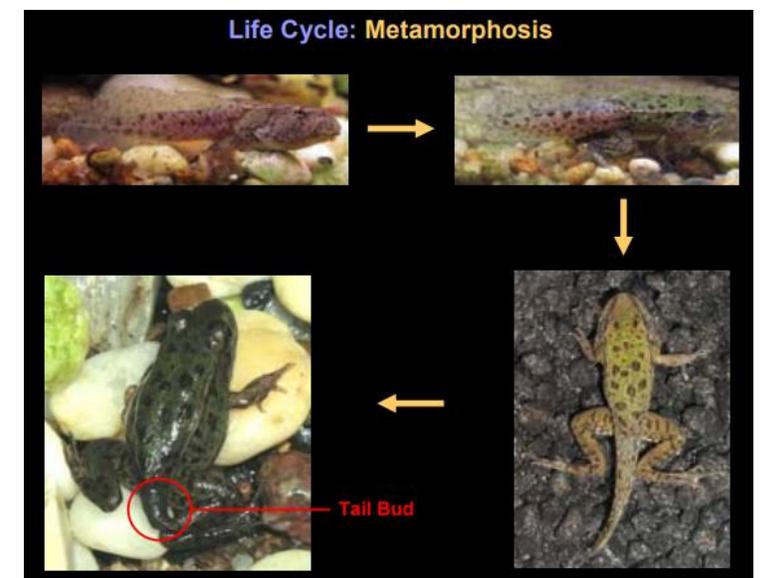
Green Frogs

Metamorphs

- No intersex
- No differences in biological metrics, sex ratio, abnormalities or parasite prevalence

Adults

- Intersex = 1%: degraded; 0% reference
- Sex ratios male dominated but no differences among site types
- No differences in biological metrics, abnormalities or parasite prevalence



Frog metamorphosis, credit: John Bunnell, New Jersey Pinelands Commission.



Green frog, photo credit: John Bunnell, New Jersey Pinelands Commission.

Summary

- Contaminant mixtures related to nonpoint sources from upland landscape alteration
- Estrogenic endocrine disruption and parasite occurrence was site and species dependent and continued monitoring needed.
- Point and nonpoint source pollution degrade water quality and can negatively affect aquatic ecosystems.
- Small study: expand scope to understand contaminant mixtures effects on parasite prevalence/diversity in Pinelands species across gradient of landscape alteration



Acknowledgements

- Jon Cohl, Lisa Carper, Molly Schreiner and Anna Boetsma - water chemistry
- Emily Underwood and Adam Sperry - green frog necropsies
- Darlene Bowling and Pam Whittington - green frog histology slides
- Cheyenne Simpson, Adam Sperry – fish necropsies/histology

Thank you!

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Breitmeyer, S.E. et al., 2021, Organic and inorganic constituents in surface water and native and non-native fish and frog health data collected from streams, impoundments and wetlands in the New Jersey Pinelands, 2017-2020: U.S. Geological Survey data release, <https://doi.org/10.5066/P93VW8GX>

