Use of Imaging FlowCytobot (IFCB) Data to Retrospectively Characterize a Harmful Algal Bloom in Virginia Waters

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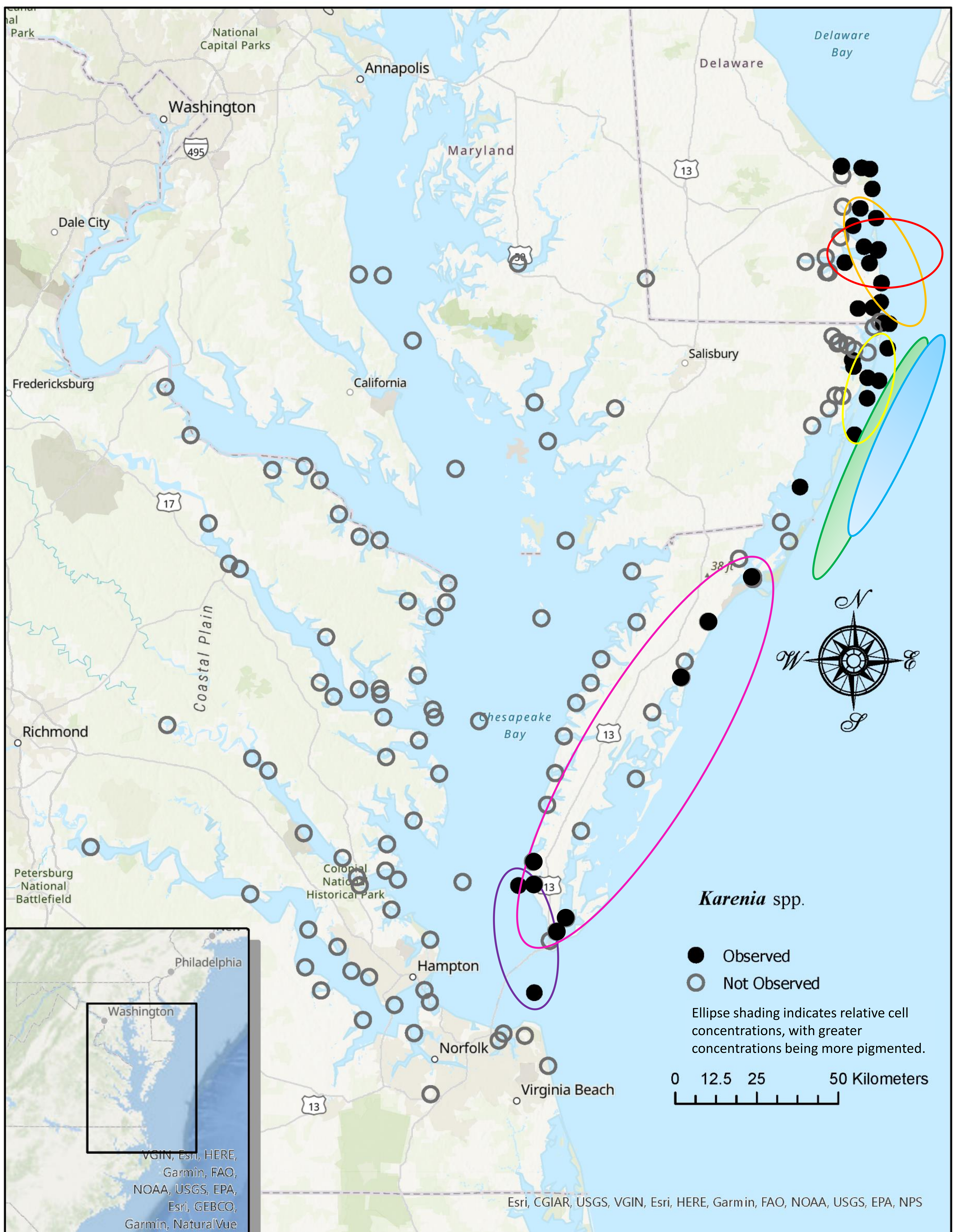
Introduction

Despite their global occurrence, bloom reports of toxigenic *Karenia* species in the United States are rare outside of the Gulf of Mexico, where long-term monitoring for the nearly annual blooms of *K. brevis*, which produces the neurotoxin brevetoxin (BTX), the causative agent of neurotoxic shellfish poisoning (NSP), has occurred since 1954 (Steidinger, 2009). Even with an increase in HAB and water quality monitoring across the United States (Anderson et al., 2021), it has only been within the past decade that blooms caused by other *Karenia* species have been documented: *K. mikimotoi* in Kachemak Bay, Alaska (Vandersea et al., 2020) and the Gulf of Maine (Record et al., 2021; Sculley et al., 2022), and *K. mikimotoi* and *K. papilionacea* in the lower Chesapeake Bay and along the Delmarva coast (Wolny et al., 2024).

Regionally, one of the biggest hurdles for monitoring offshore HABs, such as *Karenia*, is the lack of in-situ data collections. Along the Delmarva Peninsula, Delaware's HAB monitoring efforts occur approximately monthly, May through November, are volunteer-driven, and constrained to shoreline sampling (Whereat et al; 2004; Wolny et al., 2024). State agencies in Maryland conduct monthly monitoring at 16 stations within the coastal bays throughout the year and at five offshore stations April through October (Wolny et al., 2024). The Chesapeake Bay Program and Virginia state agencies conduct year-round monthly monitoring at 76 sites across the Chesapeake Bay and in Virginia's coastal bays, but there is no routine offshore monitoring (CBP, 2017; VDH, 2017; Wolny et al., 2024).

In November 2023, a late autumn bloom of *K. papilionacea* was detected in shellfish harvesting areas on Virginia's eastern shore. Here, we show that offshore in-situ data, collected via an Imaging Flowcytobot (IFCB; Sosik et al., 2014) aboard the *R/V Pisces*, documented bloom initiation/advection along the Delmarva coast on October 29, three weeks before it was detected through routine shoreline sampling (see Wolny et al. (2024) for methodology). This, coupled with a recent history of *Karenia* blooms, highlights a need for regional remote, autonomous monitoring platforms to augment monitoring efforts.

Data



Historic Data Summary

The first reported *Karenia* bloom in the Delmarva region occurred at Indian River Inlet, Delaware, August 30 – September 12, 2007.
Maximum cell concentration: 2.1 × 10^6 cells L^{-1}
Community: *K. papilionacea*, *K.* cf *brevis*

Blooms of *Karenia* were detected between Fenwick Island and the Indian River Inlet, Delaware between late August and early October in 2010 and 2016.
Maximum cell concentration: 2.3 × 10^5 cells L^{-1}
Community: *K. papilionacea*, *K.* cf *brevis*

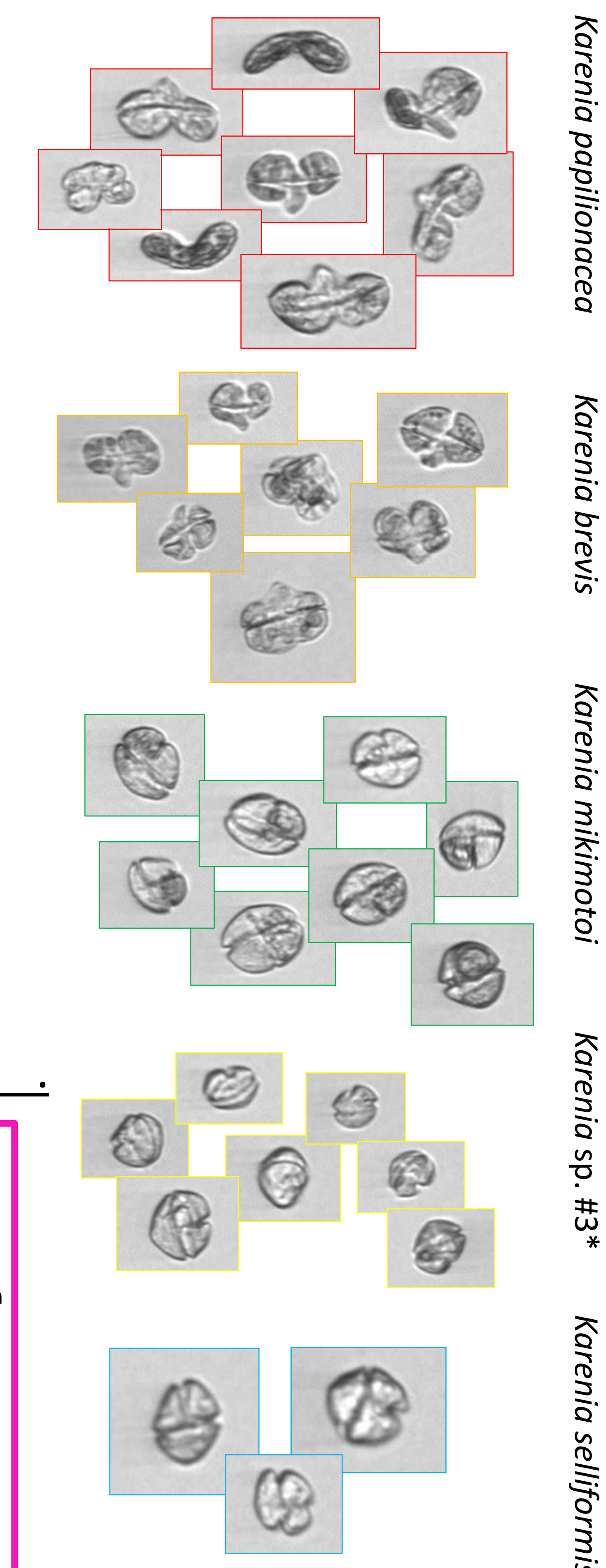
Blooms of *Karenia* were detected north and south of Ocean City, Maryland August through September 2016, 2018, and 2019.
Maximum cell concentration: 2.3 x 10^5 cells L^{-1}
Community: *K. papilionacea*, *K.* cf *brevis*, *K. mikimotoi*, *K.* sp. #3

A *K. mikimotoi* bloom was documented along the Delmarva coastline in June 2018.
Maximum cell concentration: 1.9 x 10^5 cells L^{-1}
Community: *K. mikimotoi*, *K. papilionacea*, *K. selliformis*

A *K. papilionacea* bloom was documented along the Delmarva coastline in July 2018.
Maximum cell concentration: 1.6 x 10^5 cells L^{-1}
Community: *K. papilionacea*, *K.* cf *brevis*, *K. mikimotoi*, *K.* sp. #3, *K. selliformis*

A *K. papilionacea* bloom was detected in the lower Chesapeake Bay from late August to early October in 2017.
Maximum cell concentration: 5.8 x 10^6 cells L^{-1}
No other *Karenia* species were identified.

Species



*Cells identified as *Karenia* sp. #3 are similar to those described by Steidinger et al. (2008) as "*Karenia umbella*-like". In 2018, *K. umbella* and *K. longicanalis* were synonymized, with *K. longicanalis* having the name priority (Wang et al., 2018). To date, populations of this species from the Delmarva region and the Gulf of Mexico, have not been critically examined with morphological and molecular techniques to determine its identity. However, the original descriptions indicate this species causes fish kills (de Salas et al. 2004; Yang et al., 2001).

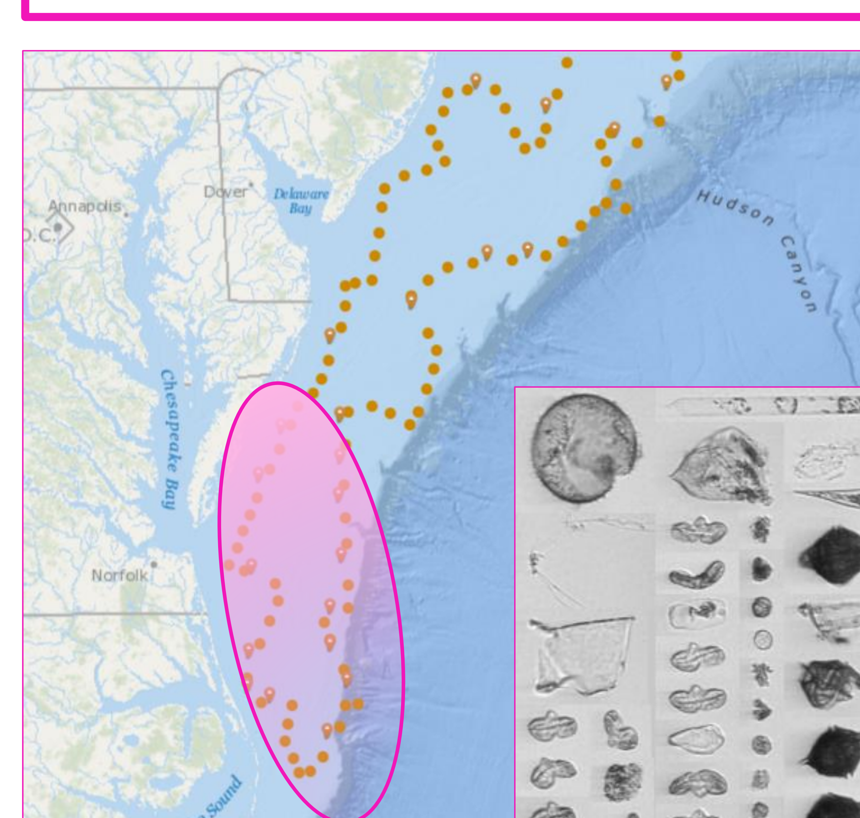
Current Data Examination

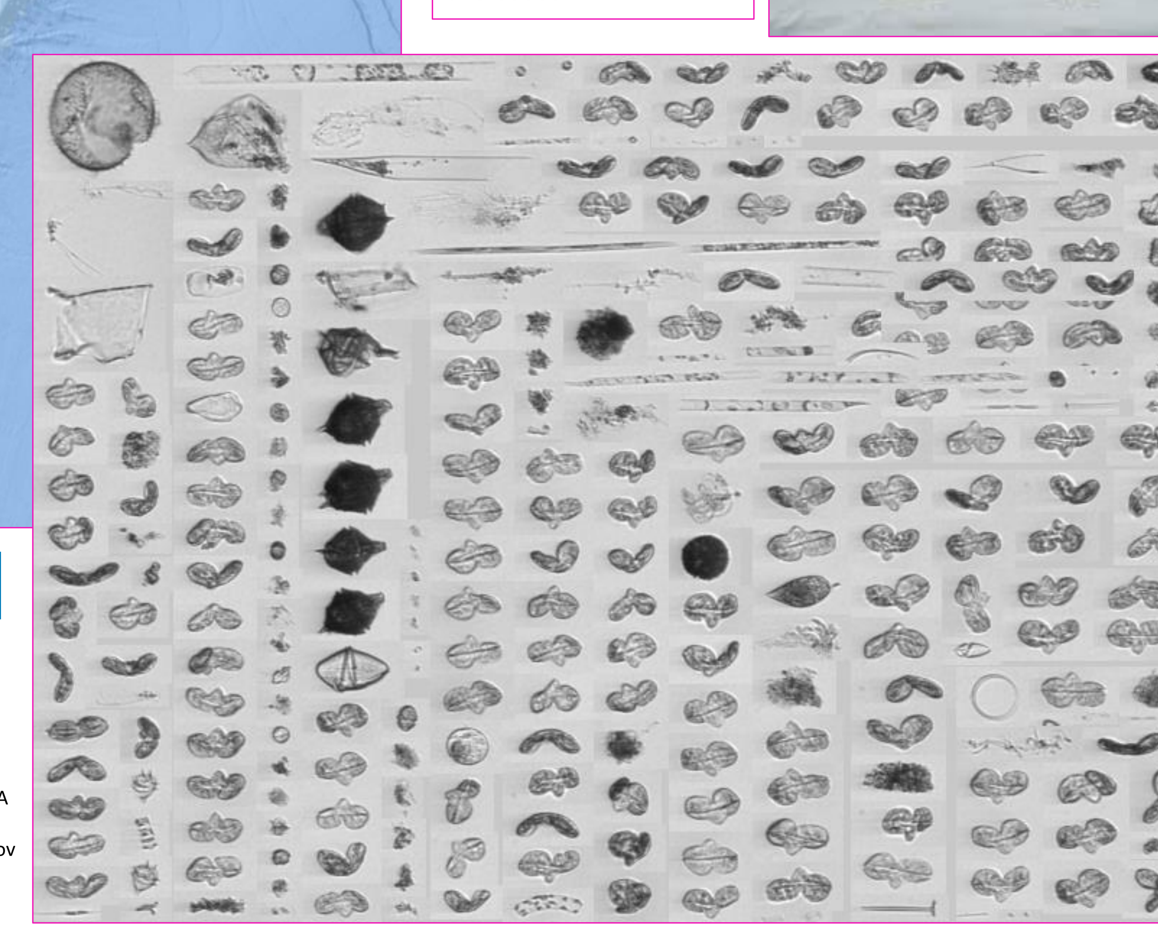
A *K. papilionacea* bloom was detected in shellfish harvesting areas on the Virginia eastern shore in mid-November 2023.
Maximum cell concentration: 3.6 x 10^5 cells L^{-1}
Community: *K. papilionacea*, *K. mikimotoi*

Analysis of IFCB data collected off the Delmarva Peninsula between October 29 – November 3, 2023 indicated a *Karenia* bloom along the coast.
Maximum cell concentration: 1.6 x 10^5 cells L^{-1}
Community: *K. papilionacea*, *K.* cf *brevis*, *K. mikimotoi*, *K.* sp. #3

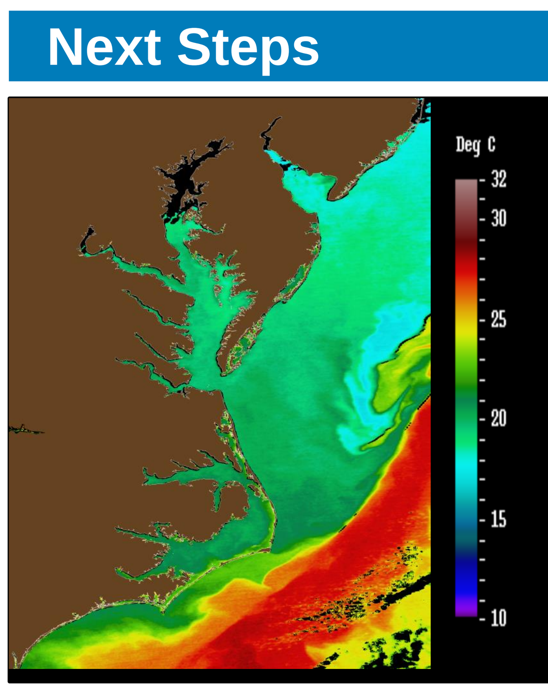
- A retrospective analysis of IFCB data collected during the October and November 2023 NESLTER EcoMon cruise revealed an offshore *K. papilionacea* bloom not detected as part of Virginia's water quality monitoring programs.
- Bloom initiation along or advection to the Delmarva Peninsula was detected via IFCB on October 29, three weeks before the bloom was detected during shoreline sampling.
- The Virginia coastal bays were not sampled for HABs and water quality until November 16, by which time *K. papilionacea* bloom concentrations (≥10^3 cells L^{-1}; Yamaguchi et al., 2016) were already present in shellfish harvesting areas.
- To date, tests of Delmarva wild populations of *K. papilionacea* for BTX have been negative (Wolny et al., 2024) but local strains have demonstrated toxicity in laboratory settings (Fowler et al., 2015). No regional cases of NSP have been identified.
- *K. mikimotoi* and *K. papilionacea* have caused adverse fishery and environmental impacts in other global regions (Amzil et al., 2021; Li et al., 2019), thus are of concern for local resource managers. To date, no assays for the hemolytic compounds produced by *K. mikimotoi* have been conducted on local populations.
- Data collected between 2007 and 2022 suggests late August into September as the typical *Karenia* bloom season for the Delmarva region. The offshore IFCB data indicates *Karenia* blooms can also be present in November, which corresponds to Virginia's molluscan shellfish harvesting season (VDH, 2017).
- *K. selliformis* and *Karenia* sp. #3, identified from IFCB images, are reported for the first time in Virginia waters. *K.* cf *brevis*, previously reported in Virginia waters in June 1980 (Marshall, 1982), was found within a bloom of *K. papilionacea*, as is common in Maryland and Delaware *Karenia* blooms.

Above: *R/V Pisces*
Right: IFCB instrument
Below: IFCB image mosaic of the 2023 *K. papilionacea* bloom.
Below Left: October – November 2023 NESLTER EcoMon cruise track (tan dots). Pink ellipse indicates the bloom area. Ellipse shading indicates relative cell abundance; higher abundances were observed along the coast.





Next Steps



- Determine persistence of *K. papilionacea* within Virginia shellfish harvesting areas by examining phytoplankton samples collected in the winter of 2024.
- Correlate *Karenia* bloom data with oceanographic parameters such as sea surface temperature and Gulf Stream intrusion.
- Examine the 2023 bloom data with respect to satellite imagery and algorithms successfully used to detect and track *Karenia* blooms in other locations (e.g., Gulf of Mexico (normalized Fluorescence Light Height; Soto et al., 2015) and the Celtic Sea (Red Band Difference; Jordan et al., 2021)). Attempts to conduct ±3-day match-ups for the 2018 offshore *Karenia* blooms were unsuccessful due to cloud cover (Wolny et al., 2024).
- Use previously collected Coastal Pioneer New England Shelf Array and NESLTER EcoMon cruise IFCB data to determine the frequency and spatial and temporal distribution of *Karenia* in the mid-Atlantic region to help guide efforts for resource management of inshore and offshore aquaculture operations and exploit research opportunities available during the mid-Atlantic Bight deployment of the Coastal Pioneer Array.

Left: Sea Surface Temperature imagery for mid-Atlantic region on October 30, 2023. Image courtesy of USF College of Marine Science Optical Oceanography Laboratory.

Acknowledgements

Heidi Sosik and Emily Peacock (WHOI) are thanked for their guidance with the IFCB data. Todd Egerton (VDH) and Ed Whereat (UD) are thanked for assisting with dataset construction. Support for this project was provided by VDH, VDEQ, EPA's Chesapeake Bay Program, and NOAA MERHAB (#NA18NOS4780176 to MM).

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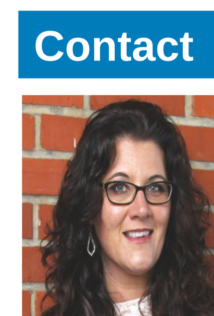

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