HAB MONITORING IN LOWER CHESAPEAKE BAY: YORK RIVER PATTERNS 2007 – PRESENT DEVELOPMENT OF NEW ASSAYS

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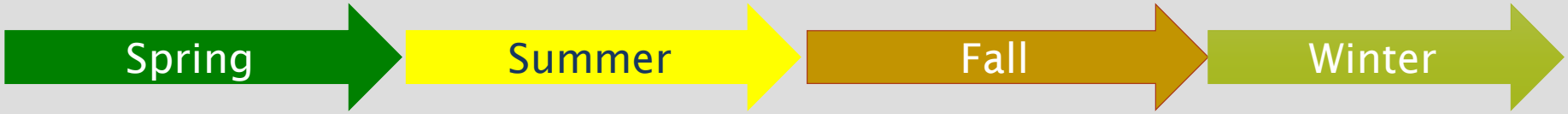
- YORK RIVER BLOOM PATTERNS
  - Overall yearly pattern
  - Late summer blooms
    - *Margalefidinium polykrikoides*
    - *Alexandrium monilatum*


York River *A. monilatum* bloom photo: W. Vogelbein

- HABs of HUMAN HEALTH CONCERN
  - Toxins detected by SPATTs
  - Molecular assays under development
    - *Dinophysis* spp.
    - *Pseudo-nitzschia* spp.
    - Azaspiracid producers

General Bloom Pattern in VA waters



Diatoms

Heterocapsa triquetra

Pseudo-nitzschia spp. (Toxin = Domoic acid) ✝

Dinophysis spp. ✝

✝ Possible human health effects
* Can be harmful to shellfish

Karlodinium veneficum (Toxin = Karlo toxin) *

Prorocentrum spp. *

Raphidophytes (Toxin = Brevetoxin?) ✝

Margalefidinium (Cochlodinium) polykrikoides *

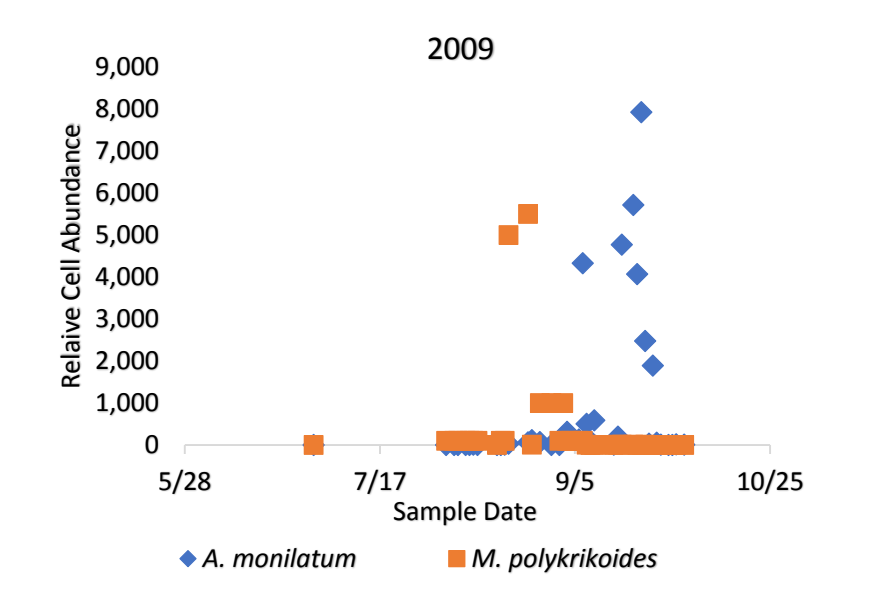
Alexandrium monilatum- toxin = goniodomin *

LATE SUMMER BLOOM AND NON-BLOOM YEARS?

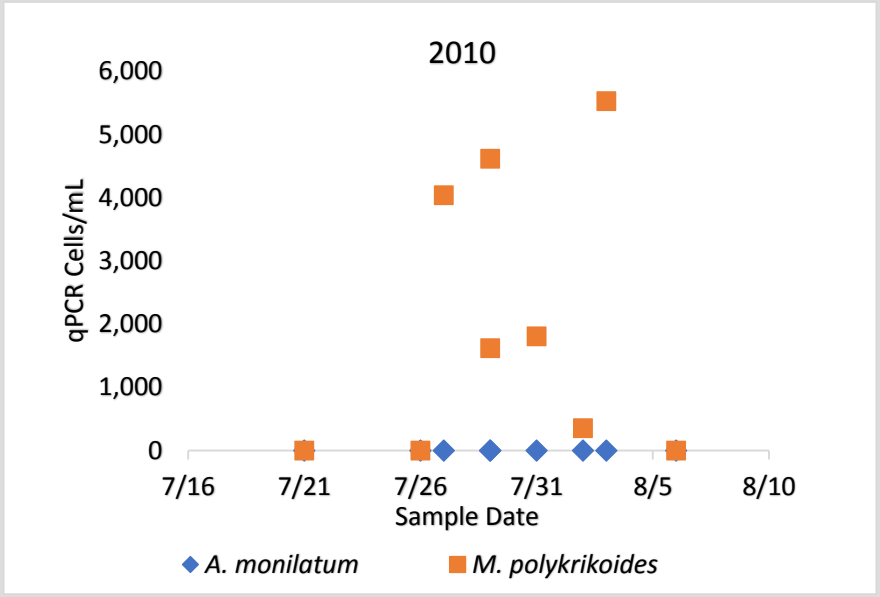
- Blooms of both species:
  - 2007-09, 2012-13, 2015-17 and 2020
    - Expansion of *A. monilatum* began in 2012, further in 2015 and 2016
- Blooms of only *M. polykrikoides*:
  - 2010-11
- No blooms in the York:
  - 2014, 2018 or 2019
- Blooms only southernmost Bay 2018

2008 AND 2009 BOTH SPECIES

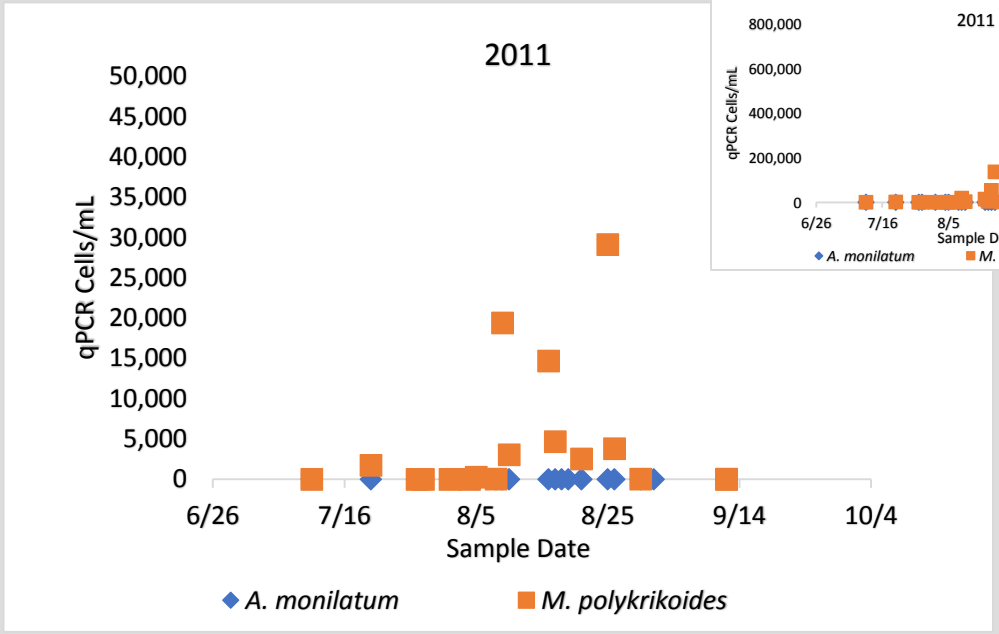
Hurricane Hanna Sept. 6, 2008



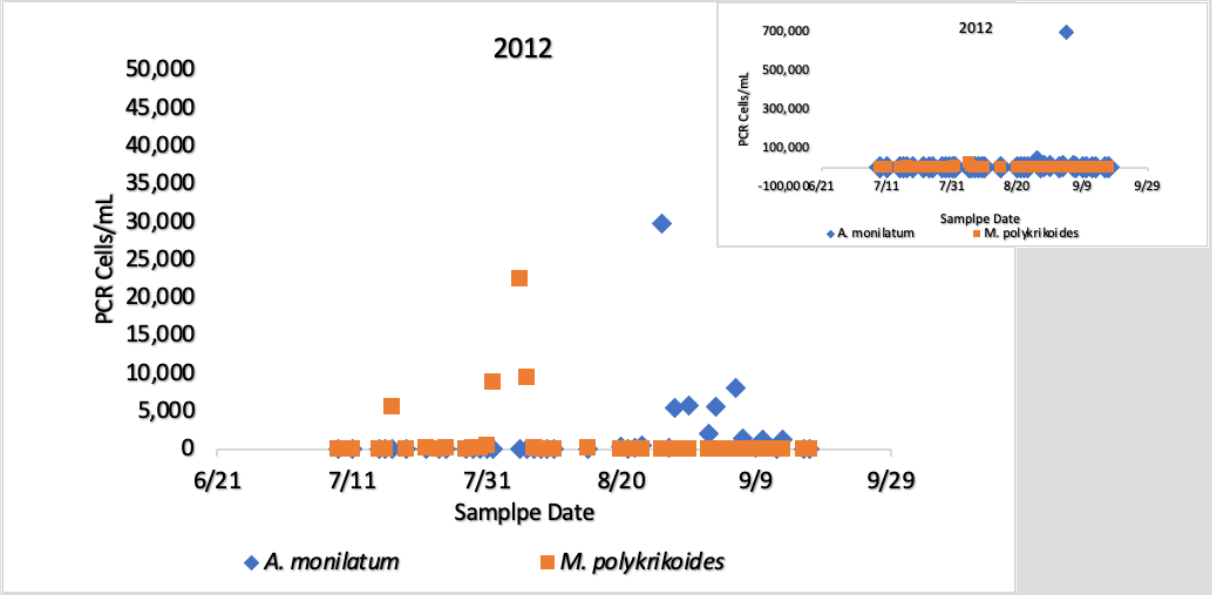
2010 AND 2011- ONLY MARGE

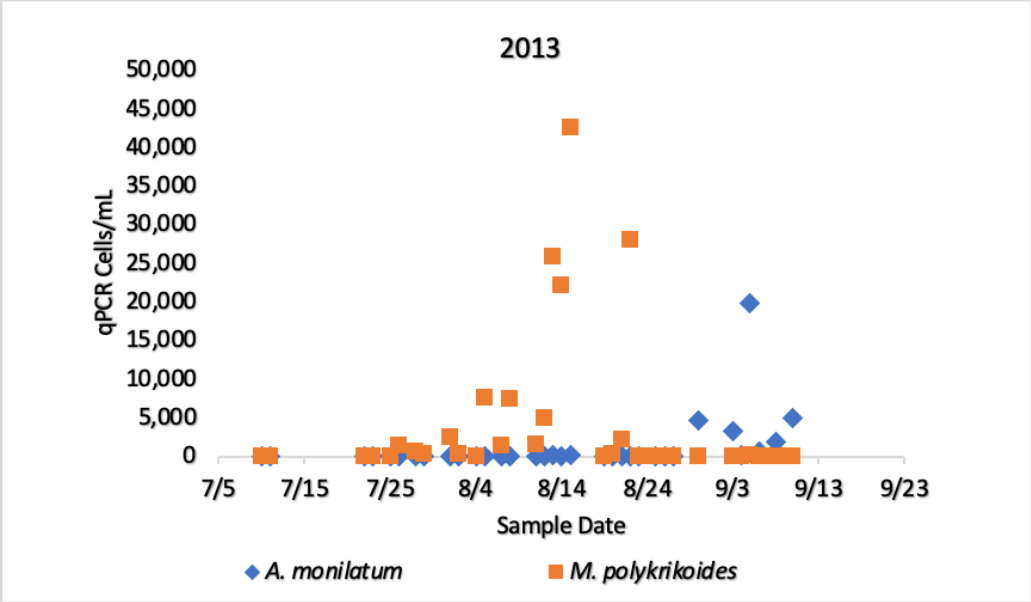


Hurricane Irene
Aug. 27, 2011
Very heavy rainfall following dry conditions



2012 AND 2013



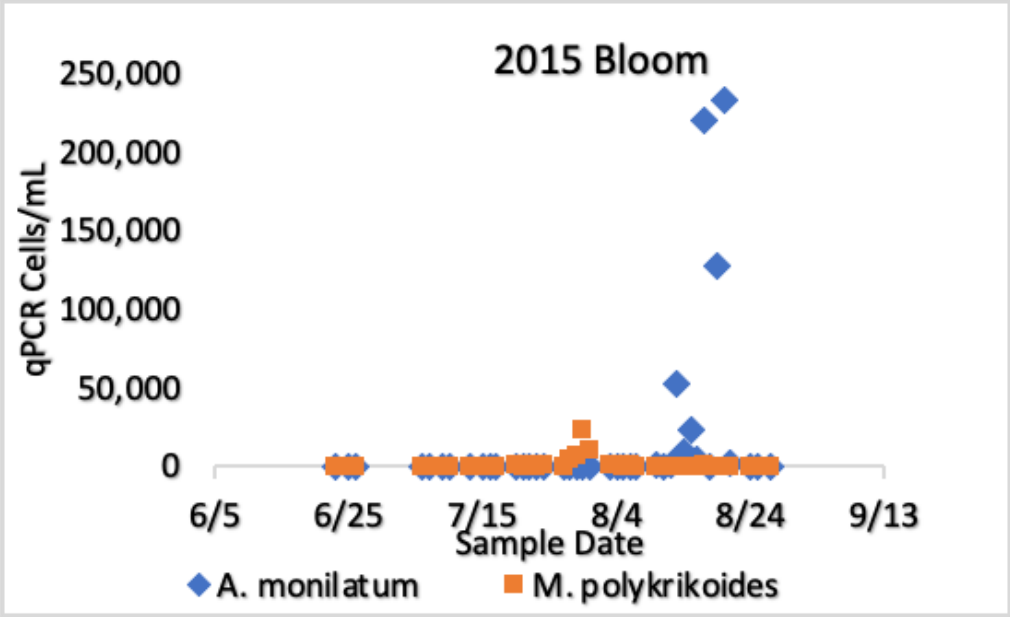


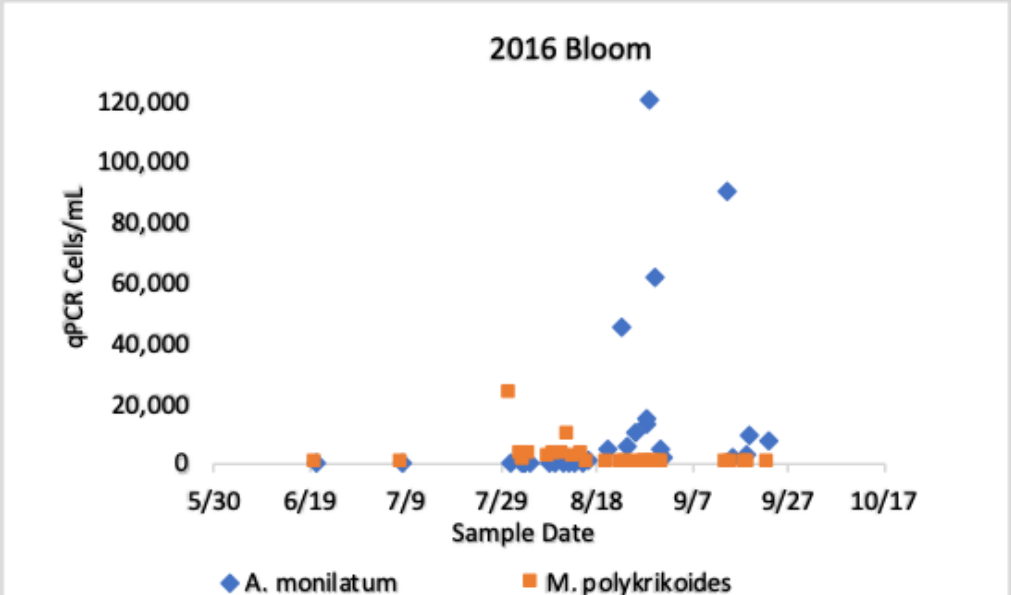
Expansion of *M. polykrikoides* and *A. monilatum* from the York River to entire lower Chesapeake Bay

- Expansion north and south of the York River region. *M.p.*-40+ years, *A.m.* 10 years
- *M.p.*: expanded in the 1990's (Marshall 1995, Marshall et al. 2005).
- *A.m.*: first recent bloom in the York River in 2007, expansion started 2012

2015 AND 2016

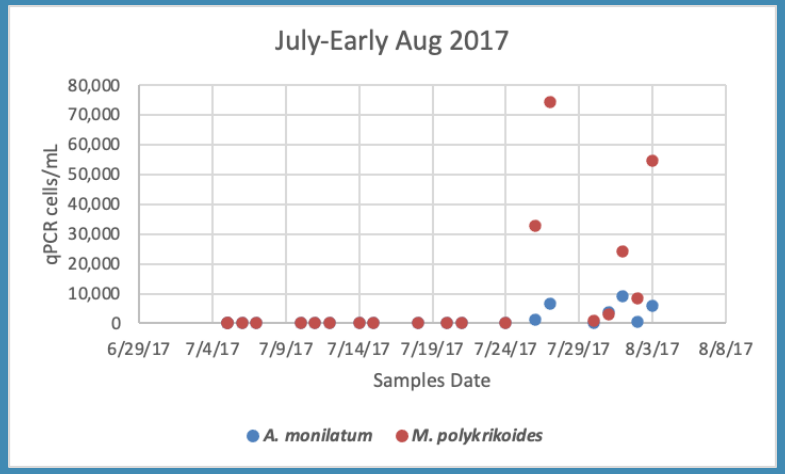
Large Alex blooms
Shellfish mortalities

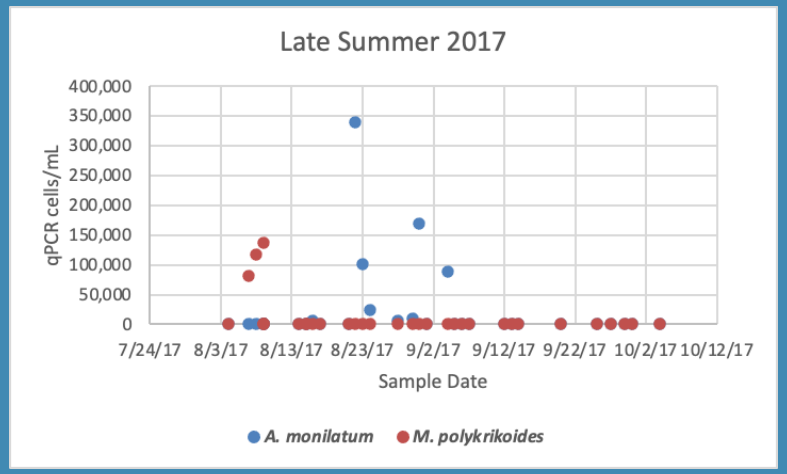


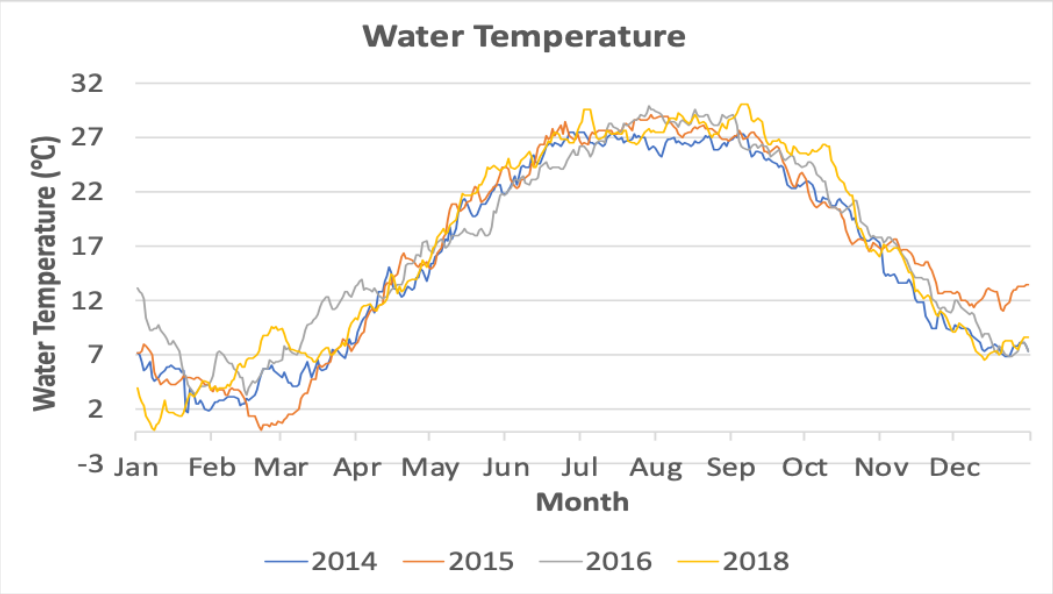


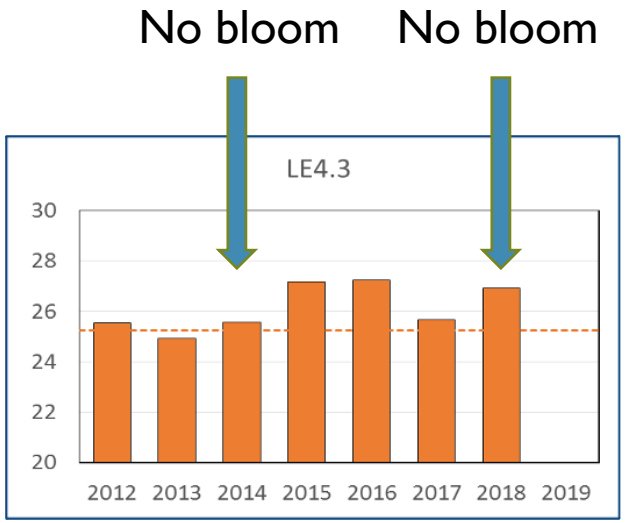
2017

MIXED → MARGE → ALEX



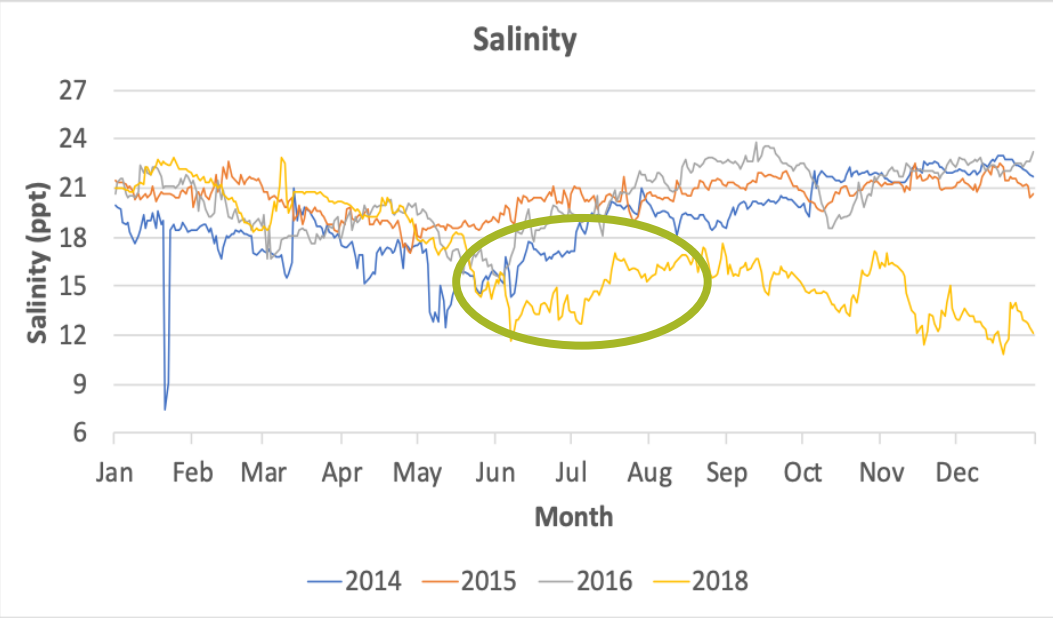


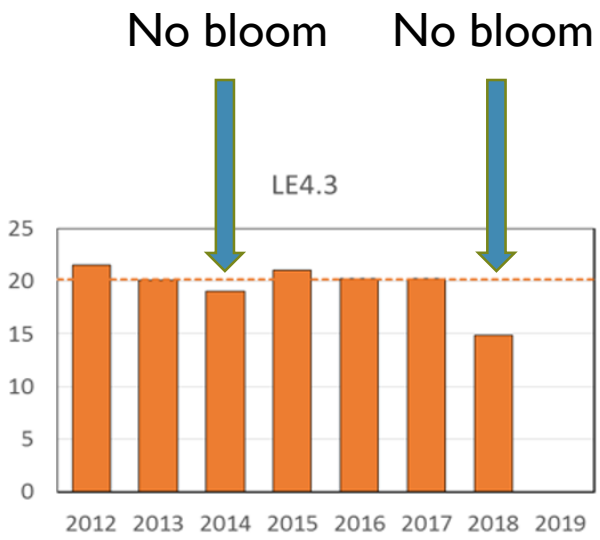




TEMPERATURE IMPACT ON BLOOM FORMATION?

No Blooms in the York River 2014, 2018 or 2019

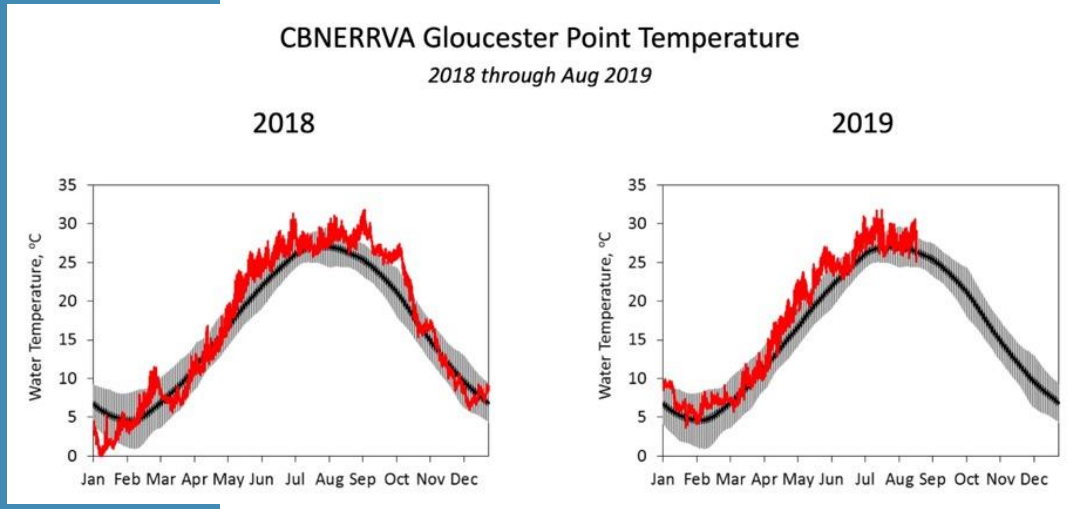


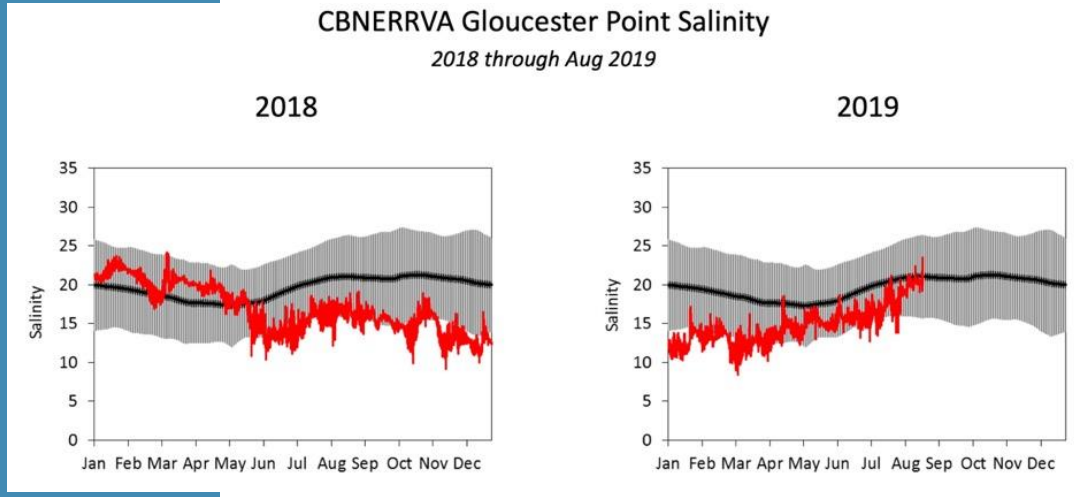


SALINITY IMPACT ON BLOOM FORMATION?

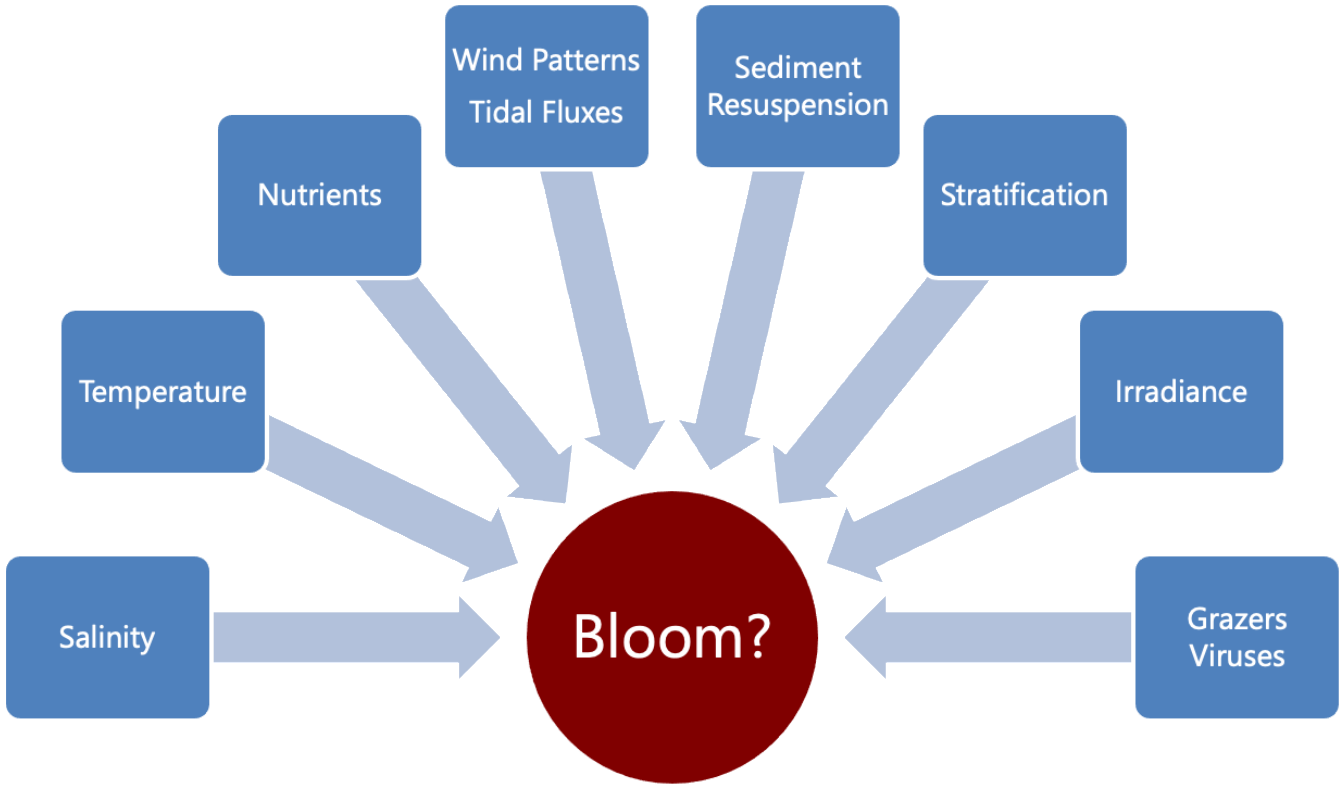
No Blooms in the York River 2014, 2018 or 2019

SALINITY AND TEMPERATURE: 2018 -2019





Marg and Alex: to bloom or not to bloom?



ADDITIONAL MODELING AND ANALYSES UNDERWAY OF BLOOM AND NON-BLOOM YEARS

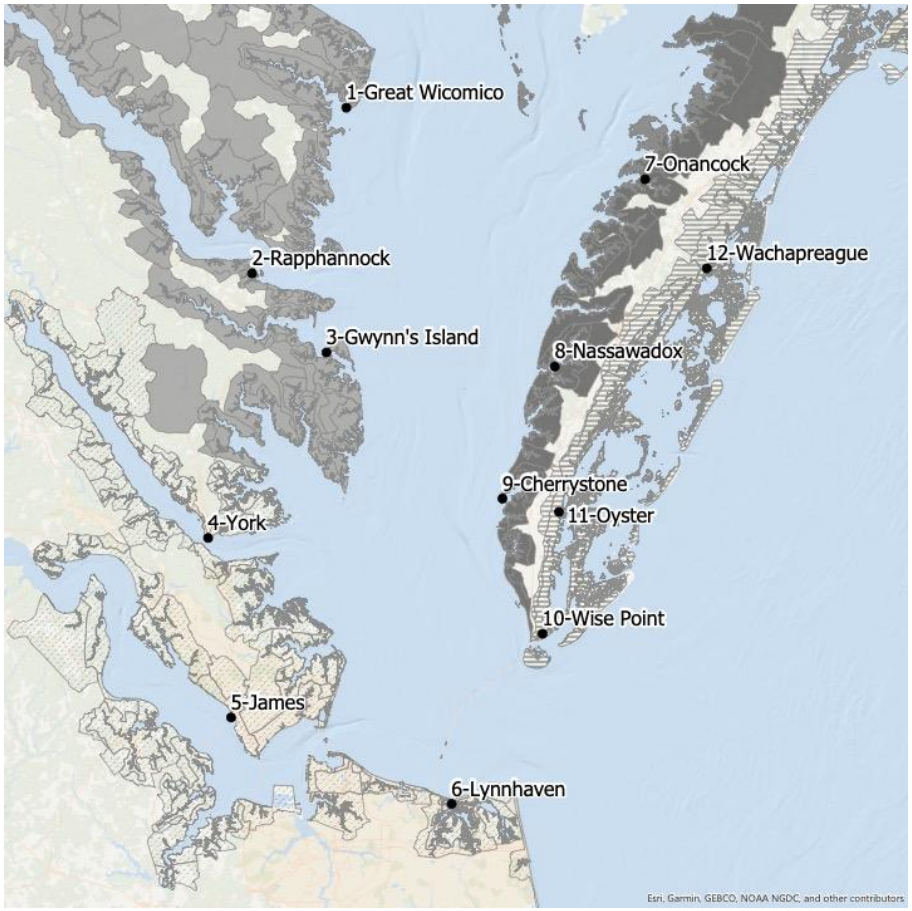
HABs OF HUMAN HEALTH CONCERN

MANUSCRIPT in revision: Onofrio et al., Spatiotemporal distribution of phycotoxins and their co-occurrence within nearshore waters. In: Harmful Algae

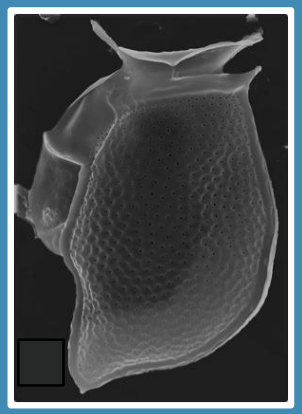
A Solid Phase Adsorption Toxin Tracking (or SPATT) disc after it has been removed from the water. Credit: S. Pease/VIMS

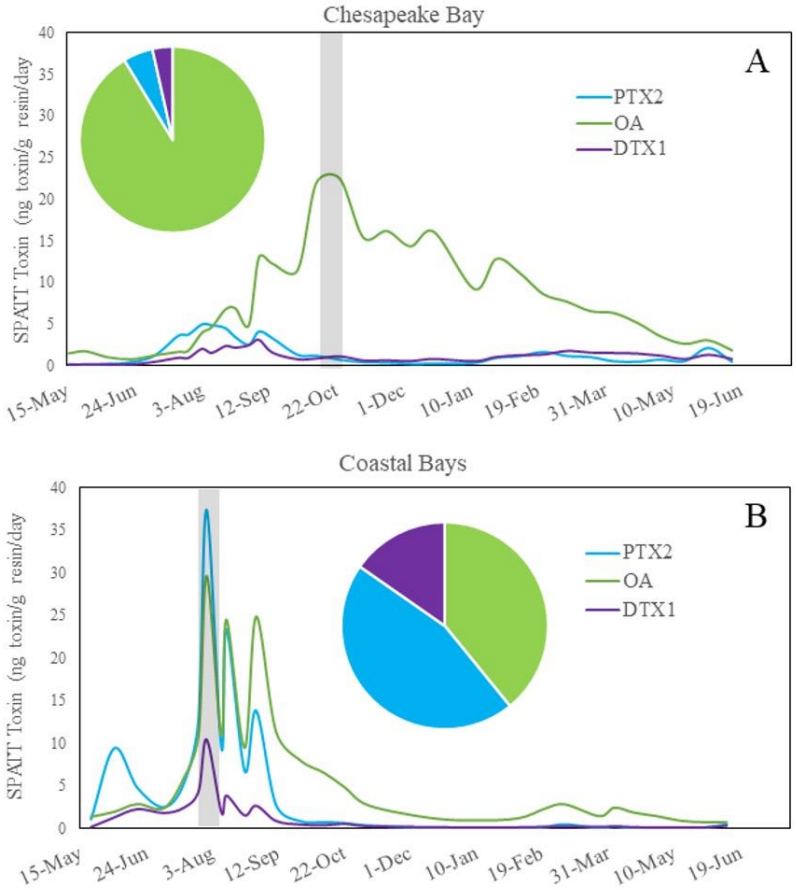
Collaborative study detecting phytotoxins in Chesapeake Bay region
CBTOX: VIMS, VDH, ODU, UMD

SAMPLING SITES

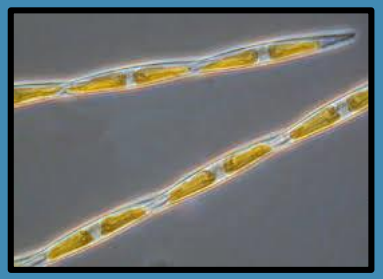


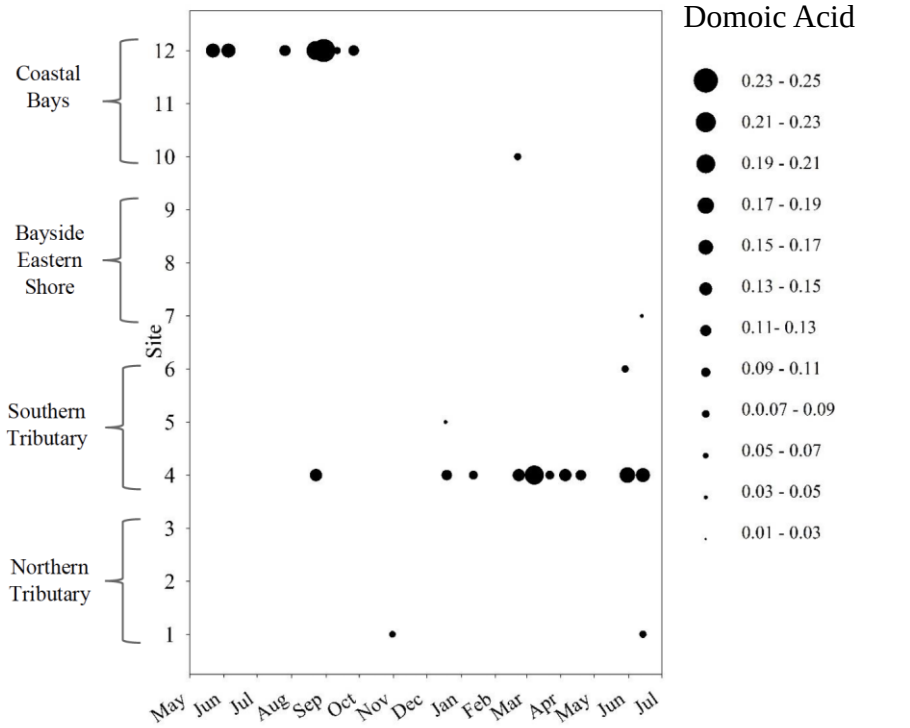
DINOPHYSIS spp. TOXINS (also some *PROROCENTRUM* spp.)





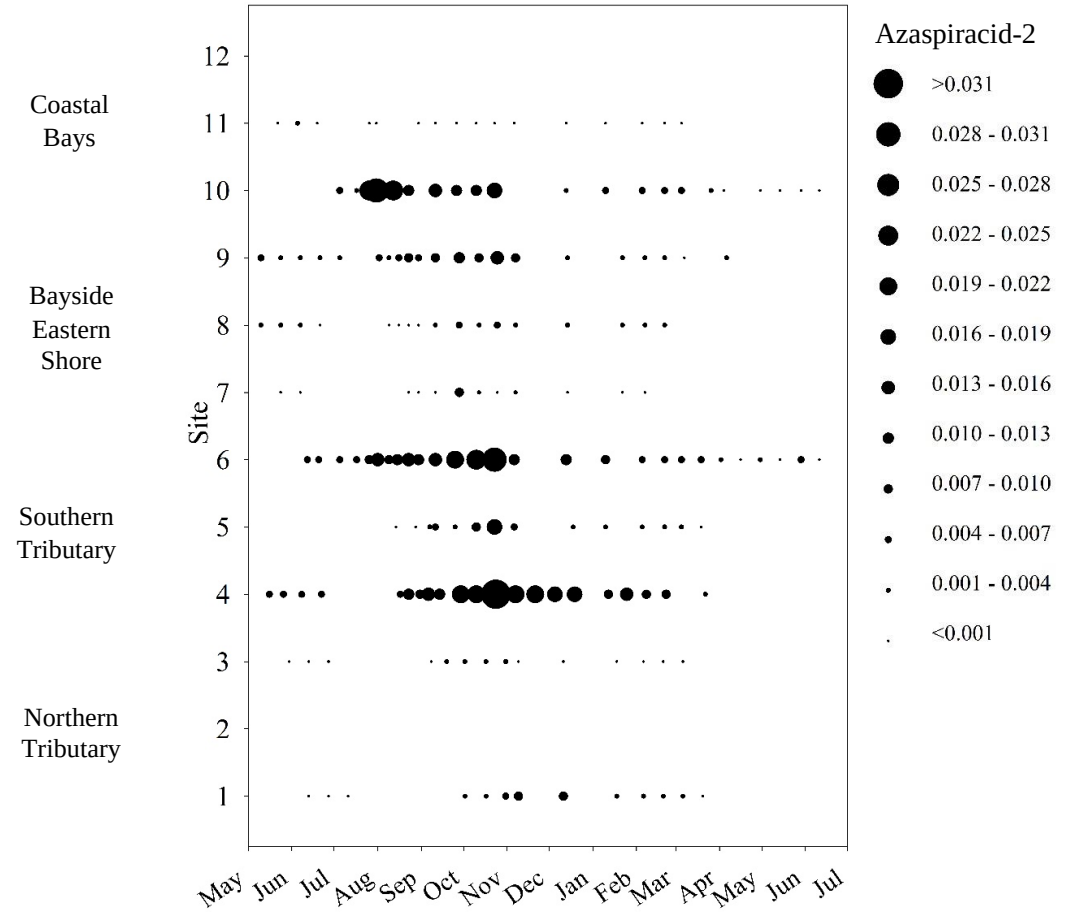
PSEUDO-NITZSCHIA spp.





AZADINIUM and *AMPHIDOMA* spp.

Photo: Salas et al. 2011



DEVELOPMENT OF MOLECULAR ASSAYS TO DETECT HABs OF HUMAN HEALTH CONCERN

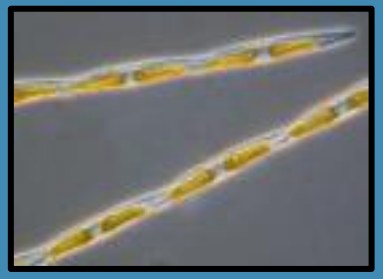
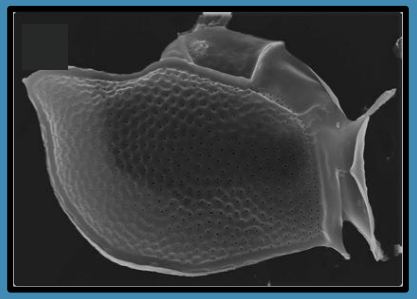
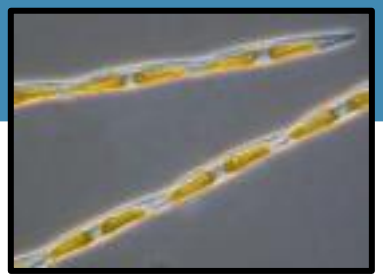


Photo: Salas et al. 2011





Pseudo-nitzschia spp.

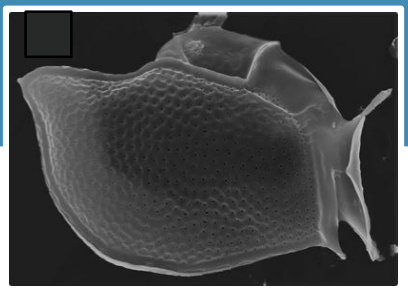
- We are in the process of screening the VDH samples using the PnGenus assay (Fitzpatrick et al. 2010) with *P. artica* as standard control material.

- We have screened >80 samples to date.

- Samples of note so far (>10 cells/ml):

#445	3/31/20	Wise Point	CBTOX-9	43 cells/ml
#289	7/7/20	Wise Point	CBTOX-9	40 cells/ml
#701	8/6/20	Cunjer Chanel	98-21	66 cells/ml
#720	8/10/20	Fisherman's Inlet	92-6	29 cells/ml
#723	8/18/20	Mosquito Creek	100-1	414 cells/ml
#702	8/20/20	Kegotank Bay	99-1	840 cells/ml
#724	8/26/20	Rudee Inlet	73-1	15 cells/ml
#714	9/8/20	Metomkin Bay	98-1	209 cells/ml

VA Beach and seaside Eastern Shore



Dinophysis spp.

- A genus PCR primer set (ITS1-dino, Penna et al. 2007) was used to amplify DNA fragments for sequencing.

- Fragments amplifed from 2020 samples where *Dinophysis* was visually identified.
  - York River (4-CBTOX) 4/15/19
  - Folly Creek (97-15) 7/7/20
  - Cunjer Channel (96-21) 7/8/20
  - Kegotank Bay (99-1) 7/21/20
  - Dividing Creek (15-9) 7/30/20
  - Metompkin Bay (98-1) 8/5/20

- Cloned and sequenced the fragments and currently analyzing the sequences.

- Sequence data will be used to design a qPCR assay for screening samples

AZA2 Toxin Producer(s)?

Photo: Salas et al. 2011

The Problem:

- "Fishing" expedition. No visual identification of an AZA2 producing organism in this area.
- SPATTs that detected the AZA2 toxin were in place for 2 weeks. Water samples for qPCR were collected at a discreet time – **was** the AZA2 producer present?
- Not *Azadinium spinosum* or *A. poporum,* the most common AZA2 producers, based on species-specific qPCR results
- Sequencing of the qPCR fragment from a family-level assay are inconclusive due to lack of sequence variation in this region for the entire Amphidomataceae family.

AZA2 Toxin Producer(s)

Results of the Amphidomataceae family qPCR assay screening DNA from water samples taken in 2017 at sites where the collected SPATT was positive for AZA2

Photo: Salas et al. 2011

HAB #	Date Collected	AZA2 (ng/g resin/day)	Site	Amphidomataceae based on melting curve analysis
482	7/31/17	0.0297	Wise Point	POS
616	8/12/17	0.0232	Wise Point	NEG
784	9/11/17	0.0149	Wise Point	POS
797	9/14/17	0.0121	York	POS
838	9/26/17	0.0109	Wise Point	POS
839	9/25/17	0.0206	Lynnhaven	NEG
843	9/29/17	0.0200	York	NEG
870	10/10/17	0.0218	York	POS
883	10/10/17	0.0112	Wise Point	POS
884	10/10/17	0.0235	Lynnhaven	POS
904	10/24/17	0.0431	York	NEG
916	10/23/17	0.0169	Wise Point	NEG
917	10/23/17	0.0282	Lynnhaven	NEG
937	11/7/17	0.0196	York	POS
943	11/6/17	0.0122	Lynnhaven	NEG
949	11/21/17	0.0211	York	NEG
950	12/5/17	0.0168	York	POS
958	12/13/17	0.0130	Lynnhaven	NEG
962	12/19/17	0.0166	York	NEG

AZA2 Toxin Producer(s)

Results of the Amphidomataceae family qPCR assay screening DNA from 2020 water samples with cell counts equal to or greater than 0.1 cell/ml

Photo: Salas et al. 2011

HAB #	Date Collected	Sample Site	qPCR cells/mL
273	7/6/20	44-4 Severn River	0.16
287	7/8/20	96-21 Cunjer Channel	0.16
281	7/16/20	62-1 James River	0.17
399	7/22/20	101-3B Chincoteague Channel	0.18
400	7/22/20	102-28 Assateague Channel	0.13
476	7/23/20	31-3 Locklies Creek	0.87
430	8/11/20	9-1 York River	0.72
432	8/11/20	9-3 York River	0.71
699	9/14/20	15-9 Dividing Creek	0.57
854	9/14/20	Onancock Creek	0.26
855	9/15/20	Nandua Creek	0.39
827	9/28/20	Tangier Island	0.68
832	9/30/20	Corrotoman River	0.14
837	10/1/20	Cockrell Creek	0.16
822	10/5/20	Monroe Bay	0.12
833	10/5/20	Upper Machodoc Creek	2.10
869	10/19/20	Kings Creek	0.17
843	10/20/20	Nomini	0.10

AZA2 Toxin Producer(s)

Photo: Salas et al. 2011

Current Progress

- Sequenced DNA fragments generated using general dinoflagellate with Amphidomataceae family primers to screen samples from 2017 where the AZA2 toxin was reported. No AZA2 toxin producing species' sequences found.

- Designed 4 primer sets based on *Azadinium* and *Amphidoma* Genbank sequences.
  - Screened 2017 samples.
  - Sequences *Margalefidinium polykrikoides* and *Hetercapsa* spp. along with other species 😒

- Repeated PCR on 2017 samples with higher stringency annealing temperature: increases specificity-more likely to amplify only *Azadinium* or *Amphidoma* species

- Currently cloning and sequencing these DNA fragments.

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