2021 Virginia estuarine HABs: marine biotoxins update

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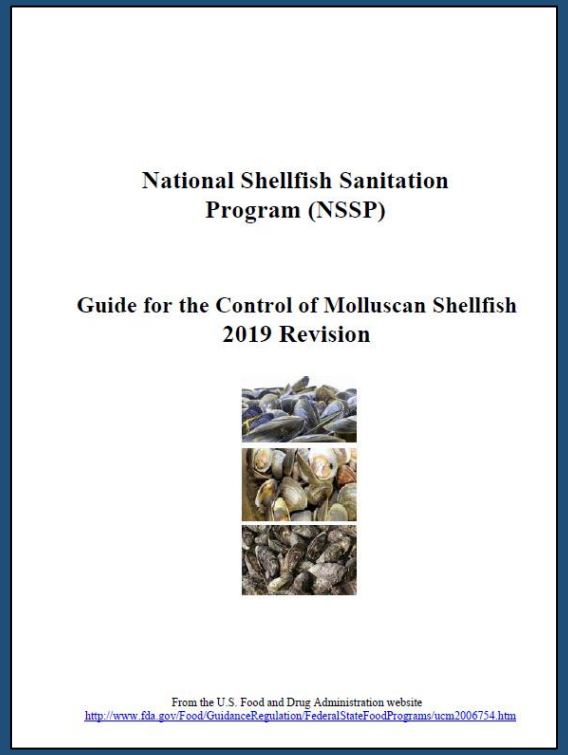
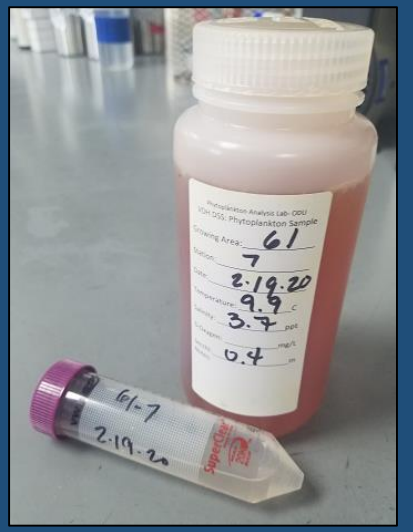
Shellfish marine biotoxin control

- Biotoxin contingency plan for:
  - Paralytic shellfish poisoning (PSP)
  - Amnesic shellfish poisoning (ASP)
  - Neurotoxic shellfish poisoning (NSP)
  - Diarrhetic shellfish poisoning (DSP)
  - Azaspiracid shellfish poisoning (AZP)
- VDH:DSS Biotoxin plan and flow chart: http://www.vdh.virginia.gov/content/uploads/sites/20/2016/05/BiotoxinControlPlan.pdf
- Monthly collections- routine fixed sites
  - Lugol's solution (250mL) – phytoplankton analyses (ODU)
    - Screened at VDH field offices
  - Unpreserved frozen sample (50mL)- targeted ELISA screening (VDH)
  - Unpreserved frozen filter sample (100mL, 3µm)- qPCR (VIMS) (all samples/stations)
- Bloom samples
  - Response to bloom reports or visual observation by field staff


National Shellfish Sanitation Program (NSSP)

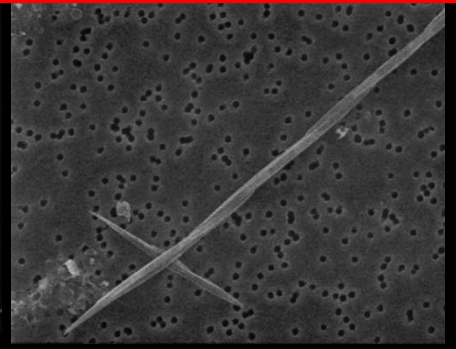
Guide for the Control of Molluscan Shellfish 2019 Revision

From the U.S. Food and Drug Administration website
http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm



Phyto Kit: Extra bottles, vials, lugol's, rubber gloves, marker

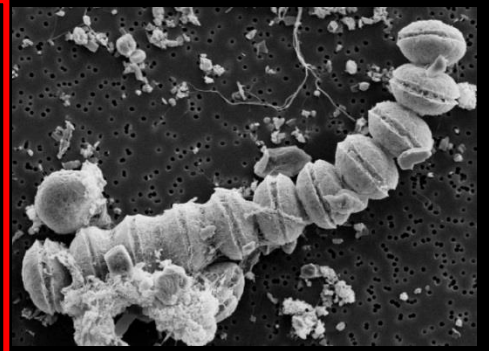
Dinophysis spp.



Pseudo-nitzschia spp.

Salas et al. 2011

Azadinium & Amphidoma spp.



Alexandrium monilatum

Margalefidinium polykrikoides

Algal species	Impacts	Main Toxin	NSSP shellfish growing area closure level (toxin w/in meat)	working regional bloom density (cell density in water column)
Alexandrium tamarense species complex	Paralytic Shellfish Poisoning	Saxitoxin	80µg /100g	presence
Karenia brevis	Neurotoxic Shellfish Poisoning	Brevetoxin	0.8mg /kg	presence
Dinophysis spp.	Diarrhetic Shellfish Poisoning	Okadaic acid	0.16 mg/kg	≥ 5 cells/ml
Pseudo-nitzschia spp.	Amnesic Shellfish Poisoning	Domoic acid	2mg/100g	*≥ 1,000 cells/ml
Azadinium & Amphidoma spp	Azaspiracid Shellfish Poisoning	Azaspiracid-a	0.16 mg/kg	TBD
Alexandrium monilatum	Fish/invertebrate mortality	Goniodomin A	NA	≥ 1,000 cells/ml
Margalefidnium polykrikoides	Fish/invertebrate mortality	icthyotoxin	NA	≥ 1,000 cells/ml
Karlodinium veneficum	Fish mortality	Karlotoxins	NA	≥ 10,000 cells/ml

2021 Updates

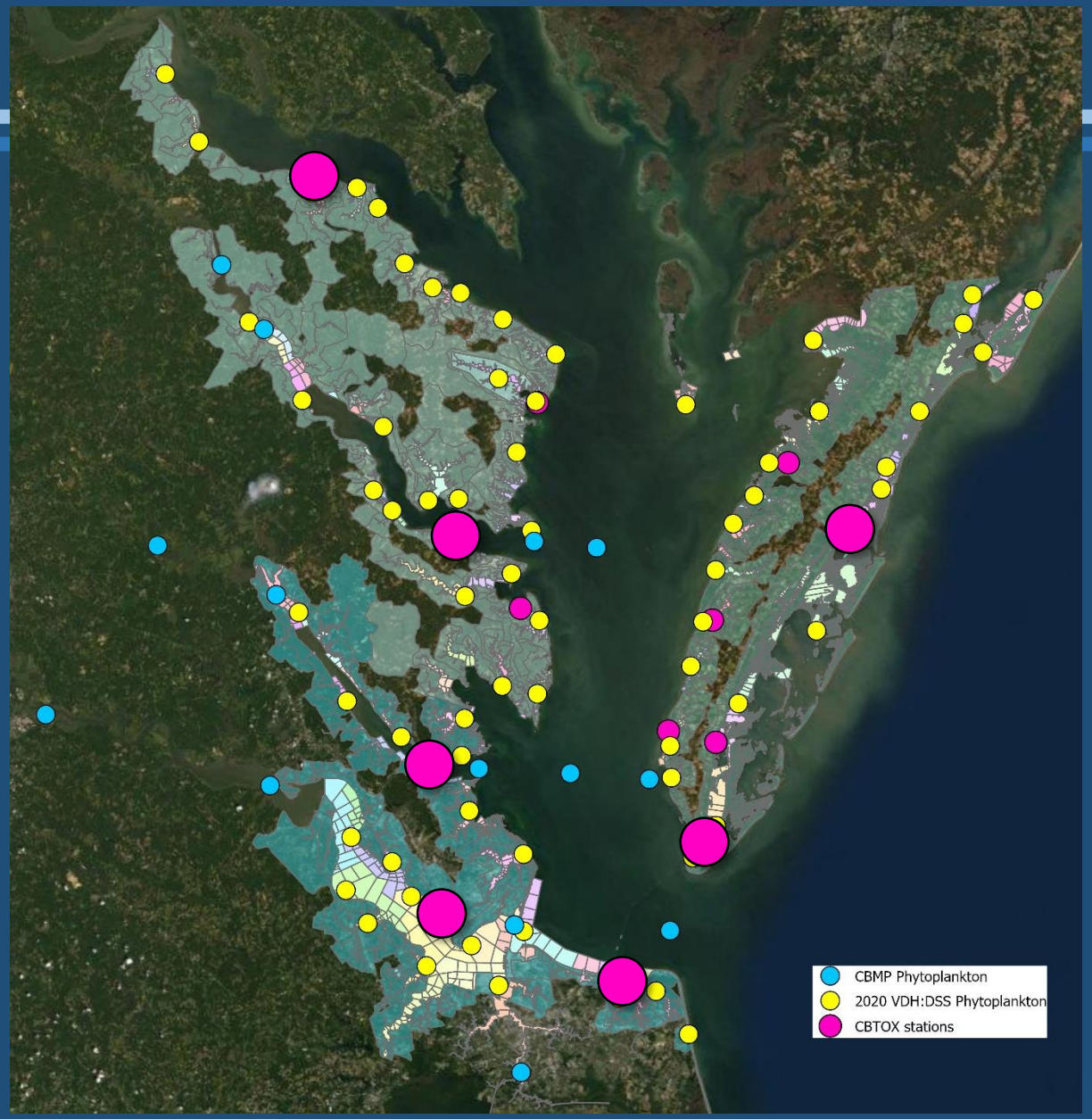
- Year-round sampling
- qPCR analyses of all DSSWH collections for marine Biotoxin producers DSP, ASP, AZP

*Differentiating thick and thin *Pseudo-nitzschia* +/- 5µm width

3µm Isopore filters
5mL Ependorf tubes

Cellvis P12-1.5H-N

Virginia Estuarine Phytoplankton monitoring

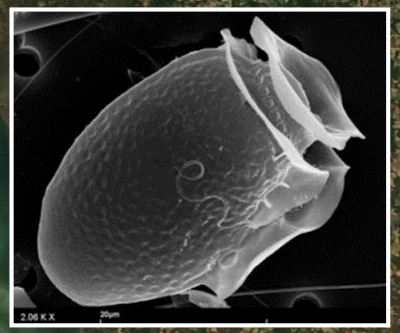


- Chesapeake Bay Monitoring Program (DEQ/ODU)
  - 14 stations
    - 7-Chesapeake Bay monthly year-round
    - 7-Tidal tributaries monthly March-October
  - Full species composition
- VDH: Shellfish (DSSWH/ ODU/VIMS)
  - 69 stations
  - Monthly year-round
  - Targeted HAB identification
  - Targeted toxin screening (based on cell counts)
  - Targeted qPCR analyses-all samples
- CBTOX (VDH:DSS/ VIMS)
  - 12 stations (2017-2018)
  - 4 stations (2019-2020)
  - 7 stations (2021-2022)
  - Bi-weekly monthly sampling
  - Full species composition
  - Routine toxin analyses

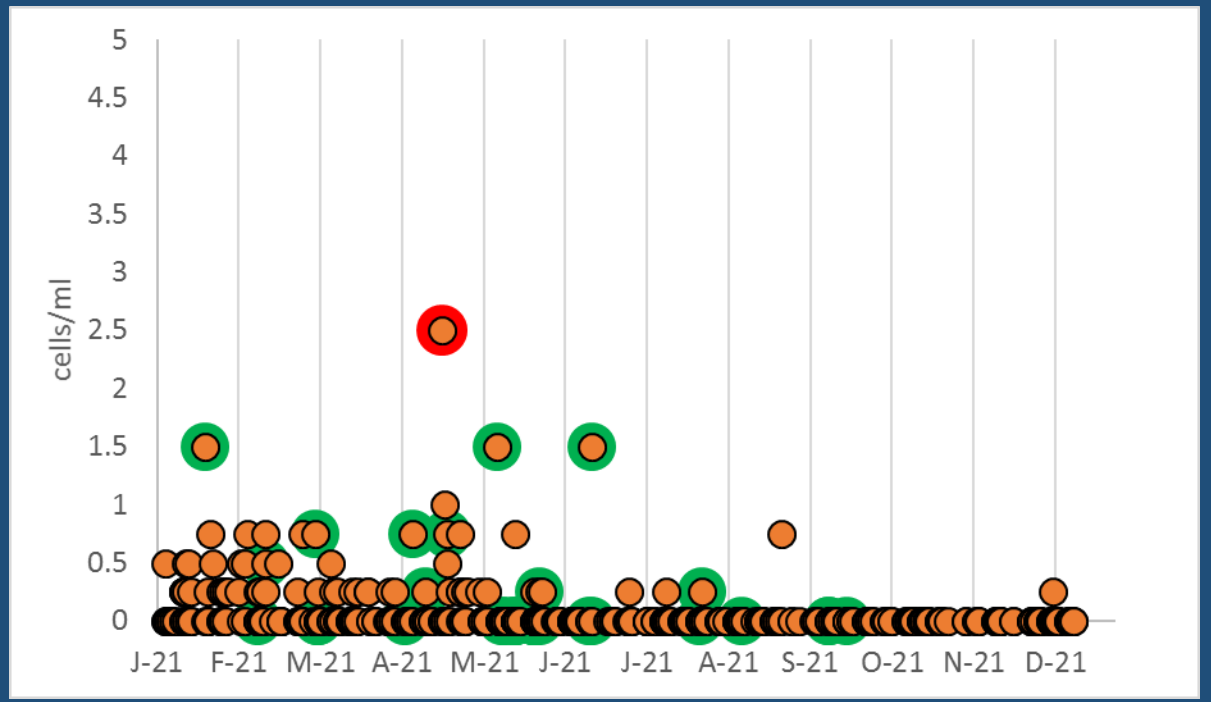
Additional monitoring: ODU and HRSD James River & research (Mulholland et al), VIMS (Reece, Smith, et al.)

DSP- *Dinophysis*

Dinophysis spp.

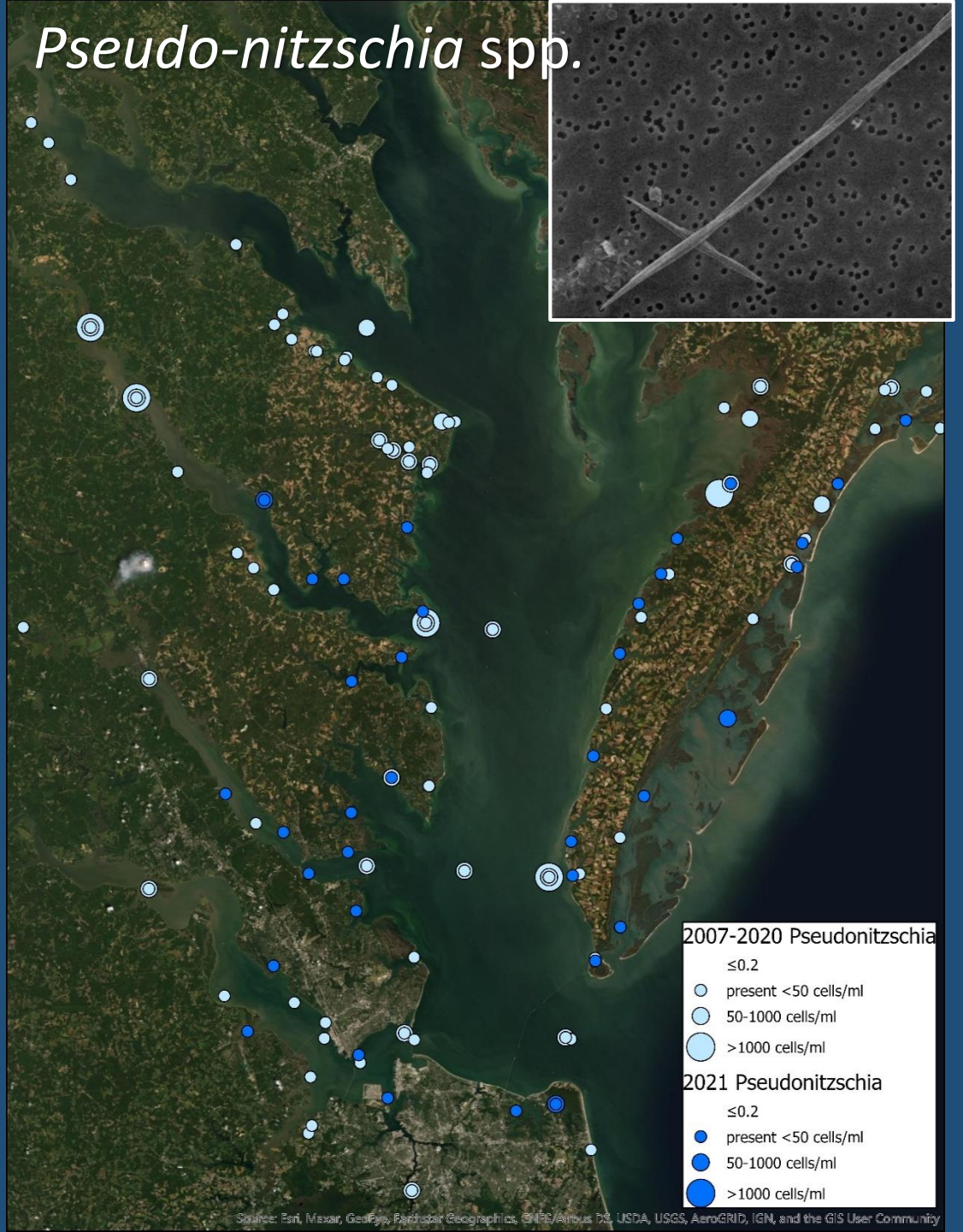


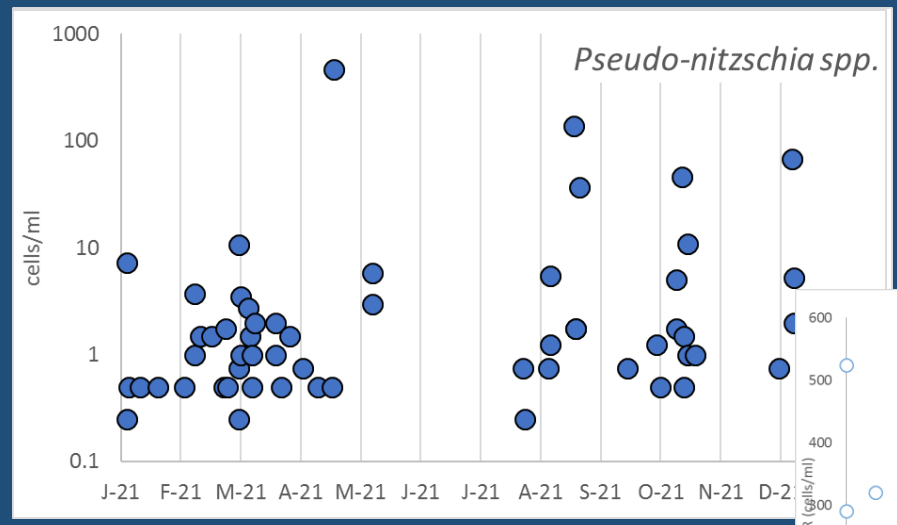
- Widespread distribution in Chesapeake Bay and seaside E. Shore
  - Generally low cell densities
  - Present in ~ 14% of 2021 samples (<0.25 cells/ml)
  - 0.25-2.5 cells/ml (1% >1/ml)
- Okadaic Acid ELISA on 40 seawater samples & 4 SPATTs
- **<u>1/44 sample above detection limit (<0.1ppb)</u>**
  - 0.13ppb: 2.5 cells/ml
  - Widespread OA/DTXs reported using SPATTs- 2017-2018 (CBTOX- Onofrio et al. 2021)

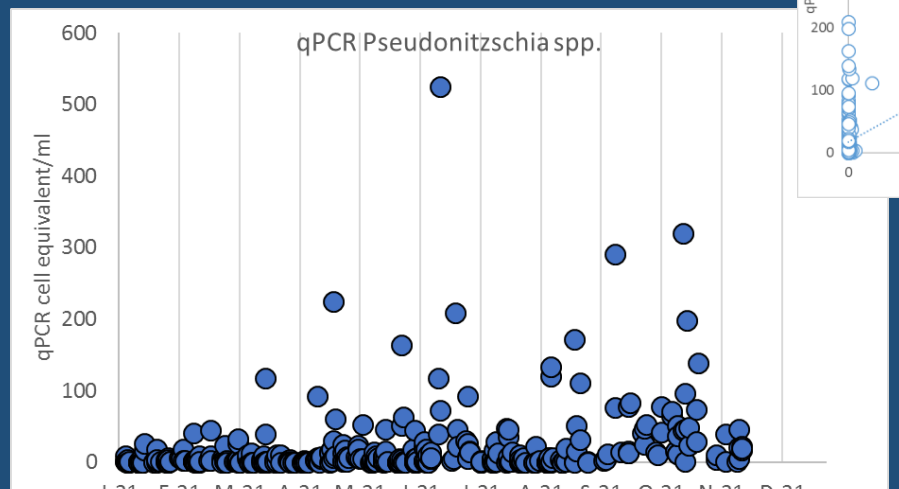


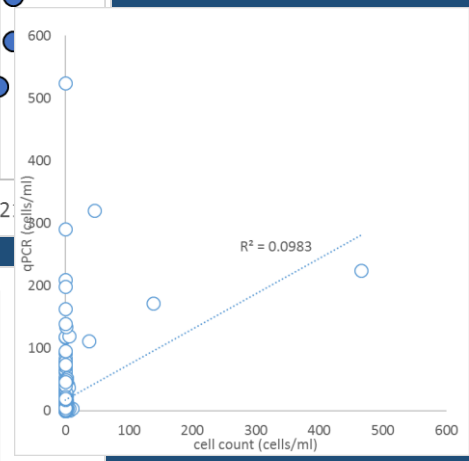
ASP- *Pseudo-nitzschia*

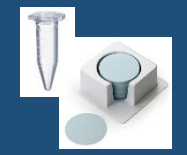
- Widespread distribution in Chesapeake Bay and Seaside E. Shore
  - Present in ~ 12% of 2020 samples: 0.5-466 cells/ml (<1% >50/ml)
- Domoic Acid ELISA on seawater samples and SPATTs
  - DA detected in 6 2020 and 1 concentrated 2018 samples and in CBTOX SPATTs 2017-2018 (Onofrio et al. 2021)

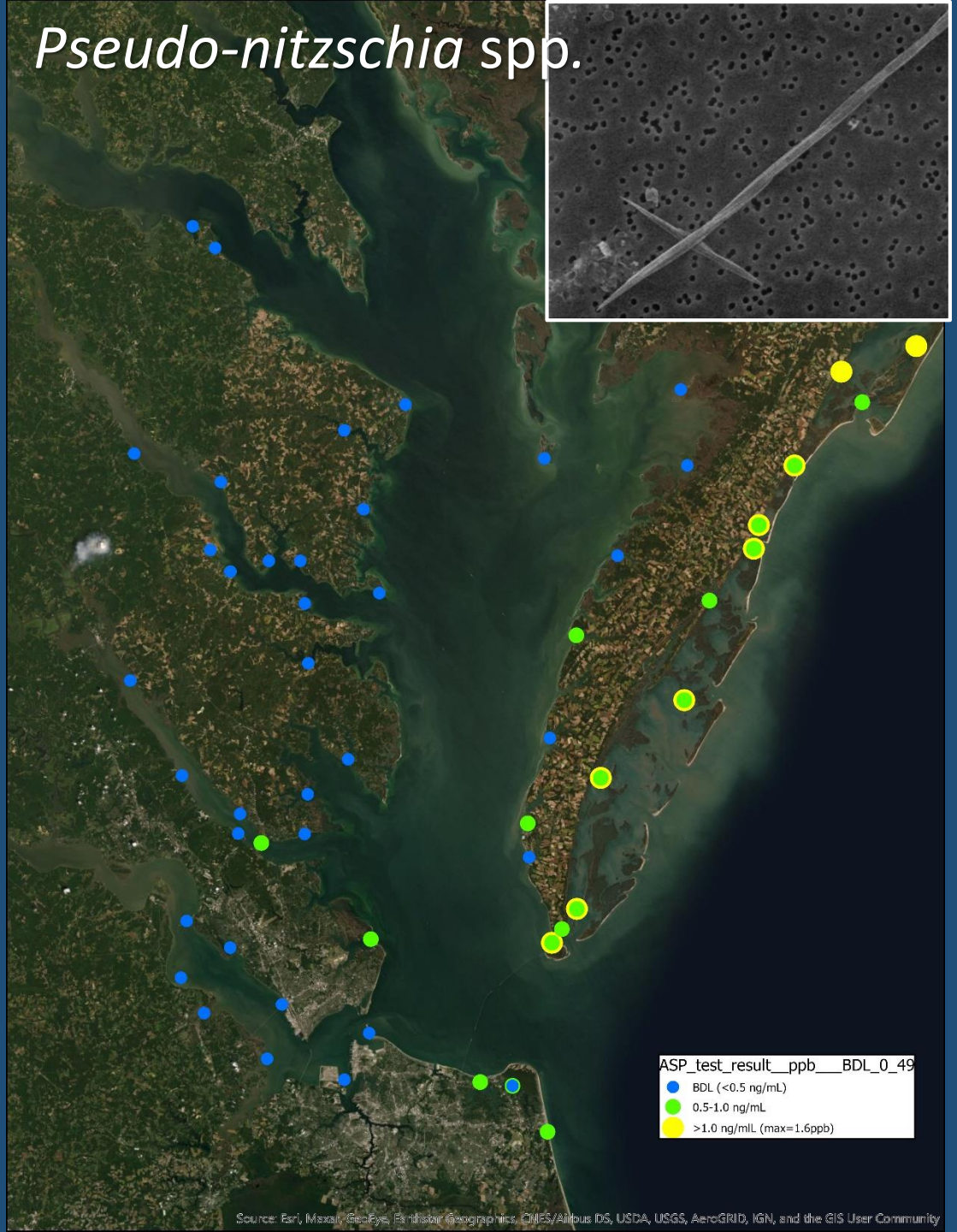












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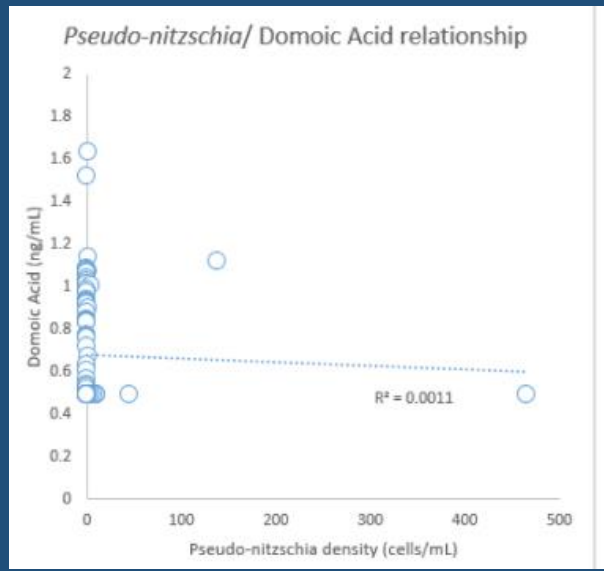
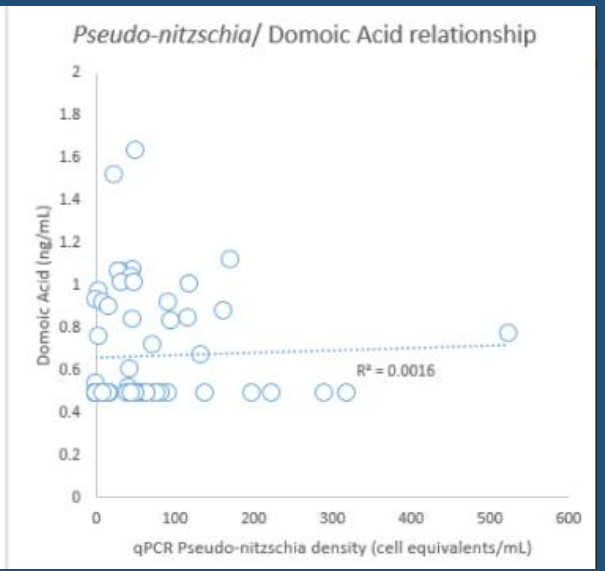
- Widespread distribution in Chesapeake Bay and Seaside E. Shore
  - Present in ~ 12% of 2020 samples: 0.5-466 cells/ml (<1% >50/ml)
- Domoic Acid ELISA on seawater samples and SPATTs
  - DA detected in 6 2020 and 1 concentrated 2018 samples and in CBTOX SPATTs 2017-2018 (Onofrio et al. 2021)
  - 40/91 samples tested 2021-2022 above detection limit (0.5ppb)
  - 0.53-1.63 ppb Domoic Acid in seawater
    - Primarily Seaside ES, throughout year
    - Similar max in seawater as measured in CBTOX study (Pease/Smith et al)
      - CBTOX shellfish DA max 0.579ppm (NSSP=20ppm)

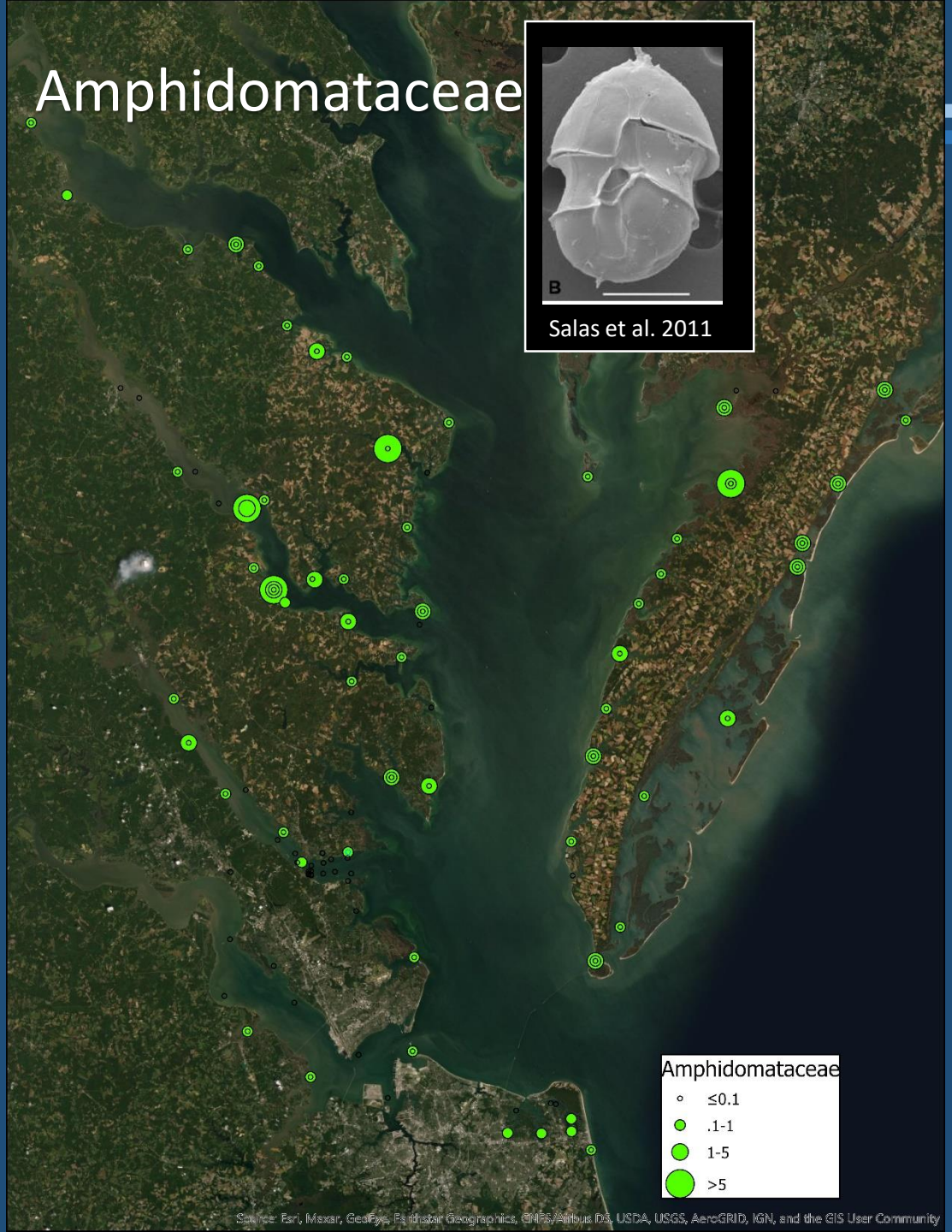
Apparent disconnect between cell density and toxin concentration

- Conflict both with cell counts & qPCR

max toxin: 1.63 ppb (1.75 cells/ml & 51.77 cell equivalents/ml)
max cells: 466 cells/ml (BDL DA), 524 qPCR (0.77 DA)



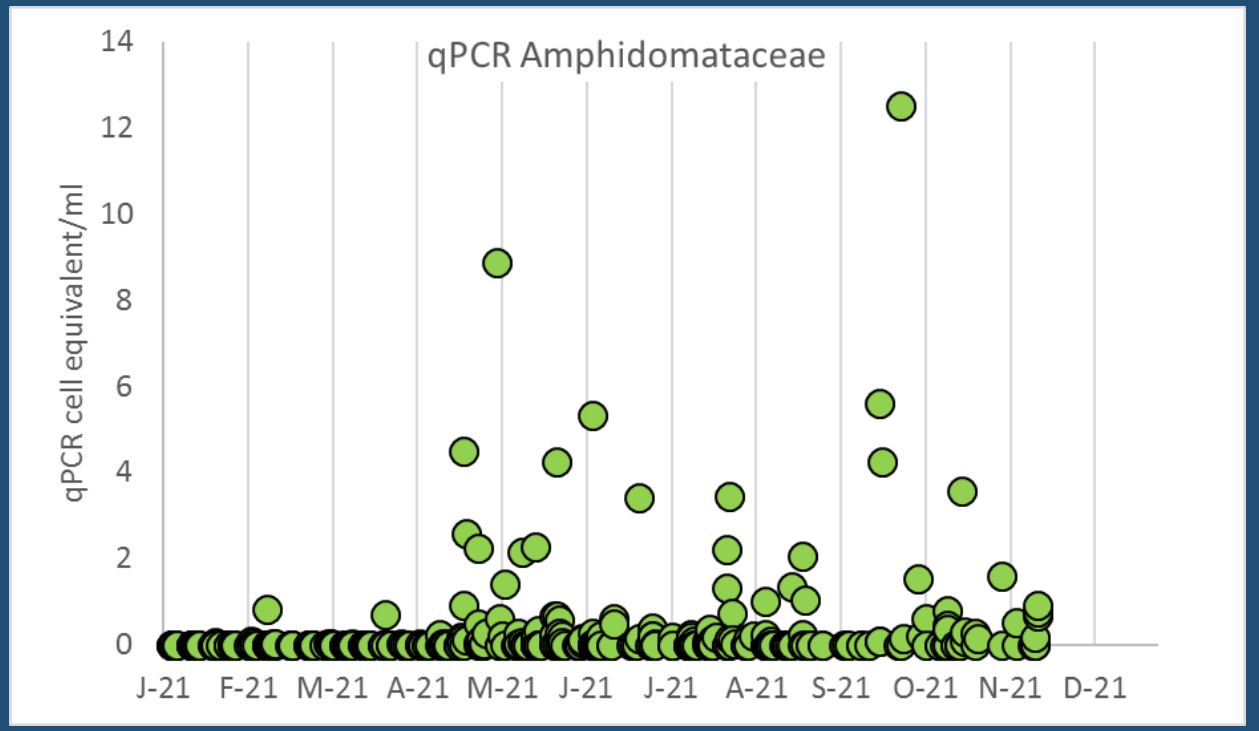




AZP- Amphidomataceae

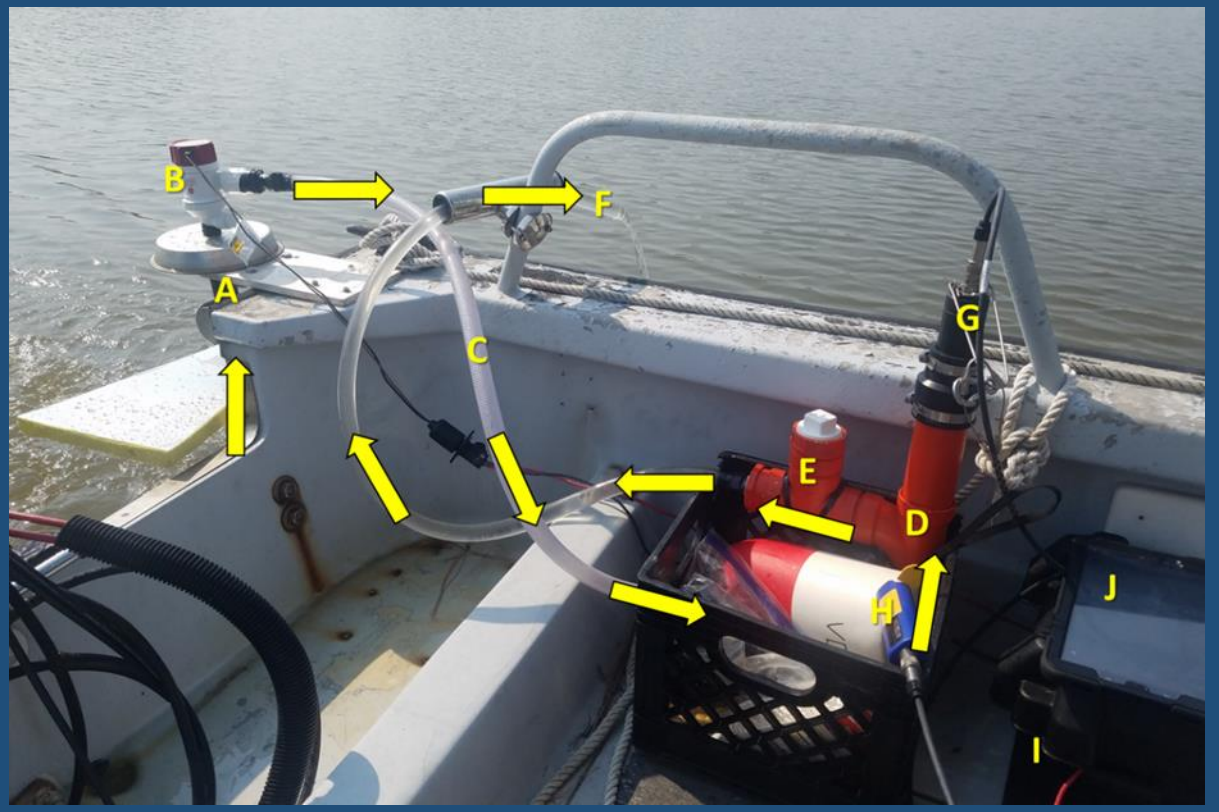
- Dinoflagellate family: *Azadinium & Amphidoma* spp.
  - Not identified through microscopy. 1st year of qPCR survey
  - qPCR >0.1 in ~ 23% of 2021 samples
  - <0.1- 12.5 cell equivalents/ml (6% >1/ml)
  - No commercial Azaspiracid test kits available

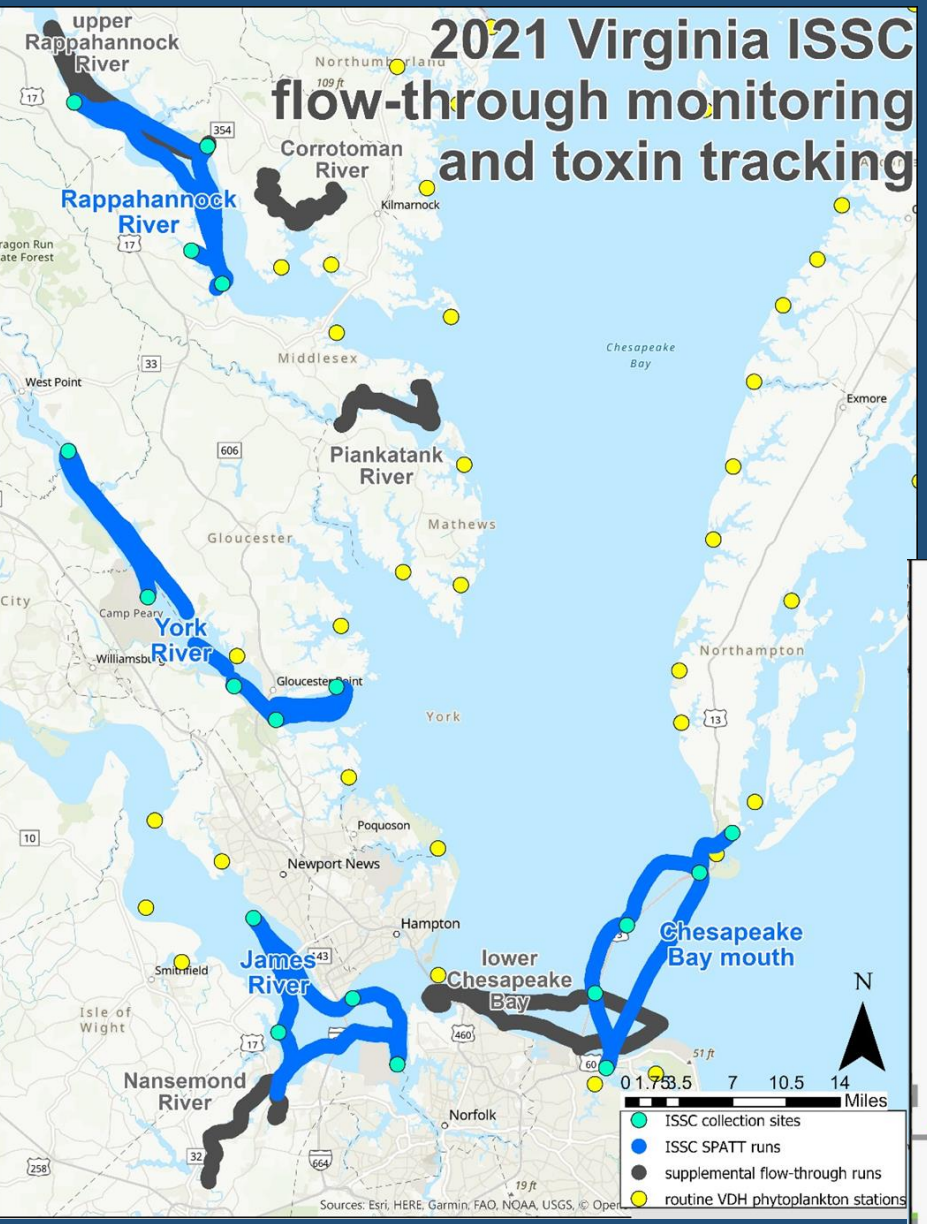
- AZA-1 reported using SPATTs- 2017-2018 (CBTOX- Onofrio et al. 2021)



ISSC grant 2020-2021

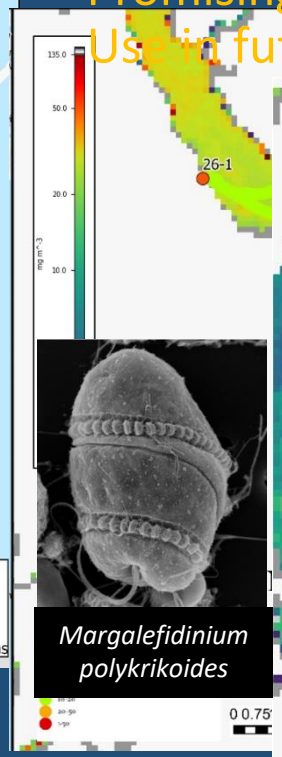
- Techniques and Tools for Toxin Management
- Biotoxin monitoring and management using flow-through real-time sampling and toxin tracking.
- Integrate spatial variability and correlated HAB/ environmental metrics
  - Flow-through
    - Chl *a* fluorescence
    - SPATT (ELISA: ASP, DSP)
    - Salinity, temperature, turbidity, DO
  - Fixed station collections
    - Cell counts
    - qPCR (VIMS)
    - ASP, DSP ELISA



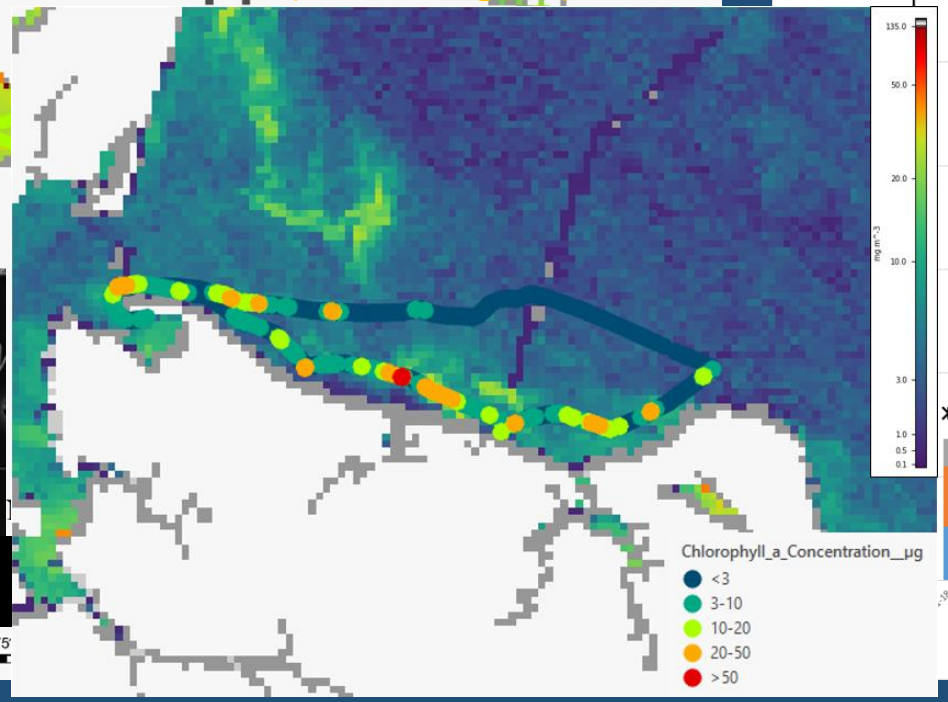


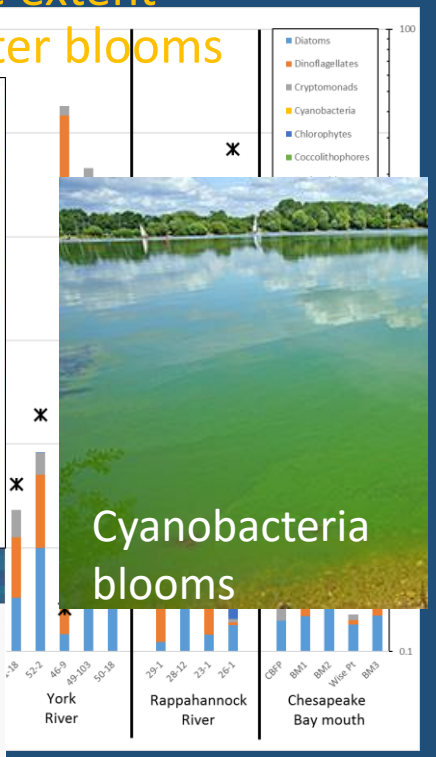
Full report available: VDH; ISSC coming soon

- System deployable on multiple VDH vessels. Improve GA characterization
  - Conducted multiple field trials in Hampton Roads and the Northern Neck regions
- Development and utilization of qPCR HAB assays (VIMS)
- 1/18 samples with visual HAB (*Pseudo-nitzschia*)
- qPCR detections of *Dinophysis, Pseudo-nitzschia* and Amphidomatacea
- All SPATTs and grab samples BDL for ASP and DSP
- Chl*a* not sig. correlated w/ HABs or total algal biomass (low)
- Promising in higher biomass blooms to characterize extent
- Use in future marine (including ES) and/or freshwater blooms



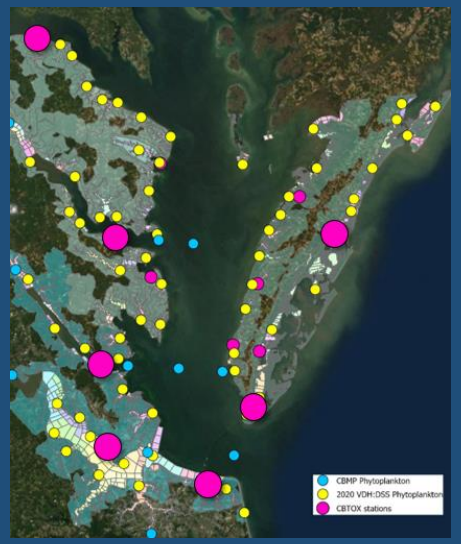
Margalefidinium polykrikoides

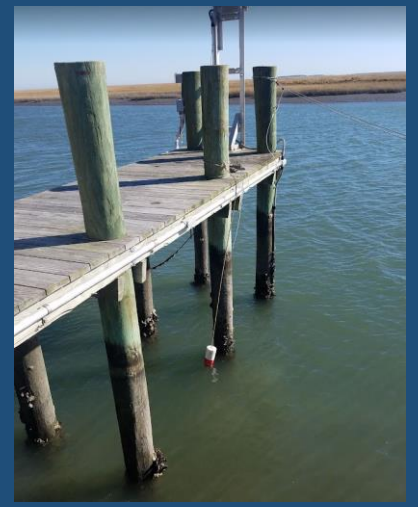


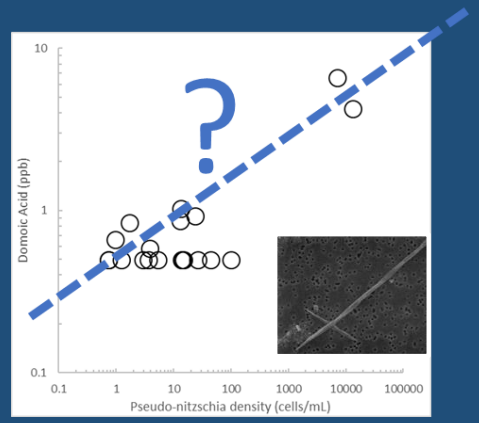


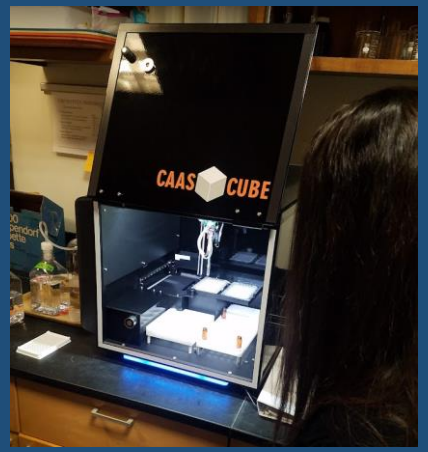
2022: ongoing and upcoming projects

- Additional shellfish deployments and toxin testing
  - Winter deployments (Dec-Feb)
  - Additional seasonal deployments- Shore
  - Additional routine seawater DA ELISAs- Shore
  - CAAS Cube
- Revisiting cell/toxin relationships and thresholds
  - qPCR vs cell counts
  - Cell densities vs toxins (seawater)
  - Toxins: SPATTs vs grab samples vs shellfish
- Flow-through system
  - Targeted blooms, additional areas (Eastern Shore)
  - Possible FW utility
- Logistics and data management
  - improved sample logs and standardized data reporting
  - database in development
- Workgroups:
  - VDH/DEQ HAB Technical Meetings (MOU development)
  - ISSC Biotoxin Committee
  - Chesapeake Bay HAB forecasting workshop steering committee (winter 2023)
  - NASA PACE phytoplankton composition subgroup









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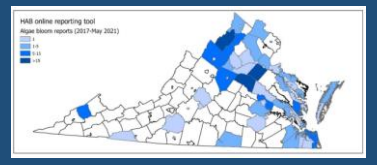
DEQ Virginia Department of Environmental Quality

NOAA

NASA

Interstate Shellfish Sanitation Conference ISSC

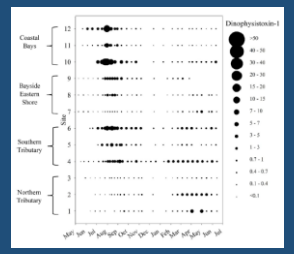
Recent publications and resources:



HARMFUL ALGAE BLOOMS IN VIRGINIA

REPORT OF THE DEPARTMENT OF ENVIRONMENTAL QUALITY

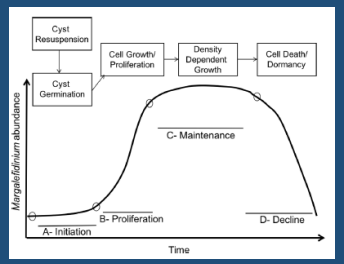
September 2021: https://rga.lis.virginia.gov/Published/2021/RD411



Spatiotemporal distribution of phycotoxins and their co-occurrence within nearshore waters

Michelle D. Onofrio [a], Todd A. Egerton [b], Kimberly S. Reece [a], Sarah K.D. Pease [a], Marta P. Sanderson [a], William Jones III [a], Evan Yeargan [b], Amanda Roach [b], Caroline DeMent [a], Adam Wood [b], William G. Reay [a], Allen R. Place [c], Juliette L. Smith [a,*]

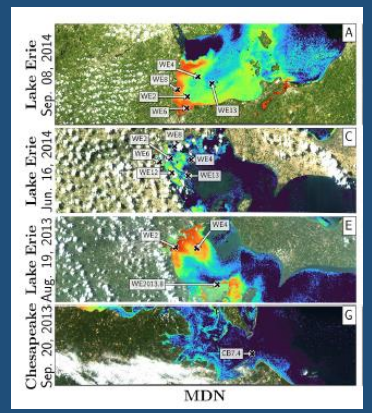
March 2021: Harmful Algae, 103, p.101993.



Understanding controls on *Margalefidinium polykrikoides* blooms in the lower Chesapeake Bay

Eileen E. Hofmann [a,*], John M. Klinck [a], Katherine C. Filippino [b,d], Todd Egerton [c], L. Brynn Davis [a], Michael Echevarría [b], Eduardo Pérez-Vega [b], Margaret R. Mulholland [b]

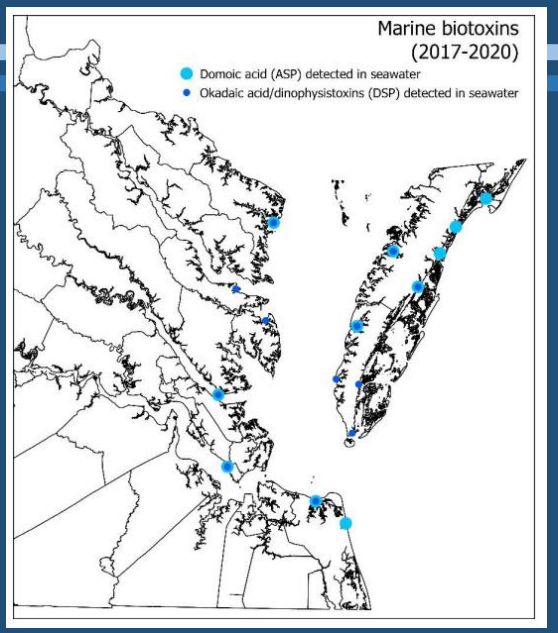
June 2021: Harmful Algae, 107, p.102064.



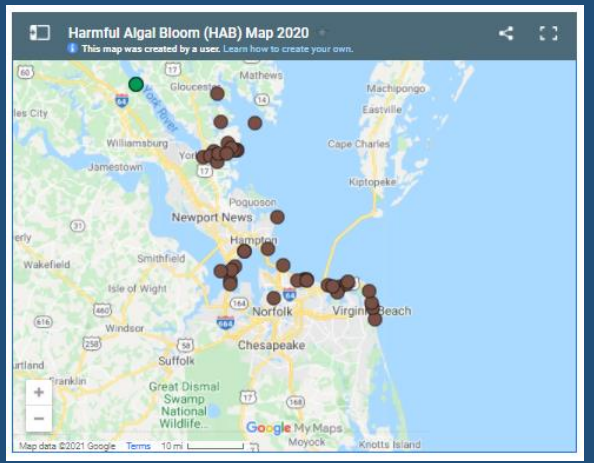
Advancing cyanobacteria biomass estimation from hyperspectral observations: Demonstrations with HICO and PRISMA imagery

Ryan E. O'Shea [a,b], Nima Pahlevan [a,b,*], Brandon Smith [a,b], Mariano Bresciani [c], Todd Egerton [d], Claudia Giardino [c], Lin Li [e], Tim Moore [f], Antonio Ruiz-Verdu [g], Steve Ruberg [h], Stefan G. H. Simis [i], Richard Stumpf [j], Diana Vaičiūtė [k]

September 2021: Remote Sensing of Environment, 266, p.112693.



https://www.SwimHealthyVA.com



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