

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

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20

21 **Abstract**

22 A bloom of *Karenia papilionacea* that occurred along the Delaware coast in late summer of  
23 2007 was the first *Karenia* bloom reported on the Delmarva Peninsula (Delaware, Maryland, and  
24 Virginia, USA). Limited spatial and temporal monitoring conducted by state agencies and citizen  
25 science groups since 2007 have documented that several *Karenia* species are an annual component  
26 of the coastal phytoplankton community along the Delmarva Peninsula, often present at background  
27 to low concentrations (100 to 10,000 cells L<sup>-1</sup>). Blooms of *Karenia* (> 10<sup>5</sup> cells L<sup>-1</sup>) occurred in  
28 2010, 2016, 2018, and 2019 in different areas along the Delmarva Peninsula coast. In late summer  
29 and early autumn of 2017, the lower Chesapeake Bay experienced a *K. papilionacea* bloom, the  
30 first recorded in Bay waters. Blooms typically occurred summer into autumn but were not  
31 monospecific; rather, they were dominated by either *K. mikimotoi* or *K. papilionacea*, with *K.*  
32 *selliformis*, *K. brevis*-like cells, and an undescribed *Karenia* species also present. Cell  
33 concentrations during these mid-Atlantic *Karenia* spp. blooms equalled concentrations reported for  
34 other *Karenia* blooms. However, the negative impacts to environmental and human health often  
35 associated with *Karenia* red tides were not observed. The data compiled here report on the presence  
36 of multiple *Karenia* species in coastal waters of the Delmarva Peninsula detected through routine  
37 monitoring and opportunistic sampling conducted between 2007 and 2022, as well as findings from  
38 research cruises undertaken in 2018 and 2019. These data should be used as a baseline for future  
39 phytoplankton community analyses supporting coastal HAB monitoring programs.

40

41 Keywords: *Karenia*, red tide, mid-Atlantic region, Delmarva Peninsula, Chesapeake Bay

42

## 43 1. Introduction

44 Blooms of the dinoflagellate *Karenia brevis* (Davis) G. Hansen & Moestrup (previously  
45 known as *Gymnodinium breve* Davis and *Ptychodiscus brevis* (Davis) Steidinger), or “red tides”,  
46 are a nearly annual occurrence in the Gulf of Mexico, where they have been documented since  
47 Spanish explorers encountered them in the 16<sup>th</sup> century (Magaña et al., 2003) and continue to be an  
48 active area of monitoring and research (Steidinger, 2009; Hu et al., 2022). *Karenia brevis* produces  
49 brevetoxin, a neurotoxin which can cause extensive fish and animal mortalities when cell  
50 concentrations exceed  $10^5$  cells L<sup>-1</sup> (Steidinger, 2009). This toxin can become aerosolized causing  
51 acute or chronic respiratory illnesses in individuals exposed to these high concentration blooms  
52 (Fleming et al., 2011). Additionally, at cell concentrations above  $10^3$  cells L<sup>-1</sup> this toxin can  
53 accumulate in shellfish tissues causing neurotoxic shellfish poisoning (NSP) in humans who  
54 consume contaminated shellfish, thus presenting a resource management concern for shellfish  
55 industries where these blooms occur (Poli et al., 2000; Steidinger, 2009).

56 Only since the 1970s have environmental and economic impacts from blooms of other  
57 *Karenia* species been documented globally. Blooms of *Karenia* spp., that do not include *K. brevis*,  
58 have been observed in diverse marine environments including Norwegian coastal waters (Dahl and  
59 Tangen, 1983); Tokyo Bay, Japan (Takayama and Adachi, 1984); the Gulf of Gabes, Tunisia  
60 (Hamza and El Abed, 1994); numerous bays in New Zealand (Chang, 1999; Haywood et al., 2004);  
61 Victoria Harbour, Hong Kong (Yang et al., 2000, 2001); Kuwait Bay (Heil et al., 2001); the  
62 coastline of South Africa (Botes et al., 2003); the coastline of eastern Tasmania, Australia (de Salas  
63 et al., 2004a,b); the Chilean coast (Mardones et al., 2020), and the region between the Kamchatka  
64 Peninsula, Russia and Hokkaido Island, Japan (Iwataki et al., 2022; Orlova et al., 2022).  
65 Observations suggest that many bloom-forming *Karenia* species can occupy diverse environments  
66 and have broader water temperature and salinity tolerances than *K. brevis*. In the United States, until  
67 recently, *Karenia* blooms have been restricted to the Gulf of Mexico and Florida’s east coast and

68 dominated by *K. brevis*, with other *Karenia* species noted as present or in low concentrations  
69 (Steidinger et al., 2008; Steidinger, 2009; Wolny et al., 2015; Harris et al., 2020). However, in the  
70 autumn of 2013 a bloom of *Karenia mikimotoi* (Miyake & Kominami ex Oda) G. Hansen &  
71 Moestrup was documented by Vandersea et al. (2020) in Kachemek Bay, Alaska, where extensive  
72 discolored water was observed, along with localized fish kills. In the late summers and early  
73 autumns of 2017 to 2020, blooms of *K. mikimotoi* caused discolored water, hypoxia, and impacts to  
74 shellfish resources in the Gulf of Maine (Record et al., 2021; Scully et al., 2022).

75 Blooms of *Karenia* have rarely been documented in the mid-Atlantic region of the United  
76 States. In 2007, a large export event of a Gulf of Mexico *K. brevis* bloom, entrapped in the Loop  
77 Current and moved eastward by the Gulf Stream, was documented by Walsh et al. (2009). This  
78 study tracked the parcel of water containing *K. brevis* using satellite remote sensing and simulate  
79 surface drifters as it moved through the Florida Straits, up Florida's east coast, and into the mid-  
80 Atlantic region. This same year marked the first observed occurrence of multiple *Karenia* species  
81 along the Delaware coastline, with blooms dominated by *Karenia papilionacea* Haywood &  
82 Steidinger (Bott, 2014). Unlike Florida's rigorous *Karenia brevis* monitoring program (described in  
83 Steidinger, 2009 and Hu et al., 2022) there is little harmful algal bloom (HAB) monitoring along the  
84 Delaware, Maryland, and Virginia (Delmarva) Atlantic seaboard outside of the region's coastal  
85 bays. Despite limited opportunities to make *in situ* observations, data collected by state agencies,  
86 universities, and citizen monitoring programs indicate that multiple *Karenia* species are seasonally  
87 part of the phytoplankton community along the coast and within the coastal bays of the Delmarva  
88 Peninsula. Here we report on the occurrence of multiple *Karenia* species in coastal waters of the  
89 Delmarva Peninsula and Chesapeake Bay detected through spatially- and temporally-limited routine  
90 monitoring and opportunistic sampling conducted between 2007 and 2022 and focused field  
91 campaigns conducted in the coastal waters of the Delmarva Peninsula in 2018 and 2019.

92

## 93 2. Methods

### 94 2.1 Overview of data collection

95 The cell abundances of *Karenia* species were measured from water samples collected as part  
96 of the Maryland and Virginia state HAB and shellfish monitoring programs and Chesapeake Bay  
97 Program (CBP) water quality monitoring activities (Figure 1). Water quality monitoring in  
98 Delaware was conducted by volunteers participating in the University of Delaware Citizen  
99 Monitoring Program (Figure 1). In 2018 and 2019 a cooperative coastal assessment agreement  
100 between the University of Maryland Center for Environmental Science (UMCES) and the  
101 Assateague Island National Park Service allowed for the collection of water samples during  
102 research cruises along the Delmarva Peninsula coastline (Figure 2).

103 State and citizen monitoring programs obtained live or 5% Lugol's iodine (#LC156725,  
104 LabChem Inc., Zelienople, PA, USA) preserved whole water samples from fixed stations or during  
105 opportunistic sampling of near surface waters (< 0.5 m) or from the depth of the chlorophyll  
106 maximum. Samples collected through the CBP followed the protocols listed in CBP (2017). Briefly,  
107 above and below pycnocline composite samples were collected and preserved with 5% Lugol's  
108 iodine. Surface and depth samples preserved with 5% Lugol's iodine were collected during the  
109 2018 and 2019 Delmarva Peninsula coastal cruises. Environmental data (e.g., temperature and  
110 salinity) were collected using Hydrolab Series 5 (Hach Environmental, Loveland, CO, USA) or YSI  
111 85, YSI 6600, Pro2030, or ProSolo (YSI, Inc., Yellow Springs, OH, USA) data sondes concurrently  
112 with phytoplankton sample collection.

113 Using light microscopy, all *Karenia* were identified and enumerated to the species level  
114 based on overall cell morphology and nuclear shape and placement as described in Yang et al.  
115 (2001), Haywood et al. (2004), de Salas et al. (2004a,b), and Steidinger et al. (2008). When  
116 identification to the species level was not possible due to staining, cell distortion, or cell orientation,  
117 cells were included in a separate category termed '*Karenia* spp.'. An undescribed *Karenia* species,

118 clearly recognizable due to the unique hypocone morphology, resembled “*Karenia* sp. #3”, as  
119 described by Steidinger et al. (2008); this naming convention was retained for this work. Cell  
120 concentrations data were plotted using ArcGIS Pro 3.0.3 (Esri, Redlands, CA, USA) and cell  
121 concentrations were binned as absent, background (100 – 1,000 cells L<sup>-1</sup>), low (>1,000 to 10,000  
122 cells L<sup>-1</sup>), medium (>10,000 to 100,000 cells L<sup>-1</sup>) and high (>100,000 cells L<sup>-1</sup>) (see Table 1).

### 123 *2.2 Delaware*

124 Between May and November 2007 – 2022, live 250-mL phytoplankton samples were  
125 collected at least monthly through the University of Delaware’s Citizen Monitoring Program (UD  
126 CMP) at 26 stations (Figure 1) located between Broadkill River (38.7904N, -75.1635W) and  
127 Fenwick Island (38.4519N -75.0493W). Sampling frequency and number of stations sampled  
128 increased during HAB events, per the UD CMP guidelines (Whereat et al., 2004). Phytoplankton  
129 samples were examined within six hours of collection using American Optical Corporation Series  
130 50 compound microscopes (American Optical Corporation, Buffalo, NY, USA) and enumerated  
131 following a modification of the Andersen and Thronsen (2003) droplet method as described in  
132 Main et al. (2018). Additional samples were collected at Delaware beaches and inlets by Delaware  
133 Department of Natural Resources and Environmental Control (DNREC) staff and preserved with  
134 5% Lugol’s iodine. For these samples, 1 – 3-mL aliquots were settled for a minimum of 30 minutes  
135 in Nunc coverglass-bottom chambers (#155379, Nunc, Rochester, NY, USA) and the entire  
136 chamber was examined using a Nikon TMS-F (Nikon Instruments Inc., Melville, NY, USA)  
137 inverted microscope.

### 138 *2.3 Maryland*

139 Monthly monitoring 1.5 km off the Maryland coastline was conducted between April and  
140 October from 2014 through 2021 at five stations that are part of the Maryland Department of  
141 Environment (MDE) shellfish classification program. Stations were located near the Maryland –  
142 Delaware state border (38.4508N, -75.0326W) to south of Ocean City (38.2111N, -75.1286W). For

143 this program, live 1-L phytoplankton samples were collected from the surface (0.5 – 1 m) and/or the  
144 depth of the chlorophyll maximum (ranging from 1.4 – 4.9 m). Between 2014 and 2020 year-round,  
145 live 1-L surface water phytoplankton samples were collected monthly from 16 stations within the  
146 Maryland coastal bays and their major tributaries by the Maryland Department of Natural  
147 Resources (MDNR) (Figure 1).

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

#### 156 *2.4 Virginia*

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

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

### 177           2.5 2018 and 2019 Delmarva Peninsula Coastal Cruises

178           Environmental parameters and samples for phytoplankton community composition analysis  
179 were collected during five research cruises in June, July, and October of 2018 and May and July  
180 2019 aboard the *R/V Rachel Carson* or the *R/V Joanne Daiber*. Two to four stations along ten  
181 transects, approximately 0.8 to 2.4 km off the Delmarva Peninsula coast, were sampled during these  
182 cruises (Figure 2). The northernmost transects started off the coast of Fenwick Island, Delaware  
183 (38.44483N, -75.0290W) and the southernmost transects ended south of Assateague Island,  
184 Virginia (37.82906N, -75.40963W). Phytoplankton samples, collected at the surface (0.5 – 1.0 m)  
185 and chlorophyll maximum depths (ranging from 2.3 – 14.5 m) using 10-L Niskin bottles on a CTD  
186 rosette, were fixed on-site with 5% Lugol’s iodine solution. Samples were examined for  
187 phytoplankton community composition and abundance at MDNR using a Zeiss Axiovert 200  
188 inverted microscope and 10-mL Utermöhl settling chambers (#435022, Hydro-Bios, Altenholz,  
189 Germany) following the modified Utermöhl method of Marshall and Alden (1990).

190

## 191   **3 Results**

### 192           3.1 Species Identification



193 Five *Karenia* species were routinely identified along the Delmarva Peninsula coastline and  
194 in the Chesapeake Bay between 2007 and 2022. Cells identified as *K. brevis*-like (Figure 3A)  
195 resembled *K. brevis* as described by Steidinger et al. (2008), particularly in cell size, nuclear shape  
196 and placement (round and located in the left hypotheca), and a slightly excavated hypotheca.  
197 However, these cells were only found during blooms of *K. papilionacea*. It is possible they were  
198 small-cell forms of *K. papilionacea* as reported by Fowler et al. (2015) or are like the *K.*  
199 *papilionacea* phylotype-I described by Yamaguchi et al. (2016), both of which have a  
200 morphological appearance more similar to *K. brevis* than *K. papilionacea* as described by Haywood  
201 et al. (2004). Large *K. papilionacea* cells (Figure 3B) were identified using the overall butterfly-like  
202 cell shape, deeply excavated hypotheca, and pointed apical carina as described by Haywood et al.  
203 (2004) and Steidinger et al. (2008), whereas small *K. papilionacea* cells were identified by the  
204 deeply excavated hypotheca and a slightly pointed apical carina (Figure 3C).

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

214 Light micrographs of living cells were not taken as part of this effort. However, high quality  
215 live micrographs of these same species can be reviewed in de Salas et al. (2004b), Haywood et al.  
216 (2004), and Steidinger et al. (2008).

217 3.2 *Karenia* Abundance and Distribution

## 218           3.2.1 Delaware

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

230           Following the 2007 *Karenia* bloom, surface waters (~0.5 m) at 26 stations along the coast  
231 and within the Delaware Inland Bays (Figure 1) were routinely monitored for the presence of  
232 *Karenia* spp. Between 2008 and 2022, *K. brevis*-like cells were rarely detected, but when observed  
233 they were in background to low concentrations (100 – 10,000 cells L<sup>-1</sup>) within a larger *K.*  
234 *papilionacea* population. *Karenia mikimotoi* has been observed in Delaware waters since 2019, but  
235 always at low cell concentrations. Neither *K. selliformis* nor *Karenia* sp. #3 have been detected in  
236 Delaware waters.

237           *Karenia papilionacea* was found in all coastal routine monitoring stations but occurred less  
238 frequently within the Delaware Inland Bays. The majority of observations (n=163) made between  
239 2007 and 2022 contained background to low concentrations of this species. Cell concentrations of  
240 *K. papilionacea* blooms that occurred in Delaware coastal waters in 2010 and 2016 were on the  
241 order of  $10^5$  cells L<sup>-1</sup> but no adverse environmental impacts were reported. The *K. papilionacea*  
242 blooms documented in all three years (2007, 2010, and 2016) were centred on the region between

243 the Indian River Inlet and Fenwick Island (38.6782N, -75.0685W to 38.4519N, -75.04933W)  
244 between late August and early October (Table 2).

### 245 3.2.2 Maryland

246 Analysis of water samples collected from surface (0.5 to 1.0 m) and/or chlorophyll  
247 maximum depths (1.8 to 22.0 m) at five offshore stations indicated the presence of multiple *Karenia*  
248 species in at least background concentrations between 2014 and 2022 (Figure 1). However, nearly  
249 monospecific blooms of *K. papilionacea*, with cell concentrations between  $10^3$  and  $10^5$  cells L<sup>-1</sup>,  
250 were noted in the late summers of 2016, 2018, and 2019. From April through June, *K. mikimotoi*  
251 (average  $5.2 \times 10^3$  cells L<sup>-1</sup>) and *K. selliformis* (average  $5.0 \times 10^2$  cells L<sup>-1</sup>) were the most  
252 commonly occurring *Karenia* species. Between July and October, *K. papilionacea* (average  $5.0 \times$   
253  $10^4$  cells L<sup>-1</sup>) was the most common species, but *K. mikimotoi*, *Karenia* sp. #3, and cells identified  
254 as *K. brevis*-like routinely co-occurred within the *K. papilionacea* blooms in background  
255 concentrations. The maximum cell concentration of *K. papilionacea* ( $2.3 \times 10^5$  cells L<sup>-1</sup>) recorded  
256 from offshore monitoring efforts in Maryland waters occurred on 15 August 2016, within a bloom  
257 extending along a broad swath of the coastline north and south of Ocean City, Maryland, from at  
258 least 38.3978N to 38.2898N latitude (Tables 1 and 2). While concentrations of *K. papilionacea*  
259 ranged from 1.1 to  $2.3 \times 10^5$  cells L<sup>-1</sup> in bloom waters, water samples were negative for the  
260 brevetoxin congener PbTx-2 (data not shown) and no adverse environmental impacts were reported.

261 Within the Maryland coastal bays, *Karenia* species have been documented since 2016 in the  
262 surface waters (~0.5 m) at four of the 16 routinely monitored stations. Background concentrations  
263 ( $\leq 1,000$  cells L<sup>-1</sup>) of *K. selliformis* have been reported in Chincoteague Bay in the months of April  
264 and May. In the Isle of Wight Bay, background (300 cells L<sup>-1</sup>) to medium ( $3.1 \times 10^4$  cells L<sup>-1</sup>) cell  
265 concentrations of *K. papilionacea*, *K. mikimotoi*, and *K. selliformis* have been reported between  
266 May and October.

### 267 3.2.3 Virginia

268 In 2017, the first observations of *K. papilionacea* on record in the Chesapeake Bay were  
269 made in samples collected near the bay mouth in August, September, and October at two CBP long-  
270 term monitoring stations (Figure 1). At station CB 7.4 (36.9933N, -76.0106W), *K. papilionacea*  
271 was found in samples collected above the pycnocline in August ( $2.1 \times 10^5$  cells L<sup>-1</sup>) and October  
272 ( $3.0 \times 10^4$  cells L<sup>-1</sup>). In October, *K. papilionacea* was also observed below the pycnocline, but at  
273 lower concentrations ( $8.9 \times 10^3$  cells L<sup>-1</sup>). The highest concentration of *K. papilionacea* recorded in  
274 Virginia waters,  $5.8 \times 10^6$  cells L<sup>-1</sup> (Table 1), was observed in a sample collected above the  
275 pycnocline at station CB 7.3E (37.2286N, -76.0542W) in September. Despite the elevated *K.*  
276 *papilionacea* cell concentrations, no environmental impacts were reported. Other *Karenia* species  
277 were not observed in these samples and no *Karenia* were observed in surface water samples  
278 collected by VDH as part of the shellfish monitoring program during that same year. Despite the  
279 bloom observed off the Delmarva coast in 2018, no *Karenia* were observed in samples collected at  
280 CBP stations or samples collected within the Virginia coastal bays by VDH. The other *Karenia*  
281 species observed in Maryland and Delaware waters have not been detected in Virginia's coastal  
282 bays or within the Chesapeake Bay since routine monitoring was started in 2007. However, *K.*  
283 *mikimotoi* was recorded in Virginia coastal waters at background concentrations off the southern  
284 end of Assateague Island during the 2018 and 2019 cruises.

#### 285 3.2.4 2018 and 2019 Delmarva Peninsula coastal cruises

286 In the summer and autumn of 2018, *Karenia* spp. were observed in Delmarva Peninsula  
287 coastal waters in both surface samples and samples collected at the depth of the chlorophyll maxima  
288 (Table 3; Figure 2). In June, the bloom was dominated by *K. mikimotoi* (maximum concentration of  
289  $1.9 \times 10^5$  cells L<sup>-1</sup>). *Karenia mikimotoi* comprised 98% of the *Karenia* population and *K.*  
290 *papilionacea* and *K. selliformis* contributed the other 2%. The *K. mikimotoi* bloom was concentrated  
291 along the Maryland coastline off of Assateague Island (Figure 2A). In July, the bloom was  
292 dominated by *K. papilionacea* (maximum concentration of  $1.6 \times 10^5$  cells L<sup>-1</sup>), which comprised

293 96% of the *Karenia* population. *Karenia brevis*-like cells, *K. mikimotoi*, *K. selliformis*, and *Karenia*  
294 sp. #3 were all documented in samples collected during this bloom. The *K. papilionacea* bloom  
295 extended over much of the Maryland coastline (Figure 2B). Severe weather prevented sampling of  
296 coastal waters in August and September. When sampling resumed in October, *Karenia* populations  
297 had decreased to background concentrations. All previously reported species were present, except  
298 cells identified as *K. brevis*-like, and more than 50% of the *Karenia* population was composed of  
299 *Karenia* sp. #3.

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

304

#### 305 4 Discussion

306 Phytoplankton communities along the eastern seaboard of the United States have been  
307 sporadically documented during research cruises since the early 20<sup>th</sup> century (Cleve, 1900; Bigelow  
308 1915; Cowles, 1930). Marshall and colleagues conducted extensive *in situ* sampling of the  
309 phytoplankton communities in the coastal waters off the Delmarva Peninsula between the 1960s  
310 and 1980s (Marshall, 1969a, 1969b, 1976, 1978, 1982, 1984, 1985, 1986, 1988, 1991; Marshall and  
311 Cohn, 1983, 1987; Marshall and Ranasinghe, 1989; Marshall and Shomers, 1990; Marshall et al.,  
312 1981; Matta and Marshall, 1984; Wagoner and Marshall, 1991). These studies observed a  
313 phytoplankton community dominated year-round by diatoms and cryptophytes with distinct winter  
314 and summer dinoflagellate populations, the latter of which increased in abundance offshore during  
315 summer blooms. However, there have been limited *in situ* phytoplankton surveillance at the species  
316 level in the past three decades. More recently, observations of phytoplankton communities have  
317 been conducted using Chemtax applications (2004 – 2009; Pan et al., 2011) and satellite remote

318 sensing (2002 – 2016; Moisan et al., 2017). A bi-weekly to monthly microscopy-based  
319 phytoplankton community analysis was conducted by Makinen and Moisan (2012) at stations 9.6  
320 km and 40.7 km off of Assateague Island between June 2005 and December 2007. Collectively,  
321 these works indicate, that at a functional group level, the phytoplankton community structure  
322 observed between 2002 and 2016 was similar to that described between the 1960s and 1980s by  
323 Marshall and colleagues.

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

338 Following this 2007 bloom, water quality and HAB monitoring programs in the Delaware  
339 and Maryland portion of the Delmarva Peninsula have routinely detected these same five *Karenia*  
340 species in summer and autumn offshore and coastal bay phytoplankton populations ranging from  
341 background (100 – 1,000 cells L<sup>-1</sup>) to bloom level (>100,000 cells L<sup>-1</sup>) concentrations. However, the  
342 origins of these populations are unknown. Using a genetic analysis, Coyne et al. (2015) detected the

343 presence of a *K. papilionacea* strain, unique from Gulf of Mexico and New Zealand strains, in the  
344 shelf waters off Delaware in June and then along the coast in July, indicating a potential regional  
345 offshore source for coastal blooms. Liu et al. (2020; 2021) showed that cyst production and  
346 sequestering in sediments is a possible source of the *K. mikimotoi* blooms in the coastal waters of  
347 China, but cysts have not been documented in other *Karenia* species (Persson et al., 2013; Feki-  
348 Sahnoun et al., 2017) and were not detected in Delaware sediments (Coyne et al., 2015). Ballast  
349 water could also be an introduction mechanism for *Karenia* spp. to the Delmarva Peninsula region.  
350 Thousands of commercial vessels visit ports in both Chesapeake Bay (Carney et al., 2017) and  
351 Delaware Bay (Altiok et al., 2012) annually. Carney et al., (2017) reported that ballast water  
352 discharges to the Chesapeake Bay increased by 374% between 2005 and 2013 and an earlier study  
353 by Drake et al. (2005) indicated that 15% of commercial vessel traffic to this region came from  
354 Florida ports. However, Garrett et al. (2011) did not find *Karenia* spp. cells or cysts in ballast water  
355 or sediment samples collected from commercial vessels docked in Tampa Bay ports during a 3-year  
356 study conducted to determine the possibility of HAB species, including *Karenia* spp., being moved  
357 into or out of Florida waters from ballast exchange operations. Finally, Tester and Steidinger  
358 (1997), Walsh et al. (2009), and Weisberg et al. (2019) demonstrated that *K. brevis* blooms (and  
359 other *Karenia* species contained therein; Wolny et al., 2015) can be exported out of the Gulf of  
360 Mexico via the Loop Current and transported along the United States east coast via the Gulf Stream.  
361 While this latter mechanism is the most likely source of the Delmarva Peninsula *Karenia* blooms,  
362 the lack of routine monitoring of coastal and shelf waters precludes a broader understanding of the  
363 origins of *Karenia* populations in the mid-Atlantic region and spatially- and temporally-rigorous  
364 sampling should be considered in future monitoring and research programs to answer this question.

365         The different environmental conditions wherein each *Karenia* species has been observed  
366 suggests that each species may have its own environmental niche in the mid-Atlantic region (Tables  
367 2 and 3). In the Gulf of Mexico, *K. brevis* is the most successful of the *Karenia* species based on its

368 numerical and spatial dominance across a range of elevated temperatures (20 – 28 °C) and salinities  
369 (31 – 37) observed there (Steidinger, 2009). Based on data collected during the 2018 and 2019  
370 Delmarva Peninsula coastal cruises and regional monitoring programs, *K. mikimotoi* occupies the  
371 broadest temperature and salinity niche space (7.0 – 26.7 °C and 19.7 – 30.9 salinity) of the *Karenia*  
372 species. However, routine monitoring data suggest that *K. papilionacea* is the most frequently  
373 detected and abundant *Karenia* species in the mid-Atlantic region of the United States (n = 239  
374 observations, maximum concentration =  $5.8 \times 10^6$  cells L<sup>-1</sup>). Targeted monitoring of coastal waters  
375 and kinetic studies using cultured isolates from contrasting environments would help shed light on  
376 the optimal niche space for each *Karenia* species as other mid-Atlantic HAB species (e.g.,  
377 *Chattonella* spp., *Heterosigma akashiwo*, *Margalefidinium polykrikoides*) are known to occupy  
378 different niche spaces than their global counterparts (Handy et al., 2005; Zhang et al., 2006;  
379 Mulholland et al., 2009).

380 Both the historical work in the mid-Atlantic region conducted by Marshall and Cohn (1983,  
381 1987) and Wagoner and Marshall (1991) and the more recent studies by Pan et al. (2011) and  
382 Moisan et al. (2017) indicate that summer dinoflagellate populations, and their fractional  
383 contribution to the total chlorophyll, increase from the coastline towards the shelf, with greatest  
384 concentrations at the mid-shelf region (Moisan et al., 2017) and to the south of the Chesapeake Bay  
385 mouth (Wagoner and Marshall, 1991). This area has largely been devoid of systematic, high  
386 frequency *in situ* monitoring of phytoplankton populations. Satellite imagery processed by Moisan  
387 et al. (2017) shows seasonally high concentrations of the carotenoids 19'-hexanoyloxyfucoxanthin  
388 and 19'-butanoyloxyfucoxanthin, pigments often used as a proxy measurement for haptophytes  
389 (Latasa, 2007), but these are also the principal accessory pigment of species within the Kareniaceae  
390 (Steidinger et al., 2008), whose presence on the shelf has not been regularly investigated. The  
391 intrusion of *Karenia* populations to the mid-Atlantic region has vast implications for resource  
392 management. The impacts of these blooms depend upon the relative concentrations of each *Karenia*



393 species, the relative rates of toxin production, and the differential toxicity to aquatic communities.  
394 Currently, resource management strategies, such as the National Shellfish Sanitation Program  
395 (NSSP) guidelines, are based on the cell abundance and brevetoxin concentrations associated with  
396 *K. brevis*, which has not yet been observed in actionable concentrations ( $\geq 5,000$  cells L<sup>-1</sup>; NSSP,  
397 2017) in the Delmarva Peninsula's shellfish harvesting areas. Using LC-MS, Fowler et al. (2015)  
398 reported that *K. papilionacea* strains established from a Delaware population produce PbTx-2,  
399 though at a significantly lower concentration per cell compared to *K. brevis*. *Karenia papilionacea*  
400 is suspected to be toxigenic in other parts of the world (Haywood et al., 2004; Amzil et al., 2021)  
401 however, whole water samples collected during nearly monospecific *K. papilionacea* blooms on the  
402 Maryland coast, with cell concentrations ( $1.1$  to  $2.3 \times 10^5$  cells L<sup>-1</sup>) at or greater than those reported  
403 as toxic by Fowler et al. (2015;  $1.0 \times 10^5$  cells L<sup>-1</sup>), were negative for brevetoxin.

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

416 The cell concentrations of mid-Atlantic *Karenia* populations ( $10^5$  cells L<sup>-1</sup>) are less than  
417 those of catastrophic *Karenia* blooms reported globally ( $10^6 - 10^7$  cells L<sup>-1</sup>; Steidinger 2009;

418 Vandersea et al., 2020; Hu et al., 2022; Iwataki et al., 2022; Orlova et al., 2022), but are of equal  
419 concentrations to the *K. mikimotoi* blooms that impacted shellfish resources in the Gulf of Maine  
420 ( $10^5$  cells L<sup>-1</sup>; Scully et al., 2022). Despite the occurrence of elevated concentrations of *Karenia*  
421 spp. on the Delmarva Peninsula coast at temperature and salinity ranges in which other *Karenia*  
422 blooms have caused fish kills (see example for *K. mikimotoi* in Figure 4), no adverse environmental  
423 or human health problems have been reported to date. This is not unlike other toxic, bloom-forming  
424 species found in the Delmarva Peninsula region. For reasons unknown, species of *Dinophysis* and  
425 *Pseudo-nitzschia* are seasonally present at cell concentrations of concern but have not been  
426 associated with negative environmental impacts due to reduced toxin production compared to their  
427 global counterparts (Thessen and Stoecker, 2008; Wolny et al., 2020a; Ayache et al., 2023). From a  
428 limited number of reports, it appears that not all *Karenia* blooms have been associated with harmful  
429 environmental impacts (Table 4). Similarly, not all cultured *Karenia* strains maintain the ability to  
430 produce toxins (Sunda et al., 2013), which Lekan and Tomas (2010) linked to inherent genetic  
431 differences between cultured strains, indicating toxicity variation within natural populations is  
432 possible. The genetic assessment conducted by Coyne et al. (2015) demonstrated that the Delaware  
433 *K. papilionacea* population was distinct from the Gulf of Mexico and New Zealand populations,  
434 which coupled with different environmental conditions, may explain the lack of or reduced toxin  
435 production by the population found in the Delmarva Peninsula region (Fowler et al., 2015)  
436 compared to *K. papilionacea* populations reported to be toxic by Haywood et al. (2004) and Amzil  
437 et al. (2021). The long-term shifting of environmental conditions may play a role in the toxicity of  
438 numerous HAB species (Griffith and Gobler, 2020; Wells et al., 2020; Anderson et al., 2021).  
439 Specifically, for *Karenia*, climate change is being correlated to changes in the toxicity of *K. brevis*  
440 and *K. selliformis* (Brandenburg et al., 2019) and *K. mikimotoi* and *K. papilionacea* (Kwok et al.,  
441 2016) blooms.

442           Until local representatives of each *Karenia* species are isolated and cultured and controls on  
443 their growth and production of bioactive compounds assessed, their potential significance as a toxin  
444 source in the current and predicted conditions for the Chesapeake Bay, Delmarva Peninsula, and  
445 mid-Atlantic shelf waters cannot be determined. Further, until we better surveil mid-Atlantic waters  
446 for the presence of *Karenia* spp., their potential impacts to aquatic and coastal communities cannot  
447 be addressed. At present, phytoplankton collections along Delaware's coast are limited to a  
448 volunteer-based HAB monitoring program and no routine offshore monitoring is currently being  
449 conducted. Maryland conducts temporally- and spatially-limited phytoplankton monitoring offshore  
450 and within its coastal bays. Virginia phytoplankton monitoring is confined primarily within  
451 Chesapeake Bay, with limited sampling occurring in the Virginia coastal bays, and no routine  
452 offshore monitoring. The data compiled and presented here are from limited *in situ* monitoring  
453 activities along the Delmarva Peninsula coast. These data suggest there has been a routine  
454 occurrence of several *Karenia* spp. for the past 15 years, although no adverse environmental  
455 impacts have been reported. Because data are sporadic, it is difficult to assess whether *Karenia* spp.  
456 blooms are of emerging concern in this region. Spatially and temporally robust HAB monitoring  
457 programs in this region would help fill data gaps and allow resource managers to assess the  
458 potential for a *Karenia* population to impact fishery and recreational resources in the mid-Atlantic  
459 region. Climate change is altering mid-Atlantic niche environments and oceanographic patterns  
460 (Saba et al., 2016), and could alter the relative distribution, success, and toxicity of *Karenia* in the  
461 mid-Atlantic region as it has for other regional harmful algal species (Vidyarathna et al., 2020).

462           The ultimate causes of the spatial and temporal patterns of *Karenia* spp. observed during the  
463 2018 Delmarva cruises are unknown, but likely reflect differences in each *Karenia* species'  
464 environmental preferences, as well as differing physical and environmental forces acting on each  
465 species. In a recent study by Kim et al. (2023) in Korean waters, co-occurring *K. mikimotoi* and *K.*  
466 *papilionacea* blooms were documented in the months of March through July, similar to the

467 observations made along the Delmarva Peninsula in 2018. Studies conducted on these species, both  
468 in the Korean Strait and the East China Sea, indicate optimal growth occurs between 20 – 24 °C, but  
469 that the growth rate of *K. papilionacea* is greater than that of *K. mikimotoi* (Baohong et al., 2021;  
470 Kim et al., 2023). Both Vandersea et al. (2020) and Scully et al. (2022) showed that the blooms of *K.*  
471 *mikimotoi* observed in Kachemak Bay, Alaska, and Cape Cod Bay, Massachusetts, respectively,  
472 coincided with these regions' maximum water column stratification period. Both studies also noted  
473 sufficient nutrient pools at depth, which were exploited by *K. mikimotoi* as the populations underwent  
474 diel vertical migration. Similar dynamics have been described for *K. papilionacea* blooms in Japanese  
475 coastal waters by Yamaguchi et al. (2016). Bottom phytoplankton populations were not evaluated as  
476 part of the 2018 cruises, however, bloom concentrations of both *K. mikimotoi* and *K. papilionacea*  
477 were noted from samples collected at the depth of the chlorophyll maximum in June (5.3 – 11.5 m)  
478 and July (6 – 7.7 m), respectively. In 2019, cruises conducted in May and July failed to find a surface  
479 population of *Karenia* but did detect background concentrations of *K. mikimotoi* and *Karenia* sp. #3  
480 in at-depth waters (3.4 – 11.9 m). Of note, *K. papilionacea* was detected in elevated concentrations  
481 at the pycnocline (5 m) in the lower Chesapeake Bay in the late summer and early autumn of 2017,  
482 indicating that perhaps a larger, undetected population was advecting into the Bay from offshore,  
483 deep waters or that the bloom was concentrated at depth to take advantage of a nutrient pool. Physical  
484 forcings, such as those proposed for the 2018 Gulf of Mexico *K. brevis* bloom by Weisberg et al.  
485 (2019), in which an overwintering population was reseeded with a new bloom population or nutrient  
486 regeneration from N<sub>2</sub> fixation, decaying fish, and other processes (Mulholland et al., 2004; 2006;  
487 2014; Walsh et al., 2009; Bronk et al., 2014; Heil et al., 2014), may play a role in the species diversity  
488 and cell concentrations seen here as in other areas where *Karenia* blooms are known to persist.

489         Dating back to 2007 in Delaware (Bott, 2014) and 2012 in Maryland (J. O'Neil and J. Wolny,  
490 unpublished data), multiple *Karenia* species have been annually reported at background to low cell  
491 concentrations (100 to < 10,000 cells L<sup>-1</sup>) in summer to early autumn samples collected as part of

492 each state's coastal HAB monitoring and offshore research programs. Based on a limited number of  
493 samples, *Karenia* appear to occur less frequently in Virginia coastal waters. However, *Karenia*  
494 blooms do occur in mid-Atlantic waters as evidenced by high concentrations of *K. papilionacea* found  
495 in the Chesapeake Bay, from samples collected near the Bay mouth and Virginia's eastern shore, and  
496 blooms of both *K. mikimotoi* and *K. papilionacea* found along the Delaware and Maryland coasts in  
497 2010, 2016, 2018, and 2019. The *K. papilionacea* bloom documented in 2016 occurred along both  
498 Delaware and Maryland coastlines but was not reported in Virginia waters. In 2017, when *K.*  
499 *papilionacea* was reported in the Chesapeake Bay for the first time, concentrations were equal to  
500 bloom concentrations of *K. brevis* in the Gulf of Mexico ( $10^6$  cells  $L^{-1}$ ; Steidinger, 2009), but no  
501 blooms were reported in either Delaware or Maryland waters. In the summer of 2018, when bloom  
502 concentrations of *Karenia* were reported in Maryland's coastal waters, cells were in low abundance  
503 in Delaware coastal waters (1,000 cells  $L^{-1}$  in June and  $\leq 7,000$  cells  $L^{-1}$  in July) and absent in the  
504 lower Chesapeake Bay and the Virginia coastal bays. The data presented here indicate that along the  
505 Delmarva Peninsula *Karenia* blooms are patchy and likely subject to estuarine outflows, prevailing  
506 wind patterns, and/or ocean currents. The inclusion of more *in situ* sampling coupled with remote  
507 sensing technologies (e.g., regionally tuned satellite algorithms, autonomous monitoring platforms,  
508 imaging flow cytobot arrays) and interagency cooperation are needed to detect and track HAB  
509 occurrences. Satellite remote sensing is proving useful for detecting and tracking non-*Karenia* HABs  
510 in this region (Wolny et al., 2020b; Xiong et al., 2023). Unfortunately, not enough data exist to  
511 determine how successful these remote sensing processes are at detecting and tracking *Karenia*  
512 blooms along the Delmarva Peninsula even though satellite remote sensing has been used successfully  
513 to monitor *Karenia* blooms in other global locations (Jordan et al., 2021; Hu et al., 2022; Iwataki et  
514 al., 2022).

## 515 **5 Conclusions**

516 *Karenia* populations are routinely present in the mid-Atlantic region, raising concerns that  
517 there are now local offshore populations or populations being introduced seasonally via the Gulf  
518 Stream more frequently than historic phytoplankton surveys have shown. Coastal HAB monitoring  
519 occurring on the Delmarva Peninsula is spatially- and temporally-limited raising the possibility that  
520 the full extent of these blooms are not being detected with current *in situ* sampling efforts. Similar  
521 shifts in HAB events have been noted for other locations on the United States eastern seaboard during  
522 the same time period of this study. These emerging HABs (*i.e.*, *Pseudo-nitzschia australis* in the Gulf  
523 of Maine (Clark et al. 2022), *Dinophysis acuminata* in Long Island Sound (Hattenrath-Lehmann et  
524 al., 2013) and Chesapeake Bay and the Delmarva Peninsula's coastal bays (Wolny et al., 2020a;  
525 Ayache et al., 2023), and *Aureoumbra lagunensis* in Florida's Indian River Lagoon (Phlips et al.,  
526 2021)) have been tied to warming water masses, ocean current variations, and changes in circulation  
527 patterns within smaller embayments that impact water residence times. The role that climatic  
528 variability (e.g., shifts in the Gulf Stream), environmental niche changes (e.g., warming water  
529 temperature), and outflow variations from the Chesapeake Bay and Delaware Bay plumes (Filippino  
530 et al., 2009; Saba et al., 2016; Jiang and Xia, 2018; Xu et al., 2020) play in establishing and  
531 concentrating *Karenia* blooms in this region of the mid-Atlantic coast are unknown. A toxin risk  
532 assessment should be conducted in Maryland and Delaware waters to safeguard aquatic resources  
533 even though no adverse events due to *Karenia* blooms have been reported to date. A toxin assessment  
534 conducted in the Virginia portion of the Chesapeake Bay in 2019 and 2020 did not find brevetoxin in  
535 whole water or shellfish tissues (Pease et al., 2023), but this study excluded Virginia's coastal bays  
536 and offshore sites, where *Karenia* would most likely occur. Investigating these questions, alongside  
537 evaluating risks to the Delmarva Peninsula's growing aquaculture and coastal fishery industries  
538 (Froehlich et al., 2022), will require focused research efforts and *in situ* water quality and HAB  
539 monitoring programs with greater spatial and temporal extent than current capacities.

540

541 **Data Availability**

542 All data are publicly available at the following websites: [www.citizen-monitoring.udel.edu](http://www.citizen-monitoring.udel.edu),  
543 [www.eyesonthebay.dnr.maryland.gov](http://www.eyesonthebay.dnr.maryland.gov), and <https://datahub.chesapeakebay.net>.

544

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550

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568

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

971		Delaware	Maryland	Virginia	Bloom Concentration
972					
973	<i>K. brevis</i> -like	50,000 (Sep)	14,000 (Aug)	n/a	10 <sup>3</sup> (Steidinger, 2009)
974	<i>K. mikimotoi</i>	10,000 (Apr, Jun, Jul)	42,000 (Aug)	2,800 (June)	10 <sup>3</sup> (Li et al., 2019)
975	<i>K. papilionacea</i>	2,062,000 (Sep)	233,300 (Aug)	5,825,300 (Sep)	10 <sup>3</sup> (Yamaguchi et al., 2016)
976	<i>K. selliformis</i>	n/a	19,900 (Jun)	100 (Oct)	10 <sup>5</sup> (Feki-Sahnoun et al., 2020)
977	<i>Karenia</i> sp. #3	n/a	9,300 (Aug)	n/a	n/a

978 Table 2. Geographic range, number of occurrences, and salinity and temperature ranges reported for each *Karenia* species detected along the Delmarva Peninsula  
 979 and within Chesapeake Bay samples collected from 2007 through 2022 via state agency and citizen science monitoring efforts.

980	981	982	983	984	985	986	987
Species	Geographic Range (north to south)	Number and (Timing) of Occurrences	Salinity (ppt)	Temperature (°C)			
<i>K. brevis</i> -like	38.79N, -75.16W to 38.32N, -75.061W	17 (Aug – Nov)	27.4 – 32.9	16.5 – 26.0			
<i>K. mikimotoi</i>	38.79N, -75.16W to 38.28N, -75.09W	25 (May – Dec)	19.0 – 30.9	7.0 – 26.7			
<i>K. papilionacea</i>	38.79N, -75.16W to 36.99N, -76.01W	239 (Jun – Nov)	23.7 – 33.5	8.9 – 26.5			
<i>K. selliformis</i>	38.45N, -75.03W to 38.09N, -75.28W	14 (Apr – Jul)	24.6 – 29.4	15.5 – 20.8			
<i>Karenia</i> sp. #3	38.37N, -75.13W to 38.21N to -75.12W	7 (Jun – Oct)	19.7 – 30.9	16.4 – 28.3			

988 Table 3. Abundance, location, timing, and the salinity and temperature ranges reported for each *Karenia* species detected offshore the Delmarva Peninsula  
 989 during the 2018 coastal cruises.

990	991	992	993	994	995	996	997
Species	Maximum cell L <sup>-1</sup>	Offshore Area of Occurrence	Timing	Salinity	Temperature (°C)		
<i>K. brevis</i> -like	1.10 × 10 <sup>3</sup>	DE, MD	July	29.2 – 30.3	24.0 – 26.4		
<i>K. mikimotoi</i>	1.91 × 10 <sup>5</sup>	DE, MD, VA	June, July, October	28.1 – 33.4	19.4 – 25.1		
<i>K. papilionacea</i>	1.59 × 10 <sup>5</sup>	DE, MD, VA	June, July, October	28.3 – 33.4	21.8 – 26.4		
<i>K. selliformis</i>	1.99 × 10 <sup>4</sup>	DE, MD, VA	June, July, October	28.5 – 33.3	21.3 – 25.4		
<i>Karenia</i> sp. #3	1.50 × 10 <sup>3</sup>	DE, MD	July, October	30.0 – 32.9	23.4 – 24.3		

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

999	Species	Maximum cell L <sup>-1</sup>	Area of Occurrence	Timing	Average Salinity	Average Temperature (°C)	Reference
1002	<i>K. longicanalis</i>	$1.8 \times 10^5$	Victoria Harbour, Hong Kong	May	30	n/a	Yang et al., 2001
1003	<i>K. mikimotoi</i>	$10^4$	English Channel	August	34.75	16.5	Zevenboom et al., 1991*
1004		$3.9 \times 10^4$	Hoketsu Bay, Japan	August	33.5	26	Koizumi et al., 1996**
1005		$1.5 \times 10^7$	Cochin Estuary, India	October	26.8	30.5	Madhu et al., 2011
1006		$1.4 \times 10^6$	English Channel	July	35	16	Hartman et al., 2014
1007		$1.9 \times 10^5$	Delmarva Coast	June	27.9	19.6	this study
1008	<i>K. papilionacea</i>	$1.8 \times 10^4$	Arabian Gulf	January	41.6	14.0	Al-Yamani et al., 2012
1009		$6.1 \times 10^3$	Kochi Prefecture, Japan	May, June	33.4	29.2	Yamaguchi et al., 2016
1010		$3.2 \times 10^7$	Kuwait Bay	April	41.6	23.8	Polikarpov et al., 2020
1011		$2.1 \times 10^6$	Delmarva Coast	July	25.5	20.9	this study
1012		$5.8 \times 10^6$	Chesapeake Bay	September	26.3	23.2	this study

1013

1014 \*Data published using *Gyrodinium aureolum* as species name. \*\*Data published using *Gymnodinium mikimotoi* as species name.

1015 Figure 1. Map of routine phytoplankton monitoring stations along the Delmarva Peninsula and Chesapeake Bay. For this study, samples were  
1016 collected between 2007 and 2022 by the UD CMP, MDE, MDNR, VDH, and ODU. Open symbols indicate stations where *Karenia* spp. were  
1017 not observed. Closed symbols indicate stations where *Karenia* spp. were observed.  
1018

1019 Figure 2. Maps of the coastal transects sampled during the 2018 and 2019 Delmarva cruises conducted by UMCES. A. Distribution and cell  
1020 concentrations of the *Karenia mikimotoi* bloom detected in June 2018. B. Distribution and cell concentrations of the *K. papilionacea* bloom  
1021 detected in July 2018.  
1022

1023 Figure 3. Light micrographs of *Karenia* species detected in water samples collected in Delmarva Peninsula waters between 2007 and 2022. A. *K.*  
1024 *brevis*-like. B. Large cell form of *K. papilionacea*. C. Small cell form of *K. papilionacea*. D. *K. mikimotoi*. E. *K. selliformis*. F. Unidentified  
1025 *Karenia* species, termed *Karenia* sp. #3, as described by Steidinger et al. (2008) and de Salas (2004).  
1026

1027 Figure 4. Scatterplot of average temperature and salinity data reported in the literature for fishery impacting (black diamonds) and non-fishery  
1028 impacting (white diamonds) *Karenia mikimotoi* blooms. Data from this study are represented in the dashed line white diamond. The data  
1029 presented here were collected from this study, the references listed in Table 4, and Matsuyama (2006), Robin et al. (2013), PIRSA (2014),  
1030 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  
1031 Iwataki et al. (2022).