

The Occurrence of *Karenia* species in mid-Atlantic coastal waters: Data from the Delmarva Peninsula, USA

Jennifer L. Wolny^{a,*}, Edward B. Whereat^b, Todd A. Egerton^c, Leah A. Gibala-Smith^d, John R. McKay^e, Judith M. O'Neil^f, Catherine E. Wazniak^a, Margaret R. Mulholland^d

^a Maryland Department of Natural Resources, Resource Assessment Service, 580 Taylor Avenue, Annapolis MD 21401 USA

^b University of Delaware, Delaware Sea Grant, 700 Pilottown Road, Lewes DE 19958 USA

^c Virginia Department of Health, Division of Shellfish Safety and Waterborne Hazards, 830 Southampton Avenue, Suite 200, Norfolk VA 23510 USA

^d Old Dominion University, Department of Ocean and Earth Sciences, 4402 Elkhorn Avenue, Norfolk VA 23508 USA

^e Maryland Department of Environment, Water and Science Administration, 416 Chinquapin Round Road, Annapolis MD 21401 USA

^f University of Maryland Center for Environmental Science, Horn Point Laboratory, 2020 Horns Point Road, Cambridge MD 21613 USA

ARTICLE INFO

Edited by: Dr Po Teen Lim

Keywords:

Karenia

Red tide

Mid-Atlantic region

Delmarva Peninsula

Chesapeake Bay

ABSTRACT

A bloom of *Karenia papilionacea* that occurred along the Delaware coast in late summer of 2007 was the first *Karenia* bloom reported on the Delmarva Peninsula (Delaware, Maryland, and Virginia, USA). Limited spatial and temporal monitoring conducted by state agencies and citizen science groups since 2007 have documented that several *Karenia* species are an annual component of the coastal phytoplankton community along the Delmarva Peninsula, often present at background to low concentrations (100 to 10,000 cells L^{-1}). Blooms of *Karenia* ($> 10^5$ cells L^{-1}) occurred in 2010, 2016, 2018, and 2019 in different areas along the Delmarva Peninsula coast. In late summer and early autumn of 2017, the lower Chesapeake Bay experienced a *K. papilionacea* bloom, the first recorded in Bay waters. Blooms typically occurred summer into autumn but were not monospecific; rather, they were dominated by either *K. mikimotoi* or *K. papilionacea*, with *K. selliformis*, *K. brevis*-like cells, and an undescribed *Karenia* species also present. Cell concentrations during these mid-Atlantic *Karenia* spp. blooms equalled concentrations reported for other *Karenia* blooms. However, the negative impacts to environmental and human health often associated with *Karenia* red tides were not observed. The data compiled here report on the presence of multiple *Karenia* species in coastal waters of the Delmarva Peninsula detected through routine monitoring and opportunistic sampling conducted between 2007 and 2022, as well as findings from research cruises undertaken in 2018 and 2019. These data should be used as a baseline for future phytoplankton community analyses supporting coastal HAB monitoring programs.

1. Introduction

Blooms of the dinoflagellate *Karenia brevis* (Davis) G. Hansen & Moestrup (previously known as *Gymnodinium breve* Davis and *Ptychodiscus brevis* (Davis) Steidinger), or “red tides”, are a nearly annual occurrence in the Gulf of Mexico, where they have been documented since Spanish explorers encountered them in the 16th century (Magaña et al., 2003) and continue to be an active area of monitoring and research (Steidinger, 2009; Hu et al., 2022). *Karenia brevis* produces brevetoxin, a neurotoxin which can cause extensive fish and animal mortalities when cell concentrations exceed 10^5 cells L^{-1} (Steidinger,

2009). This toxin can become aerosolized causing acute or chronic respiratory illnesses in individuals exposed to these high concentration blooms (Fleming et al., 2011). Additionally, at cell concentrations above 10^3 cells L^{-1} this toxin can accumulate in shellfish tissues causing neurotoxic shellfish poisoning (NSP) in humans who consume contaminated shellfish, thus presenting a resource management concern for shellfish industries where these blooms occur (Poli et al., 2000; Steidinger, 2009).

Only since the 1970s have environmental and economic impacts from blooms of other *Karenia* species been documented globally. Blooms of *Karenia* spp., that do not include *K. brevis*, have been observed in

* Corresponding author.

E-mail address: jennifer.wolny@fda.hhs.gov (J.L. Wolny).

¹ Present address: US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, 5001 Campus Drive, College Park MD 20740 USA

diverse marine environments including Norwegian coastal waters (Dahl and Tangen, 1993); Tokyo Bay, Japan (Takayama and Adachi, 1984); the Gulf of Gabes, Tunisia (Hamza and El Abed, 1994); numerous bays in New Zealand (Chang, 1999; Haywood et al., 2004); Victoria Harbour, Hong Kong (Yang et al., 2000, 2001); Kuwait Bay (Heil et al., 2001); the coastline of South Africa (Botes et al., 2003); the coastline of eastern Tasmania, Australia (de Salas et al., 2004a,b); the Chilean coast (Mardones et al., 2020), and the region between the Kamchatka Peninsula, Russia and Hokkaido Island, Japan (Iwataki et al., 2022; Orlova et al., 2022). Observations suggest that many bloom-forming *Karenia* species can occupy diverse environments and have broader water temperature and salinity tolerances than *K. brevis*. In the United States, until recently, *Karenia* blooms have been restricted to the Gulf of Mexico and Florida's east coast and dominated by *K. brevis*, with other *Karenia* species noted as present or in low concentrations (Steidinger et al., 2008; Steidinger, 2009; Wolny et al., 2015; Harris et al., 2020). However, in the autumn of 2013 a bloom of *Karenia mikimotoi* (Miyake & Kominami ex Oda) G. Hansen & Moestrup was documented by Vandersea et al. (2020) in Kachemek Bay, Alaska, where extensive discolored water was observed, along with localized fish kills. In the late summers and early autumns of 2017 to 2020, blooms of *K. mikimotoi* caused discolored water, hypoxia, and impacts to shellfish resources in the Gulf of Maine (Record et al., 2021; Scully et al., 2022).

Blooms of *Karenia* have rarely been documented in the mid-Atlantic region of the United States. In 2007, a large export event of a Gulf of Mexico *K. brevis* bloom, entrapped in the Loop Current and moved eastward by the Gulf Stream, was documented by Walsh et al. (2009).

This study tracked the parcel of water containing *K. brevis* using satellite remote sensing and simulated surface drifters as it moved through the Florida Straits, up Florida's east coast, and into the mid-Atlantic region. This same year marked the first observed occurrence of multiple *Karenia* species along the Delaware coastline, with blooms dominated by *Karenia papilionacea* Haywood & Steidinger (Bott, 2014). Unlike Florida's rigorous *Karenia brevis* monitoring program (described in Steidinger, 2009 and Hu et al., 2022) there is little harmful algal bloom (HAB) monitoring along the Delaware, Maryland, and Virginia (Delmarva) Atlantic seaboard outside of the region's coastal bays. Despite limited opportunities to make *in situ* observations, data collected by state agencies, universities, and citizen monitoring programs indicate that multiple *Karenia* species are seasonally part of the phytoplankton community along the coast and within the coastal bays of the Delmarva Peninsula. Here we report on the occurrence of multiple *Karenia* species in coastal waters of the Delmarva Peninsula and Chesapeake Bay detected through spatially- and temporally-limited routine monitoring and opportunistic sampling conducted between 2007 and 2022 and focused field campaigns conducted in the coastal waters of the Delmarva Peninsula in 2018 and 2019.

2. Methods

2.1. Overview of data collection

The cell abundances of *Karenia* species were measured from water samples collected as part of the Maryland and Virginia state HAB and

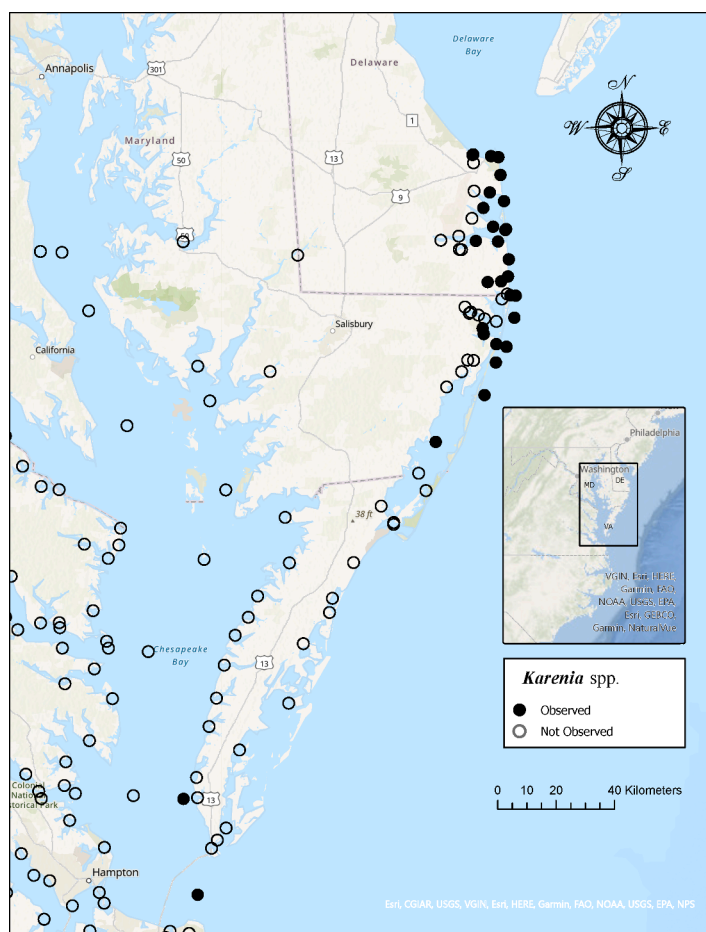


Fig. 1. Map of routine phytoplankton monitoring stations along the Delmarva Peninsula and Chesapeake Bay. For this study, samples were collected between 2007 and 2022 by the UD CMP, MDE, MDNR, VDH, and ODU. Open symbols indicate stations where *Karenia* spp. were not observed. Closed symbols indicate stations where *Karenia* spp. were observed.

shellfish monitoring programs and Chesapeake Bay Program (CBP) water quality monitoring activities (Fig. 1). Water quality monitoring in Delaware was conducted by volunteers participating in the University of Delaware Citizen Monitoring Program (Fig. 1). In 2018 and 2019 a cooperative coastal assessment agreement between the University of Maryland Center for Environmental Science (UMCES) and the Assateague Island National Park Service allowed for the collection of water samples during research cruises along the Delmarva Peninsula coastline (Fig. 2).

State and citizen monitoring programs obtained live or 5 % Lugol's iodine (#LC156725, LabChem Inc., Zelienople, PA, USA) preserved whole water samples from fixed stations or during opportunistic sampling of near surface waters (< 0.5 m) or from the depth of the chlorophyll maximum. Samples collected through the CBP followed the protocols listed in CBP (2017). Briefly, above and below pycnocline composite samples were collected and preserved with 5 % Lugol's iodine. Surface and depth samples preserved with 5 % Lugol's iodine were collected during the 2018 and 2019 Delmarva Peninsula coastal cruises. Environmental data (e.g., temperature and salinity) were collected using Hydrolab Series 5 (Hach Environmental, Loveland, CO, USA) or YSI 85, YSI 6600, Pro2030, or ProSolo (YSI, Inc., Yellow Springs, OH, USA) data sondes concurrently with phytoplankton sample collection.

Using light microscopy, all *Karenia* were identified and enumerated to the species level based on overall cell morphology and nuclear shape and placement as described in Yang et al. (2001), Haywood et al. (2004), de Salas et al. (2004a,b), and Steidinger et al. (2008). When identification to the species level was not possible due to staining, cell distortion, or cell orientation, cells were included in a separate category termed '*Karenia* spp.'. An undescribed *Karenia* species, clearly recognizable due to the unique hypocone morphology, resembled "*Karenia* sp.

#3", as described by Steidinger et al. (2008); this naming convention was retained for this work. Cell concentration data were plotted using ArcGIS Pro 3.0.3 (Esri, Redlands, CA, USA) and cell concentrations were binned as absent, background (100 – 1000 cells L^{-1}), low (>1000 to 10,000 cells L^{-1}), medium (>10,000 to 100,000 cells L^{-1}) and high (>100,000 cells L^{-1}) (see Table 1).

2.2. Delaware

Between May and November 2007 – 2022, live 250-mL phytoplankton samples were collected at least monthly through the University of Delaware's Citizen Monitoring Program (UD CMP) at 26 stations (Fig. 1) located between Broadkill River (38.7904 N, -75.1635 W) and Fenwick Island (38.4519 N, -75.0493 W). Sampling frequency and number of stations sampled increased during HAB events, per the UD CMP guidelines (Wheratt et al., 2004). Phytoplankton samples were examined within six hours of collection using American Optical Corporation Series 50 compound microscopes (American Optical Corporation, Buffalo, NY, USA) and enumerated following a modification of the Andersen and Thronksen (2003) droplet method as described in Main et al. (2018). Additional samples were collected at Delaware beaches and inlets by Delaware Department of Natural Resources and Environmental Control (DNREC) staff and preserved with 5 % Lugol's iodine. For these samples, 1 – 3-mL aliquots were settled for a minimum of 30 min in Nunc coverglass-bottom chambers (#155379, Nunc, Rochester, NY, USA) and the entire chamber was examined using a Nikon TMS-F inverted microscope (Nikon Instruments Inc., Melville, NY, USA).

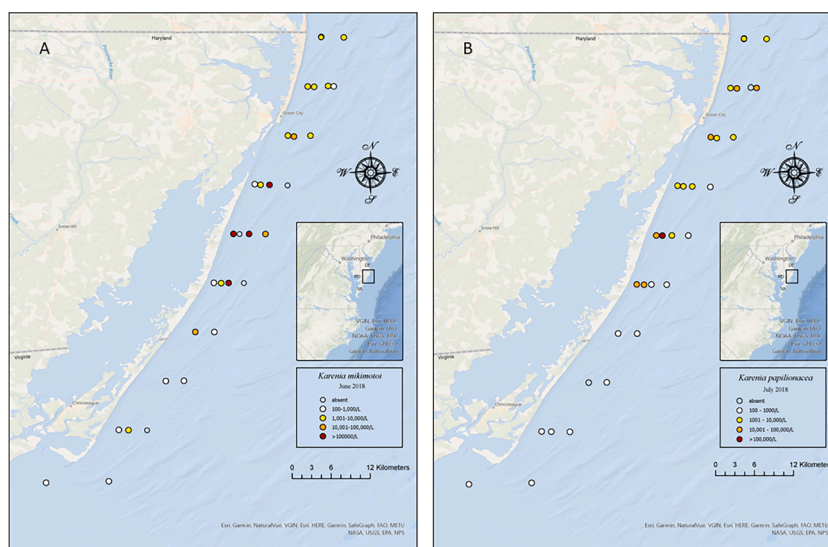


Fig. 2. Maps of the coastal transects sampled during the 2018 and 2019 Delmarva cruises conducted by UMCES. A. Distribution and cell concentrations of the *Karenia mikimotoi* bloom detected in June 2018. B. Distribution and cell concentrations of the *K. papilionacea* bloom detected in July 2018.

Table 1

Maximum cell L^{-1} concentration and (month) of observation for *Karenia* spp. detected in Delmarva Peninsula and Chesapeake Bay water samples collected between 2007 and 2022. Included are bloom concentrations for each species reported from literature describing more than five years of routine monitoring data.

	Delaware	Maryland	Virginia	Bloom Concentration
<i>K. brevis</i> -like	50,000 (Sep)	14,000 (Aug)	n/a	10 ³ (Steidinger, 2009)
<i>K. mikimotoi</i>	10,000 (Apr, Jun, Jul)	42,000 (Aug)	2,800 (June)	10 ³ (Li et al., 2019)
<i>K. papilionacea</i>	2,062,000 (Sep)	233,300 (Aug)	5,825,300 (Sep)	10 ³ (Yamaguchi et al., 2016)
<i>K. selliformis</i>	n/a	19,900 (Jun)	100 (Oct)	10 ⁵ (Feki-Sahnoun et al., 2017)
<i>Karenia</i> sp. #3	n/a	9,300 (Aug)	n/a	n/a

2.3. Maryland

Monthly monitoring 1.5 km off the Maryland coastline was conducted between April and October from 2014 through 2021 at five stations that are part of the Maryland Department of Environment (MDE) shellfish classification program. Stations were located near the Maryland–Delaware state border (38.4508 N, –75.0326 W) to south of Ocean City (38.2111 N, –75.1286 W). For this program, live 1-L phytoplankton samples were collected from the surface (0.5–1 m) and/or the depth of the chlorophyll maximum (ranging from 1.4–4.9 m). Between 2014 and 2020 year-round, live 1-L surface water phytoplankton samples were collected monthly from 16 stations within the Maryland coastal bays and their major tributaries by the Maryland Department of Natural Resources (MDNR) (Fig. 1).

All samples were examined at MDNR using Nunc coverglass-bottom chambers (#155379, Nunc, Rochester, NY, USA) and a Zeiss Axiovert 200 inverted microscope (Carl Zeiss Inc., Thornwood, NY, USA) equipped with an Olympus DP73 digital camera system (Olympus America, Center Valley, PA, USA). Nunc chambers were used to observe live material the day of collection to resolve species identifications. After observations of live material, samples were preserved with 5 % Lugol's iodine and 3-mL aliquots were settled in Nunc chambers for a minimum of 30 min before enumeration. Cells were enumerated using the modified Utermöhl method (Utermöhl, 1958) described by Marshall and Alden (1990).

2.4. Virginia

As part of the CBP monitoring program, on-going from 1985 to present, depth-integrated water samples are collected monthly from above and below the pycnocline at seven stations in the Virginia portion of the Chesapeake Bay mainstem and seven stations in the tidal portions of its tributaries (see CBP (2017) for full sampling methodology) (Fig. 1). Phytoplankton samples (500 mL) were preserved with 5 % Lugol's iodine and returned to the Old Dominion University Phytoplankton Analysis Laboratory (ODU PAL) for analysis. Phytoplankton were concentrated using a series of siphoning and settling steps (Marshall and Alden, 1990; CBP, 2017). Cells were identified and enumerated in 25-mL Utermöhl settling chambers (#435023, Hydro-Bios, Altenholz, Germany) using a Nikon Eclipse TS 100 inverted microscope equipped with a Nikon DS-Fi digital camera system (Nikon Instruments Inc., Melville, NY, USA) following the modified Utermöhl method described by Marshall and Alden (1990).

Between 2007 and 2021, the Virginia Department of Health's Division of Shellfish Safety and Waterborne Hazards (VDH DSSWH) collected 250-mL phytoplankton samples monthly from surface waters (0.5–1 m) at 69 stations located in shellfish growing areas in the Virginia coastal bays and the Chesapeake Bay and its tributaries (Fig. 1). Samples were preserved with 5 % Lugol's iodine solution and returned to the ODU PAL for identification and enumeration of a targeted group of HAB species. For these samples, 4-mL samples were placed in Cellvis 12-well glass bottom plates (#P12–1.5H–N, Cellvis, Mountain View, CA, USA) and screened using an Olympus CK41 inverted microscope equipped with an Olympus SC30 digital camera (Olympus America, Center Valley, PA, USA) following the protocol described in Pease et al. (2023).

2.5. 2018 and 2019 Delmarva Peninsula Coastal cruises

Environmental parameters and samples for phytoplankton community composition analysis were collected during five research cruises in June, July, and October of 2018 and May and July 2019 aboard the R/V *Rachel Carson* or the R/V *Joanne Daiber*. Two to four stations along ten transects, approximately 0.8 to 2.4 km off the Delmarva Peninsula coast, were sampled during these cruises (Fig. 2). The northernmost transects started off the coast of Fenwick Island, Delaware (38.44483 N, –75.0290 W) and the southernmost transects ended south of Assateague

Island, Virginia (37.82906 N, –75.40963 W). Phytoplankton samples, collected at the surface (0.5–1.0 m) and chlorophyll maximum depths (ranging from 2.3 to 14.5 m) using 10-L Niskin bottles on a CTD rosette, were fixed on-site with 5 % Lugol's iodine solution. Samples were examined for phytoplankton community composition and abundance at MDNR using a Zeiss Axiovert 200 inverted microscope and 10-mL Utermöhl settling chambers (#435022, Hydro-Bios, Altenholz, Germany) following the modified Utermöhl method of Marshall and Alden (1990).

3. Results

3.1. Species identification

Five *Karenia* species were routinely identified along the Delmarva Peninsula coastline and in the Chesapeake Bay between 2007 and 2022. Cells identified as *K. brevis*-like (Fig. 3A) resembled *K. brevis* as described by Steidinger et al. (2008), particularly in cell size, nuclear shape and placement (round and located in the left hypotheca), and a slightly excavated hypotheca. However, these cells were only found during blooms of *K. papilionacea*. It is possible they are small-cell forms of *K. papilionacea* as reported by Fowler et al. (2015) or are like the *K. papilionacea* phylotype-I described by Yamaguchi et al. (2016), both of which have a morphological appearance more similar to *K. brevis* than *K. papilionacea* as described by Haywood et al. (2004). Large *K. papilionacea* cells (Fig. 3B) were identified using the overall butterfly-like cell shape, deeply excavated hypotheca, and pointed apical carina as described by Haywood et al. (2004) and Steidinger et al. (2008), whereas small *K. papilionacea* cells were identified by the deeply excavated hypotheca and a slightly pointed apical carina (Fig. 3C).

Both *K. mikimotoi* and *K. selliformis* Haywood Steidinger & MacKenzie cells were identified as described by Steidinger et al. (2008). *Karenia mikimotoi* cells (Fig. 3D) were ovoid in shape with a round to elongate nucleus located along the left side of the cell. *Karenia selliformis* cells (Fig. 3E) were recognized by a wide and deep invagination of the hypotheca and an elongated nucleus located horizontally within the hypotheca. The cells identified as *Karenia* sp. #3 (Fig. 3F) resembled the descriptions given by Steidinger et al. (2008) and de Salas (2004; as “cf. *Karenia* sp. D”). This species is smaller than the other *Karenia* spp., has a deeply invaginated hypotheca with a wide sulcus, a kidney bean shaped nucleus in the epitheca, and few peripherally located chloroplasts.

Light micrographs of living cells were not taken as part of this effort. However, high quality live micrographs of these same species can be reviewed in de Salas et al. (2004b), Haywood et al. (2004), and Steidinger et al. (2008).

3.2. *Karenia* abundance and distribution

3.2.1. Delaware

The first *Karenia* spp. bloom documented in the Delmarva Peninsula region was observed beginning on 30 August 2007 in the Indian River Inlet area of Delaware where medium concentrations of *K. papilionacea* (6.7×10^4 cells L^{-1}) were detected. Monitoring of Delaware coastal and Inland Bay waters continued for one month thereafter even though the bloom appeared to have dissipated from the region after 12 September. The peak *K. papilionacea* concentration during this bloom (2.1×10^6 cells L^{-1}) occurred on 6 September, 1.6 km east of the Indian River Inlet. The bloom was patchy spatially and temporally along the Delaware coastline, but where present, the average *K. papilionacea* cell concentration was 1.3×10^5 cells L^{-1} . Of note, *K. brevis*-like cells were observed during the peak of the *K. papilionacea* bloom (September 5–11) at an average concentration of 4.1×10^4 cells L^{-1} , and most commonly occurred in the high salinity (31–32) waters offshore of the Indian River Inlet.

Following the 2007 *Karenia* bloom, surface waters (~0.5 m) at 26 stations along the coast and within the Delaware Inland Bays (Fig. 1)

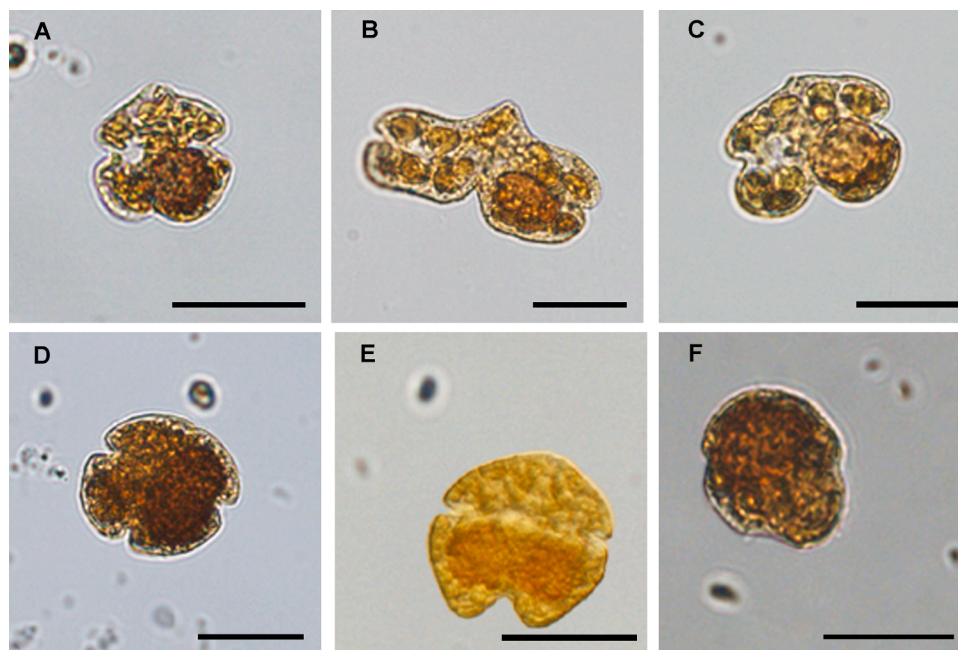


Fig. 3. Light micrographs of *Karenia* species detected in water samples collected in Delmarva Peninsula waters between 2007 and 2022. A. *K. brevis*-like. B. Large cell form of *K. papilionacea*. C. Small cell form of *K. papilionacea*. D. *K. mikimotoi*. E. *K. selliformis*. F. Unidentified *Karenia* species, termed *Karenia* sp. #3, as described by Steidinger et al. (2008) and de Salas (2004).

were routinely monitored for the presence of *Karenia* spp. Between 2008 and 2022, *K. brevis*-like cells were rarely detected, but when observed they were in background to low concentrations (100 – 10,000 cells L^{-1}) within a larger *K. papilionacea* population. *Karenia mikimotoi* has been observed in Delaware waters since 2019, but always at low cell concentrations. Neither *K. selliformis* nor *Karenia* sp. #3 have been detected in Delaware waters.

Karenia papilionacea was found in all coastal routine monitoring stations but occurred less frequently within the Delaware Inland Bays. The majority of observations ($n = 163$) made between 2007 and 2022 contained background to low concentrations of this species. Cell concentrations of *K. papilionacea* blooms that occurred in Delaware coastal waters in 2010 and 2016 were on the order of 10^5 cells L^{-1} but no adverse environmental impacts were reported. The *K. papilionacea* blooms documented in all three years (2007, 2010, and 2016) were centred on the region between the Indian River Inlet and Fenwick Island (38.6782 N, –75.0685 W to 38.4519 N, –75.04933 W) between late August and early October (Table 2).

3.2.2. Maryland

Analysis of water samples collected from surface (0.5 to 1.0 m) and/or chlorophyll maximum depths (1.8 to 22.0 m) at five offshore stations indicated the presence of multiple *Karenia* species in at least background concentrations between 2014 and 2022 (Fig. 1). However, nearly monospecific blooms of *K. papilionacea*, with cell concentrations between 10^3 and 10^5 cells L^{-1} , were noted in the late summers of 2016, 2018, and 2019. From April through June, *K. mikimotoi* (average $5.2 \times$

10^3 cells L^{-1}) and *K. selliformis* (average 5.0×10^2 cells L^{-1}) were the most commonly occurring *Karenia* species. Between July and October, *K. papilionacea* (average 5.0×10^4 cells L^{-1}) was the most commonly occurring species, but *K. mikimotoi*, *Karenia* sp. #3, and cells identified as *K. brevis*-like routinely co-occurred within *K. papilionacea* blooms in background concentrations. The maximum cell concentration of *K. papilionacea* (2.3×10^5 cells L^{-1}) recorded from offshore monitoring efforts in Maryland waters occurred on 15 August 2016, within a bloom extending along a broad swath of the coastline north and south of Ocean City, Maryland, from at least 38.3978 N to 38.2898 N latitude (Tables 1 and 2). While concentrations of *K. papilionacea* ranged from 1.1 to 2.3×10^5 cells L^{-1} in bloom waters, water samples were negative for the brevetoxin congener PbTx-2 (data not shown) and no adverse environmental impacts were reported.

Within the Maryland coastal bays, *Karenia* species have been documented since 2016 in the surface waters (~ 0.5 m) at four of the 16 routinely monitored stations. Background concentrations (≤ 1000 cells L^{-1}) of *K. selliformis* have been reported in Chincoteague Bay in the months of April and May. In the Isle of Wight Bay, background (300 cells L^{-1}) to medium (3.1×10^4 cells L^{-1}) cell concentrations of *K. papilionacea*, *K. mikimotoi*, and *K. selliformis* have been reported between May and October.

3.2.3. Virginia

In 2017, the first observations of *K. papilionacea* on record in the Chesapeake Bay were made in samples collected near the bay mouth in August, September, and October at two CBP long-term monitoring

Table 2

Geographic range, number of occurrences, and salinity and temperature ranges reported for each *Karenia* species detected along the Delmarva Peninsula and within Chesapeake Bay samples collected from 2007 through 2022 via state agency and citizen science monitoring efforts.

Species	Geographic Range (north to south)	Number and (Timing) of Occurrences	Salinity (ppt)	Temperature ($^{\circ}C$)
<i>K. brevis</i> -like	38.79 N, –75.16 W to 38.32 N, –75.061W	17 (Aug – Nov)	27.4 – 32.9	16.5 – 26.0
<i>K. mikimotoi</i>	38.79 N, –75.16 W to 38.28 N, –75.09W	25 (May – Dec)	19.0 – 30.9	7.0 – 26.7
<i>K. papilionacea</i>	38.79 N, –75.16 W to 36.99 N, –76.01 W	239 (Jun – Nov)	23.7 – 33.5	8.9 – 26.5
<i>K. selliformis</i>	38.45 N, –75.03 W to 38.09 N, –75.28W	14 (Apr – Jul)	24.6 – 29.4	15.5 – 20.8
<i>Karenia</i> sp. #3	38.37 N, –75.13 W to 38.21 N to –75.12W	7 (Jun – Oct)	19.7 – 30.9	16.4 – 28.3

stations (Fig. 1). At station CB 7.4 (36.9933 N, -76.0106 W), *K. papilionacea* was found in samples collected above the pycnocline in August (2.1×10^5 cells L^{-1}) and October (3.0×10^4 cells L^{-1}). In October, *K. papilionacea* was also observed below the pycnocline, but at lower concentrations (8.9×10^3 cells L^{-1}). The highest concentration of *K. papilionacea* recorded in Virginia waters, 5.8×10^6 cells L^{-1} (Table 1), was observed in a sample collected above the pycnocline at station CB 7.3E (37.2286 N, -76.0542 W) in September. Despite the elevated *K. papilionacea* cell concentrations, no environmental impacts were reported. Other *Karenia* species were not observed in these samples and no *Karenia* were observed in surface water samples collected by VDH as part of the shellfish monitoring program during that same year. Despite the bloom observed off the Delmarva coast in 2018, no *Karenia* were observed in samples collected at CBP stations or samples collected within the Virginia coastal bays by VDH. The other *Karenia* species observed in Maryland and Delaware waters have not been detected in Virginia's coastal bays or within the Chesapeake Bay since routine monitoring was started in 2007. However, *K. mikimotoi* was recorded in Virginia coastal waters at background concentrations off the southern end of Assateague Island during the 2018 and 2019 cruises.

3.2.4. 2018 and 2019 Delmarva Peninsula coastal cruises

In the summer and autumn of 2018, *Karenia* spp. were observed in Delmarva Peninsula coastal waters in both surface samples and samples collected at the depth of the chlorophyll maxima (Table 3; Fig. 2). In June, the bloom was dominated by *K. mikimotoi* (maximum concentration of 1.9×10^5 cells L^{-1}). *Karenia mikimotoi* comprised 98 % of the *Karenia* population and *K. papilionacea* and *K. selliformis* contributed the other 2 %. The *K. mikimotoi* bloom was concentrated along the Maryland coastline off of Assateague Island (Fig. 2A). In July, the bloom was dominated by *K. papilionacea* (maximum concentration of 1.6×10^5 cells L^{-1}), which comprised 96 % of the *Karenia* population. *Karenia brevis*-like cells, *K. mikimotoi*, *K. selliformis*, and *Karenia* sp. #3 were all documented in samples collected during this bloom. The *K. papilionacea* bloom extended over much of the Maryland coastline (Fig. 2B). Severe weather prevented sampling of coastal waters in August and September. When sampling resumed in October, *Karenia* populations had decreased to background concentrations. All previously reported species were present, except cells identified as *K. brevis*-like, and more than 50 % of the *Karenia* population was composed of *Karenia* sp. #3.

Additional cruises were conducted along the same transects in May and July of 2019 but only background concentrations of *K. mikimotoi* and *Karenia* sp. #3 were detected (data not shown). During these cruises, 70 % of *Karenia* observations were from samples collected from at the depth of the chlorophyll maximum or bottom of the water column (3.4 to 11.9 m).

4. Discussion

Phytoplankton communities along the eastern seaboard of the United States have been sporadically documented during research cruises since the early 20th century (Cleve, 1900; Bigelow 1915; Cowles, 1930). Marshall and colleagues conducted extensive *in situ* sampling of the phytoplankton communities in the coastal waters off the

Delmarva Peninsula between the 1960s and 1980s (Marshall, 1969a, 1969b, 1976, 1978, 1982, 1984, 1985, 1986, 1988, 1991; Marshall and Cohn, 1983, 1987; Marshall and Ranasinghe, 1989; Marshall and Shomers, 1990; Marshall et al., 1981; Matta and Marshall, 1984; Wagoner and Marshall, 1991). These studies observed a phytoplankton community dominated year-round by diatoms and cryptophytes with distinct winter and summer dinoflagellate populations, the latter of which increased in abundance offshore during summer blooms. However, there has been limited *in situ* phytoplankton surveillance at the species level in the past three decades. More recent observations of phytoplankton communities have been conducted using Chemtax applications (2004 – 2009; Pan et al., 2011) and satellite remote sensing (2002 – 2016; Moisan et al., 2017). A bi-weekly to monthly microscopy-based phytoplankton community analysis was conducted by Makinen and Moisan (2012) at stations 9.6 km and 40.7 km off of Assateague Island between June 2005 and December 2007. Collectively, these works indicate, that at a functional group level, the phytoplankton community structure observed between 2002 and 2016 was similar to that described between the 1960s and 1980s by Marshall and colleagues.

Marshall (1982) made the first report of *Karenia* (as *Gymnodinium brevis*) from coastal waters in the mid-Atlantic region in June 1980 from samples collected between the mouth of Chesapeake Bay, Virginia and Oregon Inlet, North Carolina. In November 1987, a *K. brevis* bloom was transported to the North Carolina coast via the Gulf Stream where it persisted for approximately four months (Tester et al., 1991). This *K. brevis* bloom, reported as *G. brevis*, contained other *Gymnodinium* species (West et al., 1996), which may represent organisms now identified as other *Karenia* species (Daugbjerg et al., 2000; de Salas, 2004; Haywood et al., 2004). Two decades later a red tide, expatriated from Florida and traveling within the Gulf Stream, was transported to Delaware's coastal waters where it was observed in the late summer of 2007 (Walsh et al., 2009) and determined to be dominated by *K. papilionacea* with the presence of *K. brevis*-like cells (Bott, 2014). Portions of this same exported bloom persisted from September 2007 through January 2008 in the coastal bays on Florida's east coast (Walsh et al., 2009) and contained multiple *Karenia* species, including *K. brevis*, *K. mikimotoi*, *K. papilionacea*, *K. selliformis*, and the undescribed *Karenia* sp. #3 (Wolny et al., 2015).

Following this 2007 bloom, water quality and HAB monitoring programs in the Delaware and Maryland portion of the Delmarva Peninsula have routinely detected these same five *Karenia* species in summer and autumn offshore and coastal bay phytoplankton populations ranging from background ($100\text{--}1000$ cells L^{-1}) to bloom level ($>100,000$ cells L^{-1}) concentrations. However, the origins of these populations are unknown. Using a genetic analysis, Coyne et al. (2015) detected the presence of a *K. papilionacea* strain, unique from Gulf of Mexico and New Zealand strains, in the shelf waters off Delaware in June 2015 and then along the coast in July 2015, indicating a potential regional offshore source for coastal blooms. Liu et al. (2020; 2021) showed that cyst production and sequestering in sediments is a possible source of the *K. mikimotoi* blooms in the coastal waters of China, but cysts have not been documented in other *Karenia* species (Persson et al., 2013; Feki-Sahnoun et al., 2017) and were not detected in Delaware coastal sediments (Coyne et al., 2015). Ballast water could also be an

Table 3

Abundance, location, timing, and the salinity and temperature ranges reported for each *Karenia* species detected offshore the Delmarva Peninsula during the 2018 coastal cruises.

Species	Maximum cell L^{-1}	Offshore Area of Occurrence	Timing	Salinity	Temperature(°C)
<i>K. brevis</i> -like	1.10×10^3	DE, MD	July	29.2 – 30.3	24.0 – 26.4
<i>K. mikimotoi</i>	1.91×10^5	DE, MD, VA	June, July, October	28.1 – 33.4	19.4 – 25.1
<i>K. papilionacea</i>	1.59×10^5	DE, MD, VA	June, July, October	28.3 – 33.4	21.8 – 26.4
<i>K. selliformis</i>	1.99×10^4	DE, MD, VA	June, July, October	28.5 – 33.3	21.3 – 25.4
<i>Karenia</i> sp. #3	1.50×10^3	DE, MD	July, October	30.0 – 32.9	23.4 – 24.3

introduction mechanism of *Karenia* spp. to the Delmarva Peninsula region. Thousands of commercial vessels visit ports in both Chesapeake Bay (Carney et al., 2017) and Delaware Bay (Altiok et al., 2012) annually. Carney et al. (2017) reported that ballast water discharges to the Chesapeake Bay increased by 374 % between 2005 and 2013 and an earlier study by Drake et al. (2005) indicated that 15 % of commercial vessel traffic to this region came from Florida ports. However, Garrett et al. (2011) did not find *Karenia* spp. cells or cysts in ballast water or sediment samples collected from commercial vessels docked in Tampa Bay ports during a 3-year study conducted to determine the possibility of HAB species, including *Karenia* spp., being moved into or out of Florida waters from ballast exchange operations. Finally, Tester and Steidinger (1997), Walsh et al. (2009), and Weisberg et al. (2019) demonstrated that *K. brevis* blooms (and other *Karenia* species contained therein; Wolny et al., 2015) can be exported out of the Gulf of Mexico via the Loop Current and transported along the United States east coast via the Gulf Stream. While this latter mechanism is the most likely source of the Delmarva Peninsula *Karenia* blooms, the lack of routine monitoring of coastal and shelf waters precludes a broader understanding of the origins of *Karenia* populations in the mid-Atlantic region and spatially- and temporally-rigorous sampling should be considered in future monitoring and research programs to answer this question.

The different environmental conditions wherein each *Karenia* species have been observed suggests that each species may have its own environmental niche in the mid-Atlantic region (Tables 2 and 3). In the Gulf of Mexico, *K. brevis* is the most successful of the *Karenia* species based on its numerical and spatial dominance across a range of elevated temperatures (20 – 28 °C) and salinities (31 – 37) observed there (Steidinger, 2009). Based on data collected during the 2018 and 2019 Delmarva Peninsula coastal cruises and regional monitoring programs, *K. mikimotoi* occupies the broadest temperature and salinity niche space (7.0 – 26.7 °C and 19.7 – 30.9 salinity) of the *Karenia* species. However, routine monitoring data suggest that *K. papilionacea* is the most frequently detected and abundant *Karenia* species in the mid-Atlantic region of the United States ($n = 239$ observations, maximum concentration = 5.8×10^6 cells L^{-1}). Targeted monitoring of coastal waters and kinetic studies using cultured isolates from contrasting environments would help shed light on the optimal niche space for each *Karenia* species as other mid-Atlantic HAB species (e.g., *Chattonella* spp., *Heterosigma akashiwo*, *Margalefidinium polykrikoides*) are known to occupy different niche spaces than their global counterparts (Handy et al., 2005; Zhang et al., 2006; Mulholland et al., 2009).

Both the historical work in the mid-Atlantic region conducted by Marshall and Cohn (1983, 1987) and Wagoner and Marshall (1991) and the more recent studies by Pan et al. (2011) and Moisan et al. (2017) indicate that summer dinoflagellate populations, and their fractional contribution to the total chlorophyll, increase from the coastline towards the shelf, with the greatest concentrations at the mid-shelf region (Moisan et al., 2017) and to the south of the Chesapeake Bay mouth (Wagoner and Marshall, 1991). This area has largely been devoid of systematic, high frequency *in situ* monitoring of phytoplankton populations. Satellite imagery processed by Moisan et al. (2017) shows seasonally high concentrations of the carotenoids 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin, pigments often used as a proxy measurement for haptophytes (Latasa, 2007), but these are also the principal accessory pigment of species within the Kareniaceae (Steidinger et al., 2008), whose presence on the shelf has not been regularly investigated. The intrusion of *Karenia* populations to the mid-Atlantic region has vast implications for resource management. The impacts of these blooms depend upon the relative concentrations of each *Karenia* species, the relative rates of toxin production, and the differential toxicity to aquatic communities. Currently, resource management strategies, such as the National Shellfish Sanitation Program (NSSP) guidelines, are based on the cell abundance and brevetoxin concentrations associated with *K. brevis*, which has not yet been observed in actionable concentrations (≥ 5000 cells L^{-1} ; NSSP, 2017) in the

Delmarva Peninsula's shellfish harvesting areas. Using LC-MS, Fowler et al. (2015) reported that *K. papilionacea* strains established from a Delaware population produce PbTx-2, though at a significantly lower concentration per cell compared to *K. brevis*. *Karenia papilionacea* is suspected to be toxigenic in other parts of the world (Haywood et al., 2004; Amzil et al., 2021) however, whole water samples collected during nearly monospecific *K. papilionacea* blooms on the Maryland coast, with cell concentrations (1.1 to 2.3×10^5 cells L^{-1}) at or greater than those reported as toxic by Fowler et al. (2015; 1.0×10^5 cells L^{-1}), were negative for brevetoxin.

The toxicity of local *K. mikimotoi* blooms has not been assessed, although hemolytic activity has been reported in cells cultured from Texas blooms (Neely and Campbell, 2006) and *K. mikimotoi* blooms from other regions have caused significant fish kills and economic losses (Li et al., 2019; Sakamoto et al., 2021). Since the 1990s, *K. selliformis* has caused significant fish kills and shellfish toxicity events in Mediterranean waters due to the presence of gymnodimines (Feki et al., 2013). More recently, massive marine life mortality events have been associated with *K. selliformis* blooms in Chilean coastal waters, around Hokkaido Island, Japan, and the Kamchatka, Russia coastline but the mode of toxicity has not been elucidated (Mardones et al., 2020; Iwataki et al., 2022; Orlova et al., 2022). The toxin profile of *K. selliformis* populations found in US waters has not been explored. Culturing and characterizing these species, along with the commonly occurring, yet unidentified *Karenia* sp. #3, is necessary to better relate toxin production by species to environmental variables and to establish a risk management strategy.

The cell concentrations of mid-Atlantic *Karenia* populations (10^5 cells L^{-1}) are less than those of catastrophic *Karenia* blooms reported globally ($10^6 - 10^7$ cells L^{-1} ; Steidinger 2009; Vandersea et al., 2020; Hu et al., 2022; Iwataki et al., 2022; Orlova et al., 2022), but are of equal concentrations to the *K. mikimotoi* blooms that impacted shellfish resources in the Gulf of Maine (10^5 cells L^{-1} ; Scully et al., 2022). Despite the occurrence of elevated concentrations of *Karenia* spp. on the Delmarva Peninsula coast at temperature and salinity ranges in which other

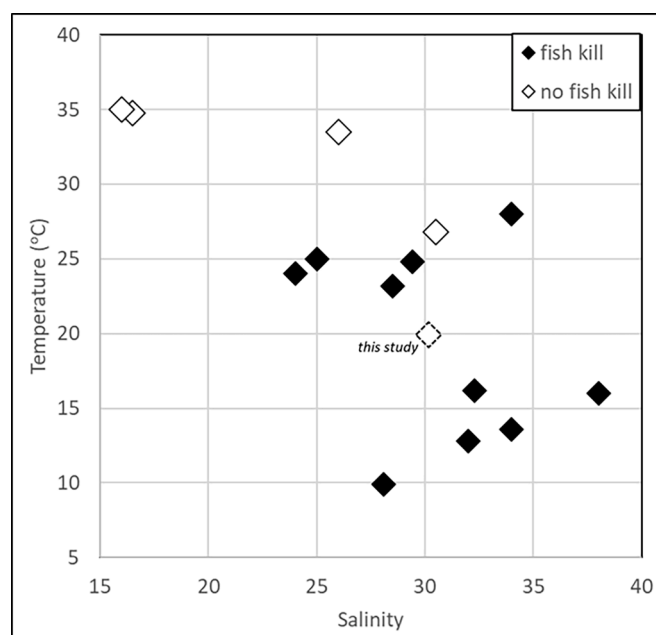


Fig. 4. Scatterplot of average temperature and salinity data reported in the literature for fishery impacting (black diamonds) and fishery non-impacting (white diamonds) *Karenia mikimotoi* blooms. Data from this study are represented in the dashed line white diamond. The data presented here were collected from this study, the references listed in Table 4, and Matsuyama (2006), Robin et al. (2013), PIRSA (2014), O'Boyle et al. (2016), Shimada et al. (2016), Aoki et al. (2017), Vandersea et al. (2020), Baohong et al. (2021), (Onitsuka et al. (2021), and Iwataki et al. (2022).

Karenia blooms have caused fish kills (see example for *K. mikimotoi* in Fig. 4), no adverse environmental or human health problems have been reported to date. This is not unlike other toxic, bloom-forming species found in the Delmarva Peninsula region. For reasons unknown, species of *Dinophysis* and *Pseudo-nitzschia* are seasonally present at cell concentrations of concern but have not been associated with negative environmental impacts due to reduced toxin production compared to their global counterparts (Thessen and Stoecker, 2008; Wolny et al., 2020a; Ayache et al., 2023). From a limited number of reports, it appears that not all *Karenia* blooms have been associated with harmful environmental impacts (Table 4). Similarly, not all cultured *Karenia* strains maintain the ability to produce toxins (Sunda et al., 2013), which Lekan and Tomas (2010) linked to inherent genetic differences between cultured strains, indicating toxicity variation within natural populations is possible. The genetic assessment conducted by Coyne et al. (2015) demonstrated that the Delaware *K. papilionacea* population was distinct from the Gulf of Mexico and New Zealand populations, which coupled with different environmental conditions, may explain the lack of or reduced toxin production by the population found here (Fowler et al., 2015) compared to *K. papilionacea* populations reported to be toxic by Haywood et al. (2004) and Amzil et al. (2021). The long-term shifting of environmental conditions may play a role in the toxicity of numerous HAB species (Griffith and Gobler, 2020; Wells et al., 2020; Anderson et al., 2021). Specifically, for *Karenia*, climate change is being correlated to changes in the toxicity of *K. brevis* and *K. selliformis* (Brandenburg et al., 2019) and *K. mikimotoi* and *K. papilionacea* (Kwok et al., 2016) blooms.

Until local representatives of each *Karenia* species are isolated and cultured and controls on their growth and production of bioactive compounds assessed, their potential significance as a toxin source in the current and predicted conditions for the Chesapeake Bay, Delmarva Peninsula, and mid-Atlantic shelf waters cannot be determined. Further, until we better surveil mid-Atlantic waters for the presence of *Karenia* spp., their potential impacts to aquatic and coastal communities cannot be addressed. At present, phytoplankton collections along Delaware's coast are limited to a volunteer-based HAB monitoring program and no routine offshore monitoring is being conducted. Maryland conducts temporally- and spatially-limited phytoplankton monitoring offshore and within its coastal bays. Virginia phytoplankton monitoring is confined primarily within Chesapeake Bay, with limited sampling occurring in the Virginia coastal bays, and no routine offshore monitoring. The data compiled and presented here are from limited *in situ* monitoring activities along the Delmarva Peninsula coast. These data suggest there has been a routine occurrence of several *Karenia* spp. for the past 15 years, although no adverse environmental impacts have been reported. Because data are sporadic, it is difficult to assess whether *Karenia* spp. blooms are of emerging concern in this region. Spatially and temporally robust HAB monitoring programs in this region would help fill data gaps and allow resource managers to assess the potential for a *Karenia* population to impact fishery and recreational resources in the

mid-Atlantic region. Climate change is altering mid-Atlantic niche environments and oceanographic patterns (Saba et al., 2016), and could alter the relative distribution, success, and toxicity of *Karenia* in the mid-Atlantic region as it has for other regional harmful algal species (Vidyarathna et al., 2020).

The ultimate causes of the spatial and temporal patterns of *Karenia* spp. observed during the 2018 Delmarva cruises are unknown, but likely reflect differences in each *Karenia* species' environmental preferences, as well as differing physical and environmental forces acting on each species. In a recent study by Kim et al. (2023) in Korean waters, co-occurring *K. mikimotoi* and *K. papilionacea* blooms were documented in the months of March through July, similar to the observations made along the Delmarva Peninsula in 2018. Studies conducted on these species, both in the Korean Strait and the East China Sea, indicate optimal growth occurs between 20 – 24 °C, but that the growth rate of *K. papilionacea* is greater than that of *K. mikimotoi* (Baohong et al., 2021; Kim et al., 2023). Both Vandersea et al. (2020) and Scully et al. (2022) showed that the blooms of *K. mikimotoi* observed in Kachemak Bay, Alaska, and Cape Cod Bay, Massachusetts, respectively, coincided with these regions' maximum water column stratification period. Both studies also noted sufficient nutrient pools at depth, which were exploited by *K. mikimotoi* as the populations underwent diel vertical migration. Similar dynamics have been described for *K. papilionacea* blooms in Japanese coastal waters by Yamaguchi et al. (2016). Bottom phytoplankton populations were not evaluated as part of the 2018 cruises, however, bloom concentrations of both *K. mikimotoi* and *K. papilionacea* were noted from samples collected at the depth of the chlorophyll maximum in June (5.3-11.5 m) and July (6-7.7 m), respectively. In 2019, cruises conducted in May and July failed to find a surface population of *Karenia* but did detect background concentrations of *K. mikimotoi* and *Karenia* sp. #3 in at-depth waters (3.4-11.9 m). Of note, *K. papilionacea* was detected in elevated concentrations at the pycnocline (5 m) in the lower Chesapeake Bay in the late summer and early autumn of 2017, indicating that perhaps a larger, undetected population was advecting into the Bay from offshore, deep waters or that the bloom was concentrated at depth to take advantage of a nutrient pool. Physical forcings, such as those proposed for the 2018 Gulf of Mexico *K. brevis* bloom by Weisberg et al. (2019), in which an over-wintering population was reseeded with a new bloom population or nutrient regeneration from N₂ fixation, decaying fish, and other processes (Mulholland et al., 2004; 2006; 2014; Walsh et al., 2009; Bronk et al., 2014; Heil et al., 2014), may play a role in the species diversity and cell concentrations seen here as in other areas where *Karenia* blooms are known to persist.

Dating back to 2007 in Delaware (Bott, 2014) and 2012 in Maryland (J. O'Neil and J. Wolny, unpublished data), multiple *Karenia* species have been annually reported at background to low cell concentrations (100 to < 10,000 cells L⁻¹) in summer to early autumn samples collected as part of each state's coastal HAB monitoring and offshore research programs. Based on a limited number of samples, *Karenia* appear to

Table 4
Published reports of non-harmful *Karenia* blooms.

Species	Maximum cell L ⁻¹	Area of Occurrence	Timing	Average Salinity	Average Temperature (°C)	Reference
<i>K. longicanalis</i>	1.8 × 10 ⁵	Victoria Harbour, Hong Kong	May	30	n/a	Yang et al., 2001
<i>K. mikimotoi</i>	10 ⁴	English Channel	August	34.75	16.5	Zevenboom et al., 1991*
	3.9 × 10 ⁴	Hoketsu Bay, Japan	August	33.5	26	Koizumi et al., 1996**
<i>K. papilionacea</i>	1.5 × 10 ⁷	Cochin Estuary, India	October	26.8	30.5	Madhu et al., 2011
	1.4 × 10 ⁶	English Channel	July	35	16	Hartman et al., 2014
	1.9 × 10 ⁵	Delmarva Coast	June	27.9	19.6	this study
	1.8 × 10 ⁴	Arabian Gulf	January	41.6	14.0	Al-Yamani et al., 2012
	6.1 × 10 ³	Kochi Prefecture, Japan	May, June	33.4	29.2	Yamaguchi et al., 2016
	3.2 × 10 ⁷	Kuwait Bay	April	41.6	23.8	Polikarpov et al., 2020
	2.1 × 10 ⁶	Delmarva Coast	July	25.5	20.9	this study
	5.8 × 10 ⁶	Chesapeake Bay	September	26.3	23.2	this study

*Data published using *Gyrodinium aureolum* as species name. **Data published using *Gymnodinium mikimotoi* as species name.

occur less frequently in Virginia coastal waters. However, *Karenia* blooms do occur in mid-Atlantic waters as evidenced by high concentrations of *K. papilionacea* found in the Chesapeake Bay, from samples collected near the Bay mouth and Virginia's eastern shore, and blooms of both *K. mikimotoi* and *K. papilionacea* found along the Delaware and Maryland coasts in 2010, 2016, 2018, and 2019. The *K. papilionacea* bloom documented in 2016 occurred along both Delaware and Maryland coastlines but was not reported in Virginia waters. In 2017, when *K. papilionacea* was reported in the Chesapeake Bay for the first time, concentrations were equal to bloom concentrations of *K. brevis* in the Gulf of Mexico (10^6 cells L^{-1} ; Steidinger, 2009), but no blooms were reported in either Delaware or Maryland waters. In the summer of 2018, when bloom concentrations of *Karenia* were reported in Maryland's coastal waters, cells were in low abundance in Delaware coastal waters (1000 cells L^{-1} in June and ≤ 7000 cells L^{-1} in July) and absent in the lower Chesapeake Bay and the Virginia coastal bays. The data presented here indicate that along the Delmarva Peninsula *Karenia* blooms are patchy and likely subject to estuarine outflows, prevailing wind patterns, and/or ocean currents. The inclusion of more *in situ* sampling coupled with remote sensing technologies (e.g., regionally tuned satellite algorithms, autonomous monitoring platforms, imaging flow cytobot arrays) and interagency cooperation are needed to detect and track HAB occurrences. Satellite remote sensing is proving useful for detecting and tracking non-*Karenia* HABs in this region (Wolny et al., 2020b; Xiong et al., 2023). Unfortunately, not enough data exist to determine how successful these remote sensing processes are at detecting and tracking *Karenia* blooms along the Delmarva Peninsula even though satellite remote sensing has been used successfully to monitor *Karenia* blooms in other global locations (Jordan et al., 2021; Hu et al., 2022; Iwataki et al., 2022).

5. Conclusions

Karenia populations are routinely present in the mid-Atlantic region, raising concerns that there are now resident offshore populations or populations being introduced seasonally via the Gulf Stream more frequently than historic phytoplankton surveys have shown. Coastal HAB monitoring occurring on the Delmarva Peninsula is spatially- and temporally-limited raising the possibility that the full extent of these blooms are not being detected with current *in situ* sampling efforts. Similar shifts in HAB events have been noted for other locations on the United States eastern seaboard during the same time period of this study. These emerging HABs (i.e., *Pseudo-nitzschia australis* in the Gulf of Maine (Clark et al., 2022), *Dinophysis acuminata* in Long Island Sound (Hattenrath-Lehmann et al., 2013) and Chesapeake Bay and the Delmarva Peninsula's coastal bays (Wolny et al., 2020a; Ayache et al., 2023), and *Aureoumbra lagunensis* in Florida's Indian River Lagoon (Phlips et al., 2021)) have been tied to warming water masses, ocean current variations, and changes in circulation patterns within smaller embayments that impact water residence times. The role that climatic variability (e.g., shifts in the Gulf Stream), environmental niche changes (e.g., warming water temperature), and outflow variations from the Chesapeake Bay and Delaware Bay plumes (Filippino et al., 2009; Saba et al., 2016; Jiang and Xia, 2016; Xu et al., 2020) play in establishing and concentrating *Karenia* blooms in this region of the mid-Atlantic coast are unknown. A toxin risk assessment should be conducted in Maryland and Delaware waters to safeguard aquatic resources even though no adverse events due to *Karenia* blooms have been reported to date. A toxin assessment conducted in the Virginia portion of the Chesapeake Bay in 2019 and 2020 did not find brevetoxin in whole water or shellfish tissues (Pease et al., 2023), but this study excluded Virginia's coastal bays and offshore sites, where *Karenia* would most likely occur. Investigating these questions, alongside evaluating risks to the Delmarva Peninsula's growing aquaculture and coastal fishery industries (Froehlich et al., 2022), will require focused research efforts and *in situ* water quality and HAB monitoring programs with greater spatial and temporal extent than

current capacities.

CRedit authorship contribution statement

Jennifer L. Wolny: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing, Visualization. **Edward B. Whereat:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Todd A. Egerton:** Data curation, Formal analysis, Resources, Visualization, Writing – original draft, Writing – review & editing. **Leah A. Gibala-Smith:** Data curation, Investigation, Writing – review & editing. **John R. McKay:** Investigation, Resources, Writing – review & editing. **Judith M. O'Neil:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Catherine E. Wazniak:** Funding acquisition. **Margaret R. Mulholland:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

www.citizen-monitoring.udel.edu, www.eyesonthebay.dnr.maryland.gov, and <https://datahub.chesapeakebay.net>.

Acknowledgments

This paper is dedicated to Karen A. Steidinger, mentor, dinoflagellate enthusiast, and friend. The HAB community would not be where it is today without her infectious scientific curiosity, visionary approach to leadership, and time spent making us all better students, teachers, and researchers.

Staff at DNREC's Recreational Waters and Shellfish Program, MDE's Shellfish Monitoring Program, MDNR's Annapolis Field Office, ODU's Water Quality Lab, VDH DSSWH, and the crew of the *R/V Fay Slover* are thanked for providing samples and water quality data through their environmental monitoring activities. UMCES and MDNR personnel and the crews of the *R/V Rachel Carson* and the *R/V Joanne Daiber* are acknowledged for their assistance with the 2018 and 2019 cruises. Nicole Minni from the University of Delaware's Water Resource Center is gratefully acknowledged for her assistance with mapping the data. The authors express their gratitude to the many past and present volunteers of the University of Delaware's Citizen Monitoring Program and the two anonymous reviewers whose comments improved this publication. Finally, we would be remiss to not acknowledge Carmelo Tomas (Emeritus Professor, University of North Carolina Wilmington) for his assistance with the initial *Karenia* bloom in 2007.

Funding for the University of Delaware's Citizen Monitoring Program was provided by the Delaware DNREC and Delaware Sea Grant. The Chesapeake Bay Program, Virginia Department of Environmental Quality and VDH DSSWH provided funding for water quality and HAB monitoring programs. Grant # P17AC01540 NPS - Chesapeake Watershed Cooperative Ecosystem Studies Unit; National Park Service, Assateague Island National Seashore to J.M.O. and C.E.W. provided the funding for the 2018 and 2019 coastal cruises.

References

- Al-Yamani, F., Saburova, M., Polikarpov, I., 2012. A preliminary assessment of harmful algal blooms in Kuwait's marine environment. *Aquat. Ecosyst. Health Manag.* 15, 64–72.

- Altiok, T., Almaz, O.A., Ghafoori, A., 2012. Modeling and Analysis of the Vessel Traffic in the Delaware River and Bay area Risk Assessment and mitigation: Final report. Center for Advanced Infrastructure and Transportation, p. 236, 204-RU6532p.
- Amzil, Z., Derrien, A., Terrillon, A.T., Duval, A., Connes, C., Marco-Miralles, F., Nézan, E., Mertens, K.N., 2021. Monitoring the emergence of algal toxins in shellfish: first report on detection of brevetoxins in French Mediterranean mussels. *Mar. Drugs* 19, 393. <https://doi.org/10.3390/md19070393>.
- Andersen, P., Thronsen, J., 2003. Estimating cell numbers. In: Hallegraeff, G.M., Anderson, D.A., Cembella, A.D. (Eds.), *Manual On Harmful Marine Microalgae*. UNESCO Publishing, Paris, France, pp. 99–130.
- Anderson, D.M., Fensin, E., Gobler, C.J., Hoeglund, A.E., Hubbard, K.A., Kulis, D.M., Landsberg, J.H., Lefebvre, K.A., Provoost, P., Richlen, M.L., Smith, J.L., 2021. Marine harmful algal blooms (HABs) in the United States: history, current status and future trends. *Harmful Algae* 102, 101975.
- Aoki, K., Kameda, T., Yamatogi, T., Ishida, N., Hirae, S., Kawaguchi, M., Syutou, T., 2017. Spatio-temporal variation in bloom of the red-tide dinoflagellate *Karenia mikimotoi* in Imari Bay. In: 2014: Factors Controlling Horizontal and Vertical Distribution, 124. *Marine Pollution Bulletin*, Japan, pp. 130–138.
- Ayache, N., Bill, B.D., Brosnahan, M.L., Campbell, L., Deeds, J.R., Fiorenino, J.M., Gobler, C.J., Handy, S.M., Harrington, N., Kulis, D.M., McCarron, P., Miles, C.O., Moore, S.K., Nagai, S., Trainer, V.L., Wolny, J.L., Young, C.S., Smith, J.L., 2023. A survey of *Dinophysis* spp. and their potential to cause diarrhetic shellfish poisoning in coastal waters of the United States. *J. Phycol.* 59, 658–680.
- Baohong, C., Kang, W., Huige, G., Hui, L., 2021. *Karenia mikimotoi* blooms in coastal waters of China from 1998 to 2017. *Estuar. Coast Shelf Sci.* 249, 107034 <https://doi.org/10.1016/j.ecss.2020.107034>.
- Bigelow, H.B., 1915. Exploration of the coast water between Nova Scotia and Chesapeake Bay, July and August 1913, by the U.S. Fisheries Schooner *Grampus*. *Bull. Museum Comp. Zool.* 59, 152–359.
- Botes, L., Sym, S.D., Pitcher, G.C., 2003. *Karenia cristata* sp. nov. and *Karenia bicuneiformis* sp. nov. (Gymnodiniales, Dinophyceae): two new *Karenia* species from the South African coast. *Phycologia* 42, 563–571.
- Bott, M., 2014. Marine Biotoxin Contingency Plan 2014. State of Delaware. Department of Natural Resources and Environmental Control, Division of Watershed Stewardship, p. 18 p.
- Brandenburg, K.M., Velthuis, M., Van de Waal, D.B., 2019. Meta-analysis reveals enhanced growth of marine harmful algae from temperate regions with warming and elevated CO₂ levels. *Glob. Chang Biol.* 25, 2607–2618.
- Bronk, D.A., Killberg-Thoreson, L., Sipler, R.E., Mulholland, M.R., Roberts, Q.N., Bernhardt, P.W., Garrett, M., O'Neil, J.M., Heil, C.A., 2014. Nitrogen uptake and regeneration (ammonium regeneration, nitrification and photofraction) in waters of the West Florida Shelf prone to blooms of *Karenia brevis*. *Harmful Algae* 38, 50–62.
- Carney, K.J., Minton, M.S., Holzer, K.K., Miller, A.W., McCann, L.D., Ruiz, G.M., 2017. Evaluating the combined effects of ballast water management and trade dynamics on transfers of marine organisms by ships. *PLoS One* 12, e0172468. <https://doi.org/10.1371/journal.pone.0172468>.
- Chang, H., 1999. *Gymnodinium brevisulcatum* sp. nov. (Gymnodiniales, Dinophyceae), a new species isolated from the 1998 summer toxic bloom in Wellington Harbor, New Zealand. *Phycologia* 38, 377–384.
- Chesapeake Bay Program, 2017. Methods and Quality Assurance For Chesapeake Bay Water Quality Monitoring Programs. CBP/TRS-319-17, p. 231. May 2017p.
- Clark, S., Hubbard, K.A., Ralston, D.K., McGillicuddy Jr., D.J., Stock, C., Alexander, M.A., Curchitser, E., 2022. Projected effects of climate change on *Pseudo-nitzschia* bloom dynamics in the Gulf of Maine. *J. Marine Syst.* 230, 103737 <https://doi.org/10.1016/j.marsys.2022.103737>.
- Cleve, P.T., 1900. The seasonal distribution of Atlantic plankton organisms. Göteborgs Kungliga Vetenskaps-och Vitterhets-Samhälles Handlingar Fjärde Följden 3, 1–369.
- Cowles, R.P., 1930. A Biological Study of the Offshore Waters of Chesapeake Bay, 46. *Bull. U.S. Bureau of Fisheries*, pp. 277–381.
- Coyne, K.J., Whereat, E., Farestad, M., Wang, Y., Farrell, J., Tomas, C., Rouse, D., 2015. Recurrent blooms of *Karenia papilionacea* in Delaware coastal waters. In: Eighth Symposium on Harmful Algae in the U.S., Long Beach, California, p. 114. November 15–19, 2015pg.
- Dahl, E., Tangen, K., 1993. 25 years experience with *Gyrodinium aureolum* in Norwegian waters [Eds.]. In: Smayda, T.J., Shimizu, Y. (Eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers, B.V., Amsterdam, pp. 15–21.
- Daugbjerg, N., Hansen, G., Larsen, J., Moestrup, Ø., 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmored dinoflagellates. *Phycologia* 39, 302–317.
- de Salas, M.F., 2004. Morphotaxonomy and genetic affinities of five novel, potentially fish-killing, Australian gymnodinioid dinoflagellates. PhD thesis. University of Tasmania, p. 170.
- de Salas, M.F., Bolch, C.J.S., Hallegraeff, G.M., 2004a. *Karenia asterichroma* sp. nov. (Gymnodiniales, Dinophyceae), a new dinoflagellate species associated with finfish aquaculture mortalities in Tasmania, Australia. *Phycologia* 43, 624–631.
- de Salas, M.F., Bolch, C.J.S., Hallegraeff, G.M., 2004b. *Karenia umbella* sp. nov. (Gymnodiniales, Dinophyceae), a new potentially ichthyotoxic dinoflagellate species from Tasmania, Australia. *Phycologia* 43, 166–175.
- Drake, L.A., Jenkins, P.T., Dobbs, F.C., 2005. Domestic and international arrivals of NOBOB (no ballast on board) vessels to lower Chesapeake Bay. *Mar. Pollut. Bull.* 50, 560–565.
- Feki, W., Hamza, A., Frossard, V., Abdennadher, M., Hannachi, I., Jacquot, M., Belhassen, M., Aleya, L., 2013. What are the potential drivers of blooms of the toxic dinoflagellate *Karenia selliformis*? A 10-year study in the Gulf of Gabes, Tunisia, southwestern Mediterranean Sea. *Harmful Algae* 23, 8–18.
- Feki-Sahnoun, W., Hamza, A., Njah, H., Barraj, N., Mahfoudi, M., Rebai, A., Hassen, M. B., 2017. A Bayesian network approach to determine environmental factors controlling *Karenia selliformis* occurrences and blooms in the Gulf of Gabès, Tunisia. *Harmful Algae* 63, 119–132.
- Filippino, K.C., Bernhardt, P.W., Mulholland, M.R., 2009. Chesapeake Bay plume morphology and the effects on nutrient dynamics and primary productivity in the coastal zone. *Estuaries Coasts* 32, 410–424.
- Fleming, L.E., Kirkpatrick, B., Backer, L.C., Walsh, C.J., Nierenberg, K., Clark, J., Reich, A., Hollenbeck, J., Benson, J., Cheng, Y.S., Naar, J., Pierce, R., Bourdelais, A. J., Abraham, W.M., Kirkpatrick, G., Zaias, J., Wanner, A., Mendes, E., Shalat, S., Hoagland, P., Stephan, W., Bean, J., Watkins, S., Clarke, T., Byrne, M., Baden, D., 2011. Review of Florida red tide and human health effects. *Harmful Algae* 10, 224–233.
- Fowler, N., Tomas, C., Baden, D., Campbell, L., Bourdelais, A., 2015. Chemical analysis of *Karenia papilionacea*. *Toxicon* 101, 85–91.
- Froehlich, H.E., Gentry, R.R., Lester, S.E., Rennick, M., Lemoine, H.R., Tapia-Lewin, S., Gardner, L., 2022. Piecing together the data of the U.S. marine aquaculture puzzle. *J. Environ. Manage.* 308, 114623 <https://doi.org/10.1016/j.jenvman.2022.114623>.
- Garrett, M.J., Wolny, J.L., Williams, B.J., Dirks, M.D., Brame, J.A., Richardson, R.W., 2011. Methods for sampling and analysis of marine microalgae in ship ballast tanks: a case study from Tampa Bay, Florida, USA. *Algae* 26, 181–192.
- Griffith, A.W., Gobler, C.J., 2020. Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae* 91, 101590.
- Hamza, A., El Abed, A., 1994. Red Tides in the Gulf of Gabes: Seven Years of Monitoring, 21. *Bulletin de l'Institut National Scientifique et Technique d'Océanographie et de Pêche de Salammbô*, pp. 66–77.
- Handy, S.M., Coyne, K.J., Portune, K.J., Demir, E., Doblin, M., Hare, C.E., Cary, S.C., Hutchins, D.A., 2005. Evaluating vertical migration behavior of harmful raphidophytes in the Delaware Inland Bays utilizing quantitative real-time PCR. *Aquatic Microbial Ecol.* 40, 121–132.
- Harris, R.J., Arrington, D.A., Porter, D., Lovko, V., 2020. Documenting the duration and chlorophyll pigments of an allochthonous *Karenia brevis* bloom in the Loxahatchee River Estuary (LRE), Florida. *Harmful Algae* 97, 101851. <https://doi.org/10.1016/j.hal.2020.101851>.
- Hartman, S.E., Hartman, M.C., Hydes, D.J., Smythe-Wright, D., Gohin, F., Lazure, P., 2014. The role of hydrographic parameters, measured from a ship of opportunity, in bloom formation of *Karenia mikimotoi* in the English Channel. *J. Mar. Syst.* 140, 39–49. Part A.
- Hattenrath-Lehmann, T.K., Marcoval, M.A., Berry, D.L., Fire, S., Wang, Z., Morton, S.L., Gobler, C.J., 2013. The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York waters. *Harmful Algae* 26, 33–44.
- Haywood, A.J., Steidinger, K.A., Truby, E.W., Bergquist, P.R., Bergquist, P.L., Adamson, J., MacKenzie, L., 2004. Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. *J. Phycol.* 40, 165–179.
- Heil, C.A., Dixon, L.K., Hall, E., Garrett, M., Lenes, J.M., O'Neil, J.M., Walsh, B.M., Bronk, D.A., Killberg-Thoreson, L., Hitchcock, G.L., Meyer, K.A., Mulholland, M.A., Procise, L., Kirkpatrick, G.L., Walsh, J.J., Weisberg, R.W., 2014. Blooms of *Karenia brevis* (Davis) G. Hansen & Ø. Moestrup on the West Florida Shelf: nutrient sources and potential management strategies based on a multi-year regional study. *Harmful Algae* 38, 127–140.
- Heil, C.A., Glibert, P.M., Al-Sarawi, M.A., Faraj, M., Behbehani, M., Husain, M., 2001. First record of a fish-killing *Gymnodinium* sp. bloom in Kuwait Bay, Arabian Sea: chronology and potential causes. *Mar. Ecol. Prog. Ser.* 214, 15–23.
- Hu, C., Yao, Y., Cannizzaro, J.P., Garrett, M., Harper, M., Markley, L., Villac, C., Hubbard, K., 2022. *Karenia brevis* bloom patterns on the west Florida shelf between 2003 and 2019: integration of field and satellite observations. *Harmful Algae* 117, 102289. <https://doi.org/10.1016/j.hal.2022.102289>.
- Iwataki, M., Lum, W.M., Kuwata, K., Takahashi, K., Arima, D., Kuribayashi, T., Kosaka, Y., Hasegawa, N., Watanabe, T., Shikata, T., Isada, T., Orlova, T.Y., Sakamoto, S., 2022. Morphological variation and phylogeny of *Karenia selliformis* (Gymnodiniales, Dinophyceae) in an intensive cold-water algal bloom in eastern Hokkaido, Japan. *Harmful Algae* 114, 102204. <https://doi.org/10.1016/j.hal.2022.102204>.
- Jiang, L., Xia, M., 2016. Dynamics of the Chesapeake Bay outflow plume: realistic plume simulation and its seasonal and interannual variability. *J. Geophys. Res.* 121, 1424–1445. <https://doi.org/10.1002/2015JC011191>.
- Jordan, C., Cusack, C., Tomlinson, M.C., Meredith, A., McGeary, R., Salas, R., Gregory, C., Croot, P.L., 2021. Using the Red Band Difference algorithm to detect and monitor a *Karenia* spp. bloom off the south coast of Ireland, June 2019. *Front. Mar. Sci.* 8, 638889 <https://doi.org/10.3389/fmars.2021.638889>.
- Kim, S., Cho, M., Yoo, J., Park, B.S., 2023. Application of a quantitative PCR to investigate the distribution and dynamics of two morphologically similar species, *Karenia mikimotoi* and *K. papilionacea* (Dinophyceae) in Korean coastal waters. *Toxins (Basel)* 15, 469. <https://doi.org/10.3390/toxins15070469>.
- Koizumi, Y., Uchida, T., Honjo, T., 1996. Diurnal vertical migration of *Gymnodinium mikimotoi* during a red tide in Hoketsu Bay. *J. Plankton Res* 18, 289–294.
- Kwok, C.S., Wan, W., Chan, K.K., Xu, S.J., Lee, F.W., Ho, K., 2016. *Karenia mikimotoi*, a rare species in Hong Kong waters, associated with a recent massive fish kill. *Harmful Algae News* 53, 4–5.
- Latasa, M., 2007. Improving estimations of phytoplankton class abundances using CHEMTAX. *Mar. Ecol. Prog. Ser.* 329, 13–21.
- Lekan, D.K., Tomas, C.R., 2010. The brevetoxin and brevenal composition of three *Karenia brevis* clones at different salinities and nutrient conditions. *Harmful Algae* 9, 39–47.

- Li, X., Yan, T., Yu, R., Zhou, M., 2019. A review of *Karenia mikimotoi*: bloom events, physiology, toxicity and toxic mechanism. *Harmful Algae* 90, 101702. <https://doi.org/10.1016/j.hal.2019.101702>.
- Liu, Y., Hu, Z., Deng, Y., Tang, Y.Z., 2020. Evidence for production of sexual resting cysts by the toxic dinoflagellate *Karenia mikimotoi* in clonal cultures and marine sediments. *J. Phycol.* 56, 121–134.
- Liu, Y., Deng, Y., Shang, L., Yi, L., Hu, Z., Tang, Y.Z., 2021. Geographic distribution and historical presence of the resting cysts of *Karenia mikimotoi* in the seas of China. *Harmful Algae* 109, 102121. <https://doi.org/10.1016/j.hal.2021.102121>.
- Magaña, H.A., Contreras, C., Villareal, T.A., 2003. A historical assessment of *Karenia brevis* in the western Gulf of Mexico. *Harmful Algae* 2, 163–171.
- Main, C.R., Greenfield, D.I., Doll, C., Wang, Y., Whereat, E., Mortensen, R., Pettay, D.T., Coyne, K.J., 2018. Critical comparison of molecular methods for detection and enumeration of the harmful algal species, *Heterosigma akashiwo*, in environmental water samples. *J. Appl. Phycol.* 30, 2425–2434.
- Makinen, C.P., Moisan, T.A., 2012. Phytoplankton assemblage patterns in the southern Mid-Atlantic Bight. *Botanica Marina* 55, 445–457.
- Madhu, N.V., Reny, P.D., Paul, M., Ullas, N., Resmi, P., 2011. Occurrence of red tide caused by *Karenia mikimotoi* (toxic dinoflagellate) in the Southwest coast of India. 2011. *Indian J. Geo-Marine Sci.* 40, 821–825.
- Mardones, J.I., Norambuena, L., Paredes, J., Fuenzalida, G., Dorantes-Aranda, J.J., Chang, K.J.L., Guzmán, L., Krock, B., Hallegraef, G., 2020. Unraveling the *Karenia selliformis* complex with the description of a non-gymnodimine producing Patagonian phylotype. *Harmful Algae* 98, 101892. <https://doi.org/10.1016/j.hal.2020.101892>.
- Marshall, H.G., 1969a. Observations on the spatial concentrations of phytoplankton. *Castanea* 34, 217–222.
- Marshall, H.G., 1969b. Phytoplankton distribution off the North Carolina Coast. *Am. Midl. Nat.* 82, 241–257.
- Marshall, H.G., 1976. Phytoplankton distribution along the eastern coast of the USA. I. Phytoplankton composition. *Mar. Biol.* 38, 81–89.
- Marshall, H.G., 1978. Phytoplankton distribution along the eastern coast of the USA. Part II. Seasonal assemblages north of Cape Hatteras, North Carolina. *Mar. Biol.* 45, 203–208.
- Marshall, H.G., 1982. The composition of phytoplankton within the Chesapeake Bay plume and adjacent waters off the Virginia coast. *Estuar. Coast Shelf Sci.* 15, 29–43.
- Marshall, H.G., 1984. Phytoplankton distribution along the eastern coast of the USA. Part V. Seasonal density and cell volume patterns for the northeastern continental shelf. *J. Plankton Res.* 6, 169–193.
- Marshall, H.G., 1985. Comparison of phytoplankton concentrations and cell volume measurements from the continental shelf off Cape Cod, Massachusetts, U.S.A. *Hydrobiologia* 120, 171–179.
- Marshall, H.G. 1986. The seasonal influence of Chesapeake Bay phytoplankton to the continental shelf. In: Skreslet, S. (Ed.) *The Role of Freshwater Outflow in Coastal Marine Ecosystems*. NATO ASI Series, vol 7. Springer, Berlin, Heidelberg, DOI: 10.1007/978-3-642-70886-2_23.
- Marshall, H.G., 1988. Phytoplankton composition at the entrance of the Chesapeake Bay, Virginia. *J. Elisha Mitchell Sci. Soc.* 104, 81–89.
- Marshall, H.G., 1991. Seasonal phytoplankton assemblages associated with the Chesapeake Bay plume. *J. Elisha Mitchell Sci. Soc. Chapel Hill N C* 107, 105–114.
- Marshall, H., Alden, R., 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. *Estuaries* 13, 287–300.
- Marshall, H.G., Cohn, M.S., 1983. Distribution and composition of phytoplankton in northeastern coastal waters of the United States. *Estuar. Coast Shelf Sci.* 17, 119–131.
- Marshall, H.G., Cohn, M.S., 1987. Phytoplankton distribution along the eastern coast of the USA. Part VI. Shelf waters between Cape Henry and Cape May. *J. Plankton Res.* 9, 139–149.
- Marshall, H.G., Nesius, K.K., Cibik, S.J., 1981. Phytoplankton studies within the Virginia barrier islands II. Seasonal study of phytoplankton within the barrier island channels. *Castanea* 46, 89–99.
- Marshall, H.G., Ranasinghe, J.A., 1989. Phytoplankton distribution along the eastern coast of the U.S.A. VII. Mean cell concentrations and standing crop. *Cont. Shelf Res.* 9, 153–164.
- Marshall, H.G., Shomers, C.E., 1990. Phytoplankton within the Virginia barrier island complex. *Va. J. Sci.* 41, 427–432.
- Matsuyama, Y., et al., 2006. Red tide due to the dinoflagellate *Karenia mikimotoi* in Hiroshima Bay 2002: environmental features during the red tide and associated fisheries damages to finfish and shellfish aquaculture. In: Moestrup, Ø., Doucette, G., Enevoldsen, H., Godhe, A., Hallegraef, G., Luckas, B., et al. (Eds.), *Proceedings of the 12th International Conference on Harmful Algae*. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, 2008. Copenhagen, pp. 209–211.
- Matta, J.F., Marshall, H.G., 1984. A multivariate analysis of phytoplankton assemblages in the western North Atlantic. *J. Plankton Res.* 6, 663–675.
- Moisan, T.A., Ruffy, K.M., Moisan, J.R., Linkswiler, M.A., 2017. Satellite observations of phytoplankton functional type spatial distributions, phenology, diversity, and ecotones. *Front. Mar. Sci.* 4, 189. <https://doi.org/10.3389/fmars.2017.00189>.
- Mulholland, M.R., Bernhardt, P.W., Heil, C.A., Bronk, D.A., O'Neil, J.M., 2006. Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. *Limnol. Oceanogr.* 51, 1762–1776.
- Mulholland, M.R., Bernhardt, P.W., Ozmon, I., Procise, L.A., Garrett, M., O'Neil, J.M., Heil, C.A., Bronk, D.A., 2014. Contribution of diazotrophy to nitrogen inputs supporting *Karenia brevis* blooms in the Gulf of Mexico. *Harmful Algae* 38, 20–29.
- Mulholland, M.R., Heil, C.A., Bronk, D.A., O'Neil, J.M., Bernhardt, P., 2004. Does nitrogen regeneration from the N₂ fixing cyanobacteria, *Trichodesmium* spp., fuel *Karenia brevis* blooms in the Gulf of Mexico? In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), *Harmful Algae 2022*. Florida Fish and Wildlife Conservation Commission. Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL, USA, pp. 47–49.
- Mulholland, M.R., Morse, R.E., Boneillo, G., Bernhardt, P.W., Filippino, K.C., Procise, L.A., Blanco-Garcia, J.L., Marshall, H.G., Egerton, T.A., Hunley, W.S., Moore, K.A., Berry, D.L., Gobler, C.J., 2009. Understanding causes and impacts of the dinoflagellate, *Cochlodinium polykrikoides*, blooms in the Chesapeake Bay. *Estuaries Coasts* 32, 734–747.
- National Shellfish Sanitation Program. 2017. Guide for the Control of Molluscan Shellfish 2017 Revision. Available at: <https://www.fda.gov/food/federalstate-food-programs/national-shellfish-sanitation-program-nssp> (last accessed 19 March 2023).
- Neely, T., Campbell, L., 2006. A modified assay to determine hemolytic toxin variability among *Karenia* clones isolated from the Gulf of Mexico. *Harmful Algae* 5, 592–598.
- O'Boyle, S., McDermott, G., Silke, J., Cusack, C., 2016. Potential impact of an exceptional bloom of *Karenia mikimotoi* on dissolved oxygen levels in waters off western Ireland. *Harmful Algae* 53, 77–85.
- Onitsuka, G., Suzukawa, K., Yoshie, N., Hirai, M., Takenaka, S., Yoshihara, Y., Ohnishi, H., Shimizu, S., Takeuchi, H., Ohta, K., Tomaru, Y., Sakamoto, S., Abe, K., Yamaguchi, A., Shikata, T., Yamaguchi, H., Takeoka, H., 2021. Spatio-temporal dynamics of the harmful dinoflagellate *Karenia mikimotoi* in Uwajima Bay and its adjacent waters: comparison between a bloom occurrence year and a non-occurrence year. *Nippon Suisan Gakkaishi* 87, 144–159.
- Orlova, T.Y., Aleksanin, A.I., Lepskaya, E.V., Efimova, K.V., Selina, M.S., Morozova, T.V., Stonik, I.V., Kachur, V.A., Karpenko, A.A., Vinnikov, K.A., Adrianov, A.V., Iwataki, M., 2022. A massive bloom of *Karenia* species (Dinophyceae) off the Kamchatka coast, Russia, in the fall of 2020. *Harmful Algae* 120, 102337. <https://doi.org/10.1016/j.hal.2022.102337>.
- Pan, X., Mannino, A., Marshall, H.G., Filippino, K.C., Mulholland, M.R., 2011. Remote sensing of phytoplankton community composition along the northeast coast of the United States. *Remote Sens. Environ.* 115, 3731–3747.
- Pease, S.K.D., Egerton, T.A., Reece, K.S., Sanderson, M.P., Onofrio, M.D., Yeargan, E., Wood, A., Roach, A., Huang, I.W., Scott, G.P., Place, A.R., Hayes, A.M., Smith, J.L., 2023. Co-occurrence of marine and freshwater phytochemicals in oysters, and analysis of possible predictors for management. *Toxicol X* 19, 100166. [doi.org/j.toxcx.2023.100166](https://doi.org/10.1016/j.toxcx.2023.100166).
- Persson, A., Smith, B.C., Morton, S., Shuler, A., Wikfors, G.H., 2013. Sexual life stages and temperature dependent morphological changes allow cryptic occurrence of the Florida red tide dinoflagellate *Karenia brevis*. *Harmful Algae* 30, 1–9.
- Phlips, E.J., Badyal, S., Nelson, N.G., Hall, L.M., Jacoby, C.A., Lasi, M.A., Lockwood, J. C., Miller, J.D., 2021. Cyclical patterns and a regime shift in the character of phytoplankton blooms in a restricted sub-tropical lagoon, Indian River Lagoon, Florida, United States. *Front. Mar. Sci.* 8, 730934. <https://doi.org/10.3389/fmars.2021.730934>.
- Poli, M.A., Musser, S.M., Dickey, R.W., Eilers, P.P., Hall, S., 2000. Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. *Toxicol* 38, 981–993.
- Polikarpov, I., Saburova, M., Al-Yamani, F., 2020. Decadal changes in diversity and occurrence of microalgae blooms in the NW Arabian/Persian Gulf. *Deep-Sea Res. II* 179, 104810. <https://doi.org/10.1016/j.dsr2.2020.104810>.
- Primary Industries and Regions South Australia. 2014. Fish kill investigation: Coffin Bay Harmful Algal (*Karenia mikimotoi*) Bloom February 2014. Government of South Australia, p. 33. Reference A1928649p.
- Record, N.R., Countway, P.D., Kanwit, K., Fernández-Robledo, J.A., 2021. Rise of the rare biosphere: thinking beyond climate envelopes for forecasting harmful algal blooms. *Elementa: Sci. Anthropocene* 9, 00056. <https://doi.org/10.1525/elementa.2020.00056>.
- Robin, R.S., Kanuri, V.V., Muduli, P.R., Mishra, R.K., Jaikumar, M., Karthikeyan, P., Kumar, C.S., Kumar, C.S., 2013. Dinoflagellate bloom of *Karenia mikimotoi* along the southeast Arabian Sea, bordering western India. *J. Ecosyst.*, 463720. <https://doi.org/10.1155/2013/463720>, 2013.
- Saba, V.S., Griffies, S.M., Anderson, W.G., Winton, M., Alexander, M.A., Delworth, T.L., Hare, J.A., Harrison, M.J., Rosati, A., Vecchi, G.A., Zhang, R., 2016. Enhanced warming of the Northwestern Atlantic Ocean under climate change. *J. Geophys. Res.* 121, 118–132.
- Sakamoto, S., Lim, W.A., Lu, D., Dai, X., Orlova, T., Iwataki, M., 2021. Harmful algal blooms and associated fisheries damage in East Asia: current status and trends in China, Japan, Korea and Russia. *Harmful Algae* 102, 101787. <https://doi.org/10.1016/j.hal.2020.101787>.
- Scully, M., Geyer, W.R., Borkman, D., Pugh, T., Costa, A., Nichols, O., 2022. Unprecedented summer hypoxia in southern Cape Cod Bay: an ecological response to regional climate change? *Biogeosciences* 19, 3523–3536. <https://doi.org/10.5194/bg-19-3523-2022>.
- Shimada, H., Kanamori, M., Yoshida, H., Imai, I., 2016. First record of red tide due to the harmful dinoflagellate *Karenia mikimotoi* in Hakodate Bay, southern Hokkaido, in autumn 2015. *Nippon Suisan Gakkaishi* 826, 934–938.
- Steidinger, K.A., 2009. Historical perspective on *Karenia brevis* red tide research in the Gulf of Mexico. *Harmful Algae* 8, 549–561.
- Steidinger, K.A., Wolny, J.L., Haywood, A.J., 2008. Identification of *Karenia* spp. (Dinophyceae) in the Gulf of Mexico. *Nova Hedwigia* 133, 236–284.
- Sunda, W.G., Burleson, C., Hardison, D.R., Moya, J.S., Wang, Z., Wolny, J., Corcoran, A. A., Flewelling, L.J., Van Dolah, F.M., 2013. Osmotic stress does not trigger

- brevetoxin production in the dinoflagellate *Karenia brevis*. Proc. Natl. Acad. Sci. 110, 10223–10228.
- Takayama, H., Adachi, R., 1984. *Gymnodinium nagasakiense* sp. nov., a red-tide forming dinophyte in the adjacent waters of Japan. Bull. Plankton Soc. Japan 31, 7–14.
- Tester, P.A., Steidinger, K.A., 1997. *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulations. Limnol. Oceanogr. 42, 1039–1051.
- Tester, P.A., Stumpf, R.P., Vukovich, F.M., Fowler, P.K., Turner, J.T., 1991. An expatriate red tide bloom: transport, distribution, and persistence. Limnol. Oceanogr. 36, 1053–1061.
- Thessen, A.E., Stoecker, D.K., 2008. Distribution, abundance and domoic acid analysis of the toxic diatom genus *Pseudo-nitzschia* from the Chesapeake Bay. Estuaries Coasts 31, 664–672.
- Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilung Internationale Vereinigung Fuer Theoretische Angewandte Limnologie 9, 1–38.
- Vandersea, M., Tester, P., Holderied, K., Hondolero, D., Kibler, S., Powell, K., Baird, S., Doroff, A., Dugan, D., Meredith, A., Tomlinson, M., Litaker, R.W., 2020. An extraordinary *Karenia mikimotoi* “beer tide” in Kachemak Bay Alaska. Harmful Algae 92, 101706. <https://doi.org/10.1016/j.hal.2018.06.008>.
- Vidyarathna, N.K., Papke, E., Coyne, K.J., Cohen, J.H., Warner, M.E., 2020. Functional trait thermal acclimation differs across three species of mid-Atlantic harmful algae. Harmful Algae 94, 101804. <https://doi.org/10.1016/j.hal.2020.101804>.
- Wagoner, B.B., Marshall, H.G., 1991. Observations of the phytoplankton standing crop at the shelf margin of the Mid Atlantic Bight. Va. J. Sci. 42, 353–360.
- Walsh, J.J., Weisberg, R.H., Lenes, J.M., Chen, F.R., Dieterle, D.A., Zheng, L., Carder, K. L., Vargo, G.A., Havens, J.A., Peebles, E., Hollander, D.J., He, R., Heil, C.A., Mahmoudi, B., Landsberg, J.H., 2009. Isotopic evidence for dead fish maintenance of Florida red tides, with implications for coastal fisheries over both source regions of the West Florida shelf and within downstream waters of the South Atlantic Bight. Prog. Oceanogr. 80, 51–73.
- Weisberg, R.H., Liu, Y., Lembke, C., Hu, C., Hubbard, K., Garrett, M., 2019. The coastal ocean circulation influence on the 2018 West Florida Shelf *K. brevis* red tide bloom. J Geophys Res 124, 2501–2512. <https://doi.org/10.1029/2018JC014887>.
- Wells, M.L., Karlson, B., Wulff, A., Kudela, R., Trick, C., Asnaghi, V., Berdalet, E., Cochlan, W., Davidson, K., De Rijcke, M., Dutkiewicz, S., 2020. Future HAB science: directions and challenges in a changing climate. Harmful Algae 91, 101632.
- West, T.L., Marshall, H.G., Tester, P.A., 1996. Natural phytoplankton community responses to a bloom of the toxic dinoflagellate *Gymnodinium breve* Davis off the North Carolina coast. Castanea 61, 356–368.
- Whereat, E.B., Farrell, J.G., Humphries, E.M., 2004. Volunteer phytoplankton monitoring in the Inland Bays of Delaware, USA. In: Steidinger, K.A., Landsberg, J. C., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission. Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL, USA, pp. 367–368.
- Wolny, J., Scott, P., Tustison, J., Brooks, C., 2015. Monitoring the 2007 Florida east coast *Karenia brevis* (Dinophyceae) red tide and neurotoxic shellfish poisoning (NSP) event. Algae 30, 49–58.
- Wolny, J.L., Egerton, T.A., Handy, S.M., Stutts, W.L., Smith, J.L., Whereat, E.B., Bachvaroff, T.R., Henrichs, D.W., Campbell, L., Deeds, J.R., 2020a. Characterization of *Dinophysis* spp. (Dinophyceae, Dinophysiales) from the mid-Atlantic region of the United States. J. Phycol. 56, 404–424.
- Wolny, J.L., Tomlinson, M.C., Schollaert Uz, S., Egerton, T.A., McKay, J.R., Meredith, A., Reece, K.S., Scott, G.P., Stumpf, R.P., 2020b. Current and future remote sensing of harmful algal blooms in the Chesapeake Bay to support the shellfish industry. Front. Mar. Sci. 7, 337. <https://doi.org/10.3389/fmars.2020.00337>.
- Xiong, J., Shen, J., Qin, Q., Tomlinson, M.C., Zhang, Y.J., Cai, X., Ye, F., Cui, L., Mulholland, M.R., 2023. Biophysical interactions control the progression of harmful algal blooms in Chesapeake Bay: a novel Lagrangian particle tracking model with mixotrophic growth and vertical migration. Limnol. Oceanogr. Lett. 8, 498–508.
- Xu, Y., Miles, T., Schofield, O., 2020. Physical processes controlling chlorophyll-a variability on the Mid-Atlantic Bight along northeast United States. J. Mar. Syst. 212, 103433 <https://doi.org/10.1016/j.jmarsys.2020.103433>.
- Yamaguchi, H., Hirano, T., Yoshimatsu, T., Tanimoto, Y., Matsumoto, T., Suzuki, S., Hayashi, Y., Urabe, A., Miyamura, K., Sakamoto, S., Yamaguchi, M., 2016. Occurrence of *Karenia papilionacea* (Dinophyceae) and its novel sister phylotype in Japanese coastal waters. Harmful Algae 57, 59–68.
- Yang, Z.B., Takayama, H., Matsuoka, K., Hodgkiss, I.J., 2000. *Karenia digitata* sp. nov. (Gymnodiniales, Dinophyceae), a new harmful algal bloom species from the coastal waters of west Japan and Hong Kong. Phycologia 39, 463–470.
- Yang, Z.B., Hodgkiss, I.J., Hansen, G., 2001. *Karenia longicanalis* sp. nov. (Dinophyceae): a new bloom-forming species isolated from Hong Kong, May 1998. Botanica Marina 44, 67–74.
- Zevenboom, W., Rademaker, M., Colijn, F., 1991. Exceptional algal blooms in Dutch North Sea waters. Water Sci. Technol. 24, 251–260.
- Zhang, Y., Fu, F., Whereat, E., Coyne, K.J., Hutchins, D.A., 2006. Bottom-up controls on a mixed-species HAB assemblage: a comparison of sympatric *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophyceae) isolates from the Delaware Inland Bays, USA. Harmful Algae 5, 310–320.