

Algal Toxins of the Chesapeake Bay

Thomas M. Harris

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Red Tides a.k.a. Harmful Algal Blooms (HABs)



Increasing presence of HABs in the Chesapeake Bay

Excess nutrients?

Global warming?

More attention being given to HABs?

“..and all the waters that were in the river were turned to blood. And the fish that was in the river died and the Egyptians could not drink of the water of the river and there was blood through all the land of Egypt.”

Exodus 7: 19-21

Why are we interested in HABs?

Toxicity to fish and shellfish

Economic impact

Toxicity to man

**Massive fish kills occurred in Maryland,
Virginia and North Carolina ~20 years ago**



Pfiesteria piscicida



JoAnn Burkholder
NC State

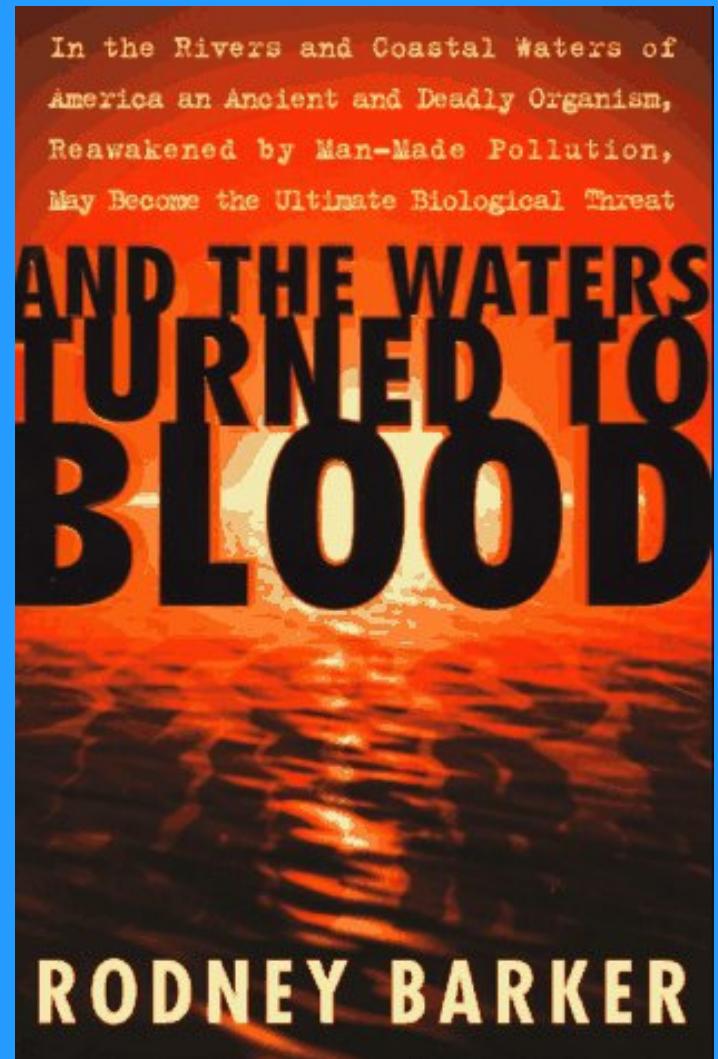
Human Toxicity

Pulmonary toxicity	Aching joints
Burning skin	Amnesia
Lachrymation	Depression
Headaches	Disorientation

Strongly suggestive that the organism produces a potent, multifunctional toxin.

1997

“Below the smooth surface of North Carolina’s eastern rivers lurks a deadly one-celled organism that is eating holes in fish and threatening people. The “cell from hell” is as frightening as the Ebola virus, but disinterested state regulators look the other way.”



Other Side of the Story - 'Pfiesteria hysteria'



Wolf Vogelbein
VIMS

- VIMS: Strong evidence that no toxin is being produced.
Fish are killed by physical attack.
- CDC: Little evidence for neurological toxicity.
- NIEHS: Little evidence for other types of toxicity.

Nature 418:967-970 (2002)

**Pfiesteria shumwayae kills fish by
micropredation not exotoxin secretion**

W. K. Vogelbein, V. J. Lovko, J. D. Shields, K. S. Reece, P. L. Mason, L. W. Haas & C. C. Walker

**The Pfiesteria situation can not be generalized;
Many HAB organisms make potent toxins.**

**The toxins frequently accumulate in fish and shellfish and can still
be present at dangerous levels long after the blooms have
dispersed.**

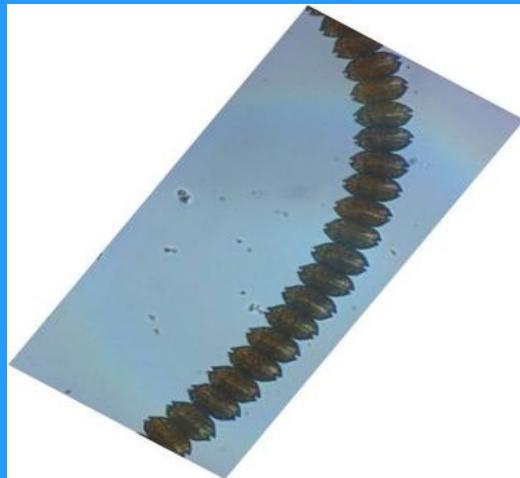
**Many of the toxins lack UV signatures and are difficult to detect.
Many of the toxins are not destroyed by cooking.**

**HAB studies at VIMS has thus far focused on the organisms.
We are now expanding these studies to the toxins they produce.**

Alexandrium monilatum



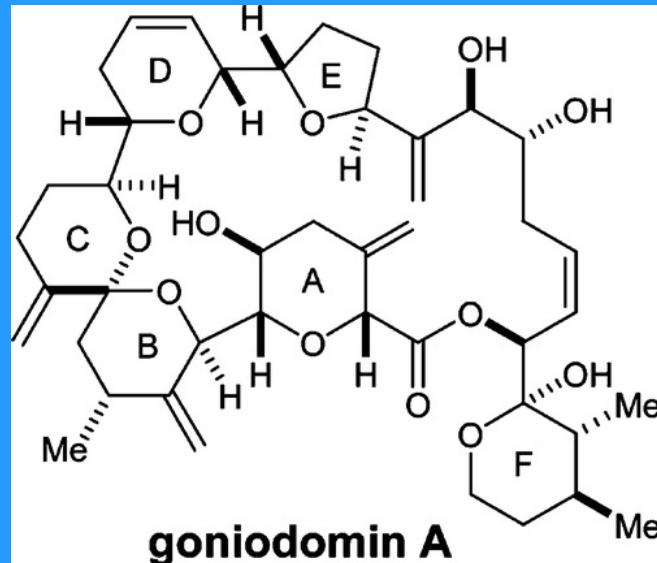
Alexandrium monilatum



Sporadically observed in the Bay for at least half a century.
In recent years appears every summer in the southern
Bay around the end of August.

Blooms occur in the York River directly in front of VIMS.
Understudied.

Goniodomin A



Toxin originally obtained from *Alexandrium hiranoi* and *pseudogonyaulax* found in Japanese waters.
Structure determined and toxicity studied by Japanese workers.

Goniodomin identified in *A. monilatum* by Moeller et al. at NOAA.

Toxicity of Goniodomin

Only limited studies have been carried out with pure toxin
Apparently lethal to fish and shellfish

Most studies have involved the organism not the toxin
Some conflicting data on shellfish

Moderate toxicity in the mouse
Strong antifungal activity

Binds actin
Potent inhibitor of angiogenesis
We suspect it is a teratogen

More detailed toxicity studies are needed on fish and shellfish to determine:

- 1) Extent of toxicity of goniodomin A**
- 2) Duration that it is retained in the marine creatures**
- 3) Upward migration through the marine foodweb**
- 4) Is it teratogenic?**

Specific Aim: Develop immunoassays for Goniodomin

Simple, inexpensive assays are needed.

- 1) A quantitative laboratory assay.**
- 2) A field-deployable automated assay.**
- 3) A low-tech method that could be carried out by
watermen and workers in processing plants.**

Collaboration

Steve Kaattari - **Immunologist**

Kim Reece - **HAB expert**

Myself - **Organic chemist**

Problem:

Antibodies are generated by immunization of mice with protein conjugates of Goniodomin.

Goniodomin is likely to kill the mouse.

Solution:

Employ protein conjugates of *fragments of Goniodomin* rather than the complete toxin.

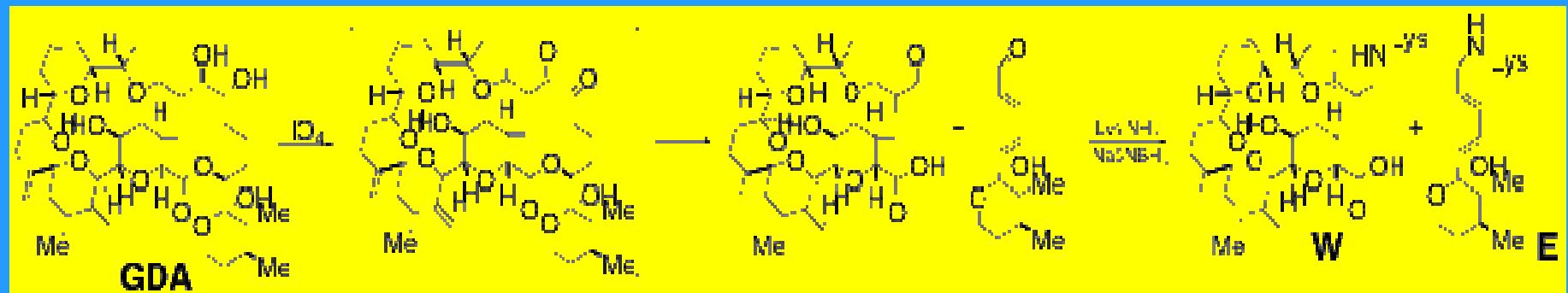
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Laboratory Method

ELISA Assay - Enzyme-Linked ImmunoSorbent Assay
A well developed biochemical technique.

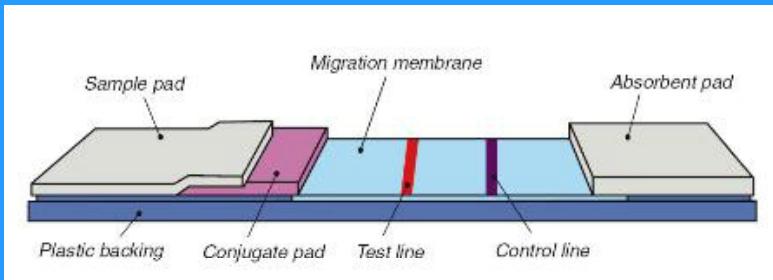


Field-Deployable Method

KinExA Assay



Low-Tech Method “Pregnancy Test”



Monoclonal antibodies devised by Dr. Kaattari for the fish pathogen, *R. salmoninarum* have been employed in user-friendly, low-tech formats for on-site diagnosis by fisheries management.



