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Succession Of The Late Summer Phytoplankton Blooms In The York River Estuary, Va

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Succession of the Late Summer Phytoplankton Blooms in the York River Estuary, VA

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ABSTRACT

The influence of bottom-up and top-down controls on the formation and persistence of phytoplankton blooms has been well studied. However, the relative importance of these bottom-up and top-down controls vary spatially and temporally. In the tidal tributaries and mainstem of Chesapeake Bay, the summer dinoflagellate population follows a succession of bloom-producing species. The dinoflagellate species *Margalefidinium polykrikoides* and *Alexandrium monilatum* are currently considered the end of this succession. These species form near-annual blooms in the lower half of Chesapeake Bay and are considered harmful algal bloom (HAB) species due to their negative ecological impacts. However, analysis of long-term monitoring data and previous field samples suggest that *Ceratium furca*, a non-toxic dinoflagellate, might be an overlooked species in this dinoflagellate succession. My objective was to explore the influence of bottom-up and top-down controls on the species succession of the late summer phytoplankton blooms in the lower Chesapeake Bay. In the laboratory I used cultures of *A. monilatum* and *C. furca* isolated from the York River to evaluate the abiotic drivers influencing the succession of the late summer bloom from *A. monilatum* to *C. furca.* These experiments showed that each species exhibited differing light and temperature preferences but neither had a preferred N:P ratio. Lower light levels and lower temperatures favor non-toxic *C. furca* over toxin-producing *A. monilatum* in the York River. This information can help oyster aquaculturists identify regions of Chesapeake Bay that are unlikely to favor *A. monilatum* and are safer for oyster grow-out practices. Next, I used a combination of weekly field sampling along with *in situ* experiments during the late summer to assess top-down control of the copepod *Acartia tonsa* on harmful *M. polykrikoides* blooms. Sampling occurred during *M. polykrikoides* blooms in the lower York River in 2021 and 2022 and prey removal experiments were conducted using the water and copepods collected. I found that at *M. polykrikoides* abundances above 2000 cells mL^{-1} *A. tonsa* experienced >50% mortality in the prey removal experiments over 24-hours. Furthermore, *A. tonsa* abundances within the lower York River declined over the course of the bloom. This suggests that at high concentrations, *M. polykrikoides* may act as its own grazing deterrent, reducing the influence of top-down control and supporting bloom proliferation and maintenance. The time it takes for the *A. tonsa* population to recover from the negative impacts of *M. polykrikoides* blooms may also result in a "window of opportunity", where a decrease in grazing pressure could support the formation of other blooms, like *A. monilatum.* This research provides insight into the bottom-up and top-down controls influencing the species succession in late summer phytoplankton blooms in the lower Chesapeake Bay. Evidence supports environmental conditions aiding in the transition from *A. monilatum* to *C. furca.* However, decreased grazing potential, as during an *M. polykrikoides* bloom, may also aid in the succession of species and provide opportunities for blooms to form. These findings can help environmental managers better predict when these blooms will occur and help to mitigate the negative impact of these blooms.

Succession of the Late Summer Phytoplankton Blooms in the York River Estuary, VA

INTRODUCTION

In marine systems, understanding the community composition and succession of phytoplankton has been a focus of ecological studies for many years (Behrenfeld et al., 2021). The phytoplankton community plays a key role in structuring the food web and drives trophic energy transfer within the ecosystem (Behrenfeld et al., 2021). In estuarine systems, like the Chesapeake Bay, phytoplankton are strongly influenced by freshwater and nutrient inputs, temperature, light availability, and tidal mixing (Harding, 1994). Phytoplankton are also highly structured by grazing impacts (Irigoien et al., 2005; Stoecker et al., 2008). These factors impact seasonal and spatial patterns within the phytoplankton community, and they may also influence the formation of phytoplankton blooms. Phytoplankton blooms can form when favorable environmental conditions (bottom-up controls) persist long enough (Thompson et al., 2008) or when a reduction in grazing pressure (top-down controls) allows for phytoplankton to grow (Mitra & Flynn, 2006). The relative importance of these bottom-up and top-down controls vary from system to system, and it is critical to study both factors to understand how these controls impact trophic energy transfer regionally (Metaxas & Scheibling, 1996).

In the tidal tributaries and mainstem of Chesapeake Bay, phytoplankton follow a seasonal succession of bloom-producing dinoflagellate species throughout the summer. Mulholland et al. (2018) suggests that the order of bloom-producing dinoflagellate species follows changes in water temperature (Figure 1.1). Currently, the two species considered the end of this succession are *Margalefidinium polykrikoides* (*cf. Cochlodinium polykrikoides*, Gómez et al., 2017) and *Alexandrium monilatum* (Figure 1.1; Mulholland et al., 2018). *M. polykrikoides* was first reported in the York River and lower Chesapeake Bay during the late 1960s and has since expanded its range to the Rappahannock, Lafayette, Elizabeth, and James Rivers (Morse et al.,

2011; Reece, 2015; Wolny et al., 2020). *M. polykrikoides* typically blooms on an annual basis starting in late July or early August. *A. monilatum* recently emerged in Chesapeake Bay in 2007 and forms blooms following the decline of the *M. polykrikoides* blooms. However, there is evidence that it was in the region as early as the 1960s (Pease, 2016; Reece, 2015; Wolny et al., 2020). *A. monilatum* and *M. polykrikoides* bloom activity has intensified throughout the region in recent years (Wolny et al., 2020). This is cause for concern as both are considered harmful algal bloom (HAB) species due to their negative ecological impacts; *M. polykrikoides* produces ichthyotoxic effects (with no toxin currently characterized) and *A. monilatum* produces the toxin goniodomin A (GDA) (Gobler et al., 2008; Hsia et al., 2006). *A. monilatum* is considered to have a successional relationship with *M. polykrikoides*, with the dissipation of *M. polykrikoides* allowing for *A. monilatum* to take over in the environment (Reece, 2015).

However, another dinoflagellate species, *Ceratium furca,* is also known to form late-summer blooms in the mid- to lower-Bay (Marshall et al., 2009; Smalley & Coats, 2002). *C. furca* is a non-toxic species and is not considered a part of the dinoflagellate bloom succession described in Figure 1.1, but due to its ability to form late-summer blooms, may play a role in the seasonal succession of phytoplankton species in the York River. *C. furca* is a relatively slow-growing species described as having a cosmopolitan distribution, tolerating a wide range of temperatures and salinities (Baek et al., 2008a; Baek et al., 2008b). Extensive studies in Sagami Bay, Japan indicate that while *C. furca* is present during all seasons, their density increases in the summer and declines after October (Baek et al., 2006; Baek et al., 2008a). Similar patterns of *C. furca* increasing in the summer are documented in the Chesapeake Bay region, however, to my knowledge, extensive *C. furca* blooms that had deleterious ecological impacts have not been recorded (Marshall et al., 2009; Mulford, 1963; Smalley & Coats, 2002).

In the summer and fall of 2020, the VIMS Phytoplankton Ecology laboratory observed an increase in the presence of *C. furca* after an *A. monilatum* bloom declined. This suggests that *A. monilatum* blooms may be succeeded by *C. furca*. Using Chesapeake Bay Program phytoplankton data from seven stations from 1992–2018, I noted that *C. furca* was present at the majority of stations during each year analyzed (Figure 1.2). When *C. furca* abundance was averaged by month, the highest abundance was found in July for all but one station. However, multiple stations also showed a secondary peak in abundance during September or October (Figure 1.3). This supports the idea that *C. furca* may increase in abundance in the early fall following the decline of *A. monilatum* blooms.

Previous Bloom Sampling

To assess *C. furca* abundances during previous *A. monilatum* blooms, I analyzed thirty-eight Lugol's preserved phytoplankton samples taken from the lower York River in 2020. These samples were collected approximately weekly from the end of July to the beginning of September as a part of the VIMS HAB research cruises. Samples were collected with a 100 mL bottle dipped below the river surface within visible bloom patches. Cell counts were conducted using a Sedgewick-Rafter counting chamber under a Zeiss Axio Imager.A2 microscope at 10x magnification. For each sample, the entire chamber was counted or until a minimum of 300 cells was reached.

From the samples counted, I did not find a strong correlation between *A. monilatum* abundance and *C. furca* abundance (Appendix, Figure A.1). However, a marked increase in both *A. monilatum* and *C. furca* abundance was noted on September 8 (Figure 1.4). Also, samples with high *C. furca* abundance typically had lower *A. monilatum* abundance, relative to the abundances for each species in other samples. This further suggests that *C. furca* may increase in

abundance around the end of *A. monilatum* blooms. However, to fully assess *C. furca's* role in the dinoflagellate bloom succession more research is needed.

The objective of my thesis research was to explore the influence of bottom-up and top-down controls on the species succession of the late summer phytoplankton blooms in the lower York River Estuary, VA. To address this objective, I divided my project into two chapters addressing two specific goals. The first was to experimentally assess the abiotic factors (bottom-up controls) influencing the succession of the late summer bloom of *A. monilatum* and *C. furca.* The second was to evaluate the role that *Acartia tonsa* copepod grazing (top-down control) has in the bloom succession of *M. polykrikoides*, *A. monilatum,* and *C. furca.* While my original intention was to assess top-down control on all three species, only *M. polykrikoides* bloomed during my two sampling seasons. Therefore, the effect of top-down control was only evaluated on *M. polykrikoides.* To address my objectives, I used a combination of laboratory experiments along with weekly field sampling during the late summer of 2021 and 2022. This research reevaluated the end of the late summer phytoplankton blooms in the lower York River Estuary and provides insight into the bottom-up and top-down controls influencing this species succession.

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Figures

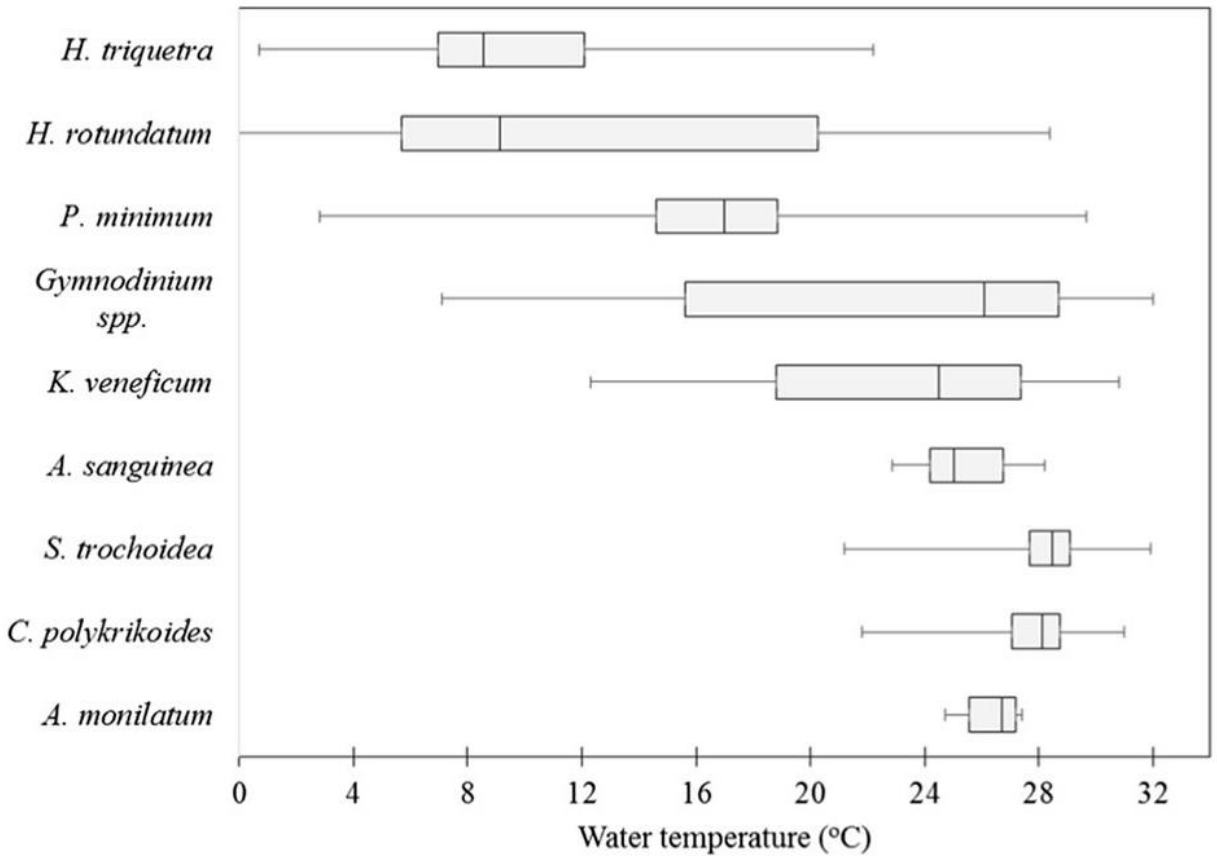

Figure 1.1: Bloom succession of dinoflagellate species with respect to water temperature in Chesapeake Bay and Virginia tidal tributaries (from Mulholland et al., 2018).

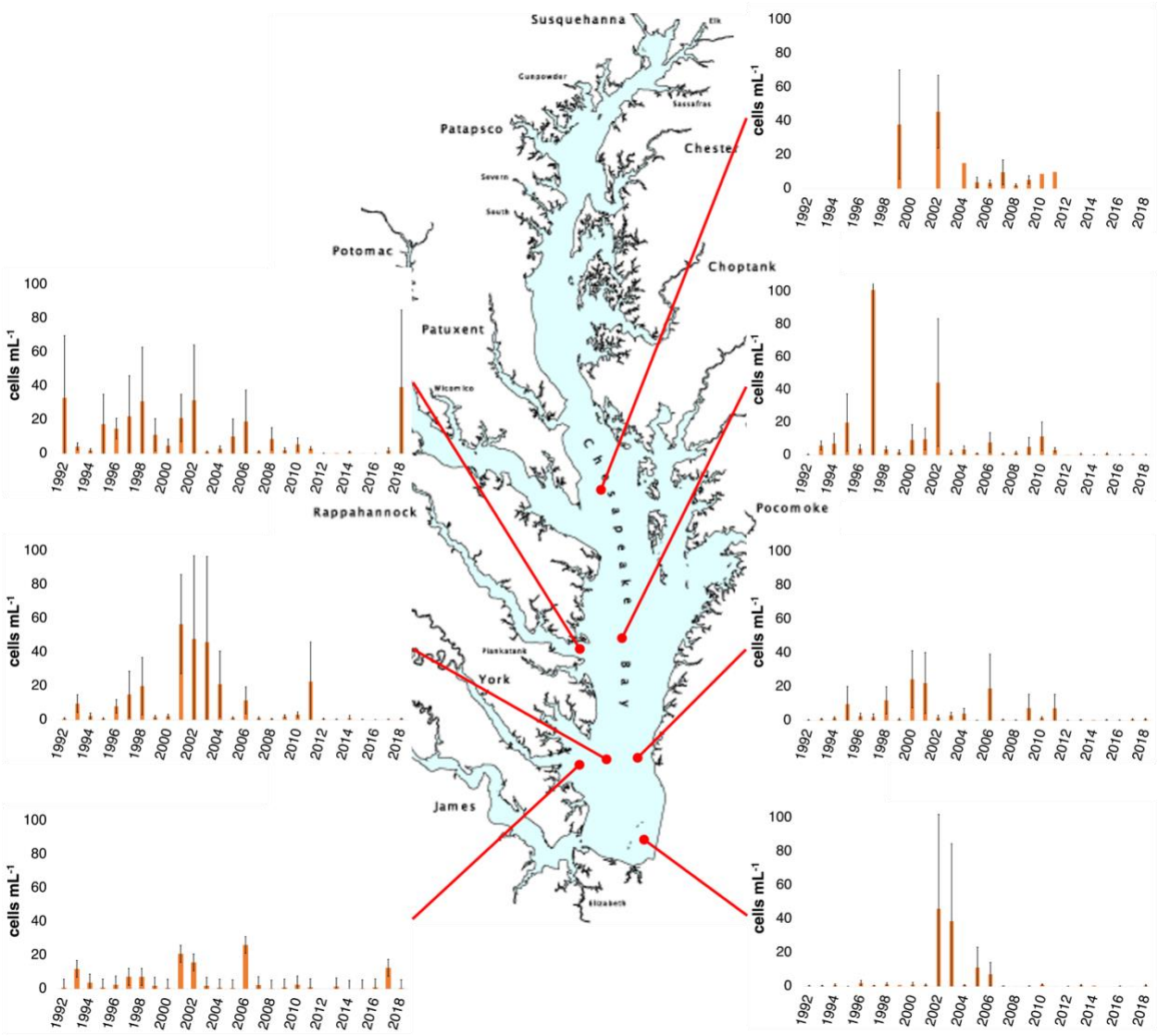

Figure 1.2: Annual *C. furca* abundance (cells mL^{-1} ± SE) from 1992–2018 at seven long-term monitoring stations within Chesapeake Bay. Tidal phytoplankton data was obtained from the Chesapeake Bay Program Baywide Plankton Database (http://www.chesapeakebay.net) and averaged by year from 1992–2018.

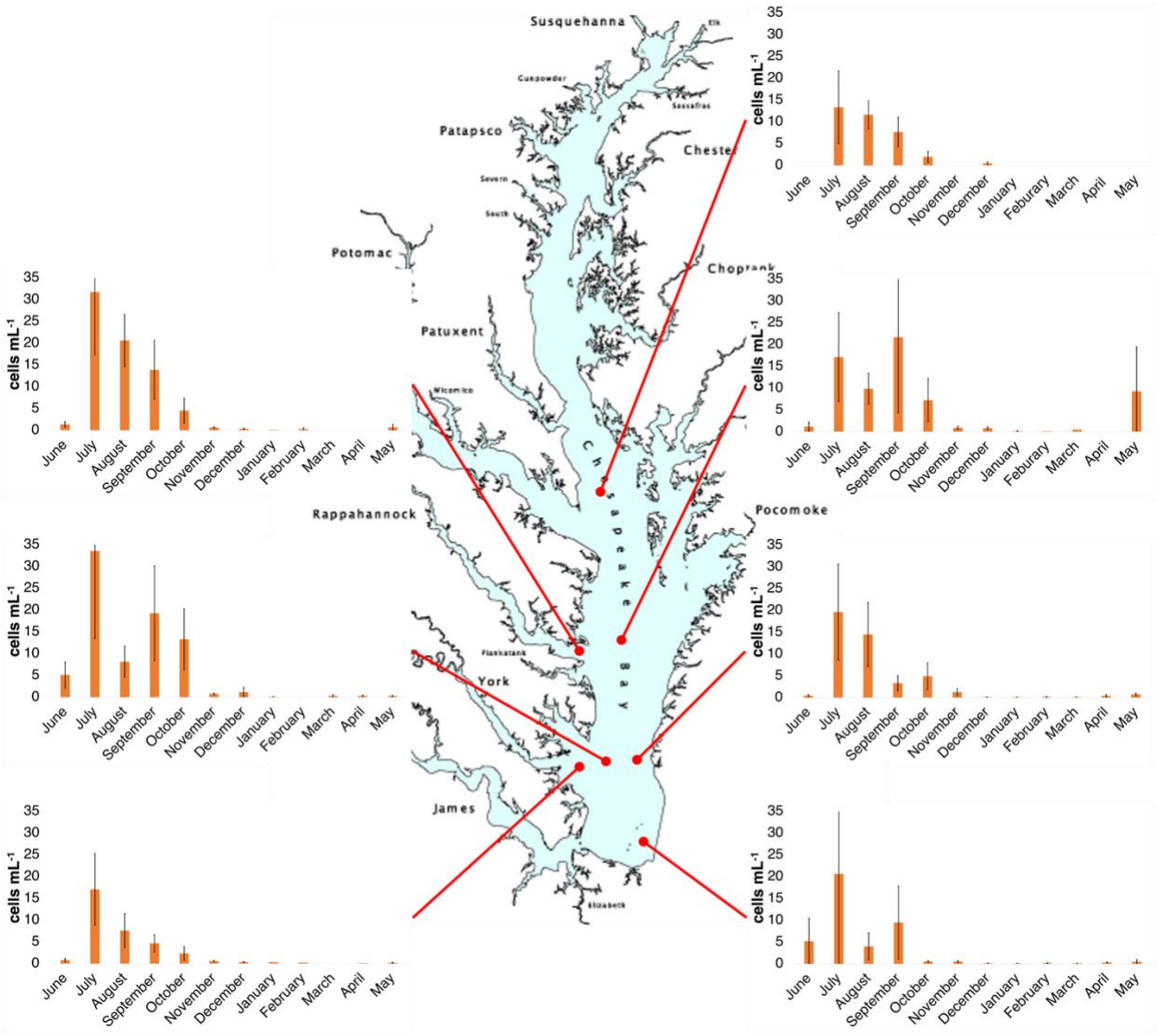

Figure 1.3: Monthly *C. furca* abundance (cells mL^{-1} ± SE) from 1992–2018 at seven long-term monitoring stations within Chesapeake Bay. Tidal phytoplankton data was obtained from the Chesapeake Bay Program Baywide Plankton Database (http://www.chesapeakebay.net) and averaged by month for the years 1992–2018.

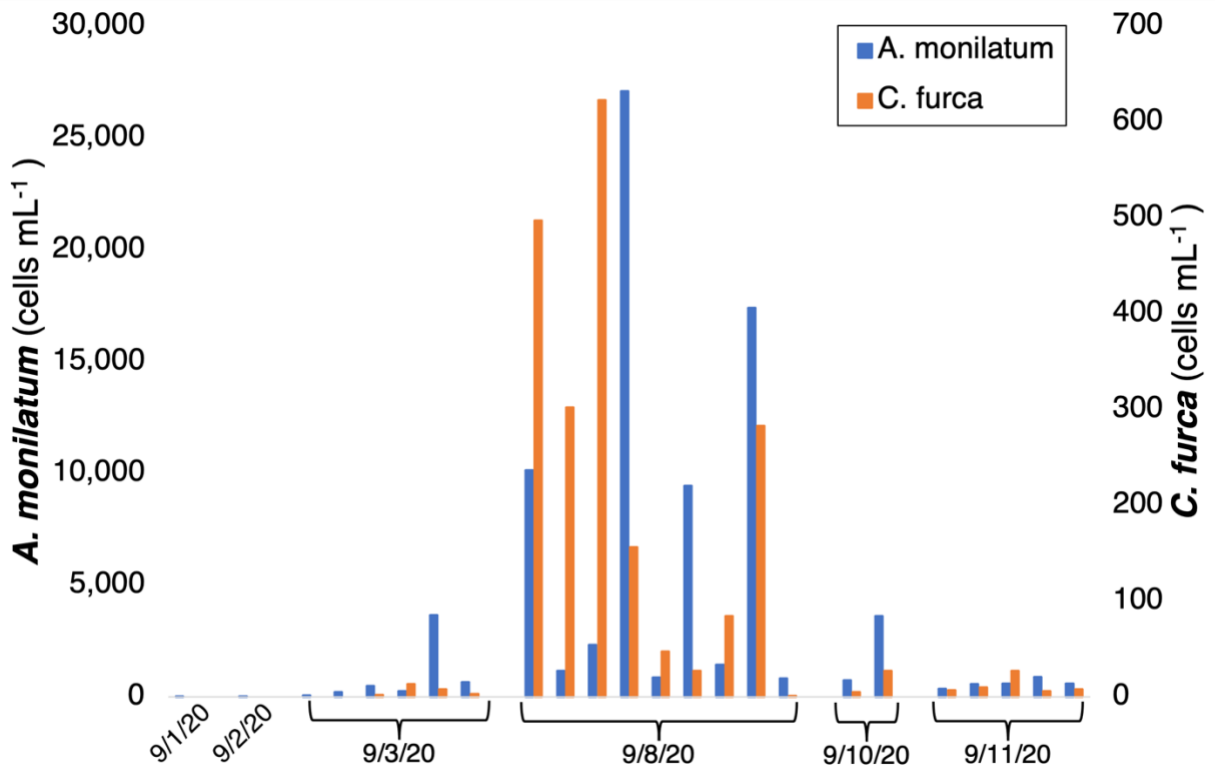

Figure 1.4: Previous HAB samples from the York River, VA. Abundance of *A. monilatum* (cells mL^{-1}) and *C. furca* (cells mL^{-1}) in the York River during 2020 bloom sampling.

Chapter 1

Abiotic drivers influencing dinoflagellate succession in the late summer bloom from *Alexandrium monilatum* to *Ceratium furca* in the York River

Introduction

Environmental controls including temperature, nutrient concentrations, and light availability are important factors contributing to phytoplankton community composition, structure, and abundance (Harding, 1994; Marshall et al., 2009; Thompson et al., 2008). Interaction of these environmental factors along with others, such as runoff, salinity, and physical mixing, leads to a spatially and seasonally heterogeneous environment. These ever-changing environmental conditions promote species diversity within the phytoplankton community, while large-scale seasonal patterns may support a semi-predicable annual species succession (Cloern & Jassby, 2010). Shifts in the dominate species at the primary trophic level will subsequently affect the biomass and composition of higher trophic levels (Marshall, 2009; Thompson et al., 2008).

In the Chesapeake Bay and its tidal tributaries, variations in environmental factors support an annual succession of phytoplankton starting with winter dinoflagellate blooms. These blooms are dominated by two *Heterocapsa* spp., depending upon the region, that have the ability to survive at low temperatures and can use mixotrophy to overcome low light (Millette et al., 2017; Millette et al., 2023). In the spring, irradiance levels start to increase, and high amounts of riverine input bring large concentrations of new inorganic nutrients into the system (Malone et al., 1996; Spilling et al., 2018). Diatoms generally dominate during this time as they favor high concentrations of nitrate (Glibert et al., 2016) and their high growth rates allow them to outgrow zooplankton grazing (Reynolds, 2006). As spring transitions to summer, nutrients are depleted, nitrate is remineralized to ammonium, and density driven stratification stabilizes the water column (Malone et al., 1996). These conditions are preferred by dinoflagellates as they can swim to the nutrient rich bottom waters and uptake inorganic nutrients before returning to the surface

waters during the day to obtain adequate light for photosynthesis (Jephson & Carlsson, 2009; Reynolds, 2006). Many dinoflagellates are also mixotrophic, meaning they can ingest prey under growth limiting conditions to keep growing (Stoecker et al., 2017). Once dinoflagellates begin to dominate the phytoplankton community, they follow a succession of species throughout the summer. Mulholland et al. (2018) attributes this succession of bloom-producing species to water temperature preferences, however, there may be other factors that have not yet been fully addressed.

It is currently thought that the succession of summer bloom-producing dinoflagellate species in Chesapeake Bay concludes with *M. polykrikoides* and *A. monilatum*. However, during the summer and fall of 2020, we observed an increase in the presence of *C. furca* after an *A. monilatum* bloom declined. This suggests that *C. furca* might be an overlooked species in this dinoflagellate succession. Understanding how *C. furca* fits into this succession is important because *C. furca* is a non-toxic dinoflagellate, while *M. polykrikoides* and *A. monilatum* are considered HABs due to their toxin production or toxin-like impacts (Baek et al., 2006; Gobler et al., 2008; Hsia et al., 2006). Therefore, understanding the conditions that cause *C. furca* to succeed an *A. monilatum* bloom will help us further understand the environmental conditions that support non-toxic blooms over HABs. While previous studies have addressed some of the preferred temperature and nutrient conditions for *A. monilatum* and *C. furca* individually, there are key data gaps. Specifically, the effect of light on *A. monilatum* growth as well as the effect of comparable N:P ratios between the two species have not been studied. Furthermore, the relative importance of each environmental factor on growth has not been determined for *A. monilatum* or *C. furca* isolates from the York River.

Factors that likely influence the growth and timing of *A. monilatum* and *C. furca* include light availability, nutrient concentrations, and water temperature. Baek et al. (2008b) showed that *C. furca* is adapted to intermediate to high light intensities with the maximum growth rate of *C. furca* found above 216 μmol m^{-2} s^{-1}. To my knowledge, the response of *A. monilatum* to varying light intensities has not been reported. If *C. furca* follows an *A. monilatum* bloom, it is possible that *C. furca* is adapted to higher light levels because high abundances of *A. monilatum* could be shading *C. furca*. Alternatively, given there is less light available in the fall, *C. furca* could be adapted to lower light levels than *A. monilatum* and may be able to tolerate a wider range of light intensities. Baek et al. (2008a) showed *C. furca* abundances increasing with nitrate concentrations. Similarly, in low nitrate *A. monilatum* cultures, Juhl (2005) showed an increase in abundance following an addition of nitrate. However, no published data are available comparing the preferred nitrate concentrations of both species. Given that nutrient concentrations are likely low at the end of *A. monilatum* blooms, *C. furca* could be adapted to lower nutrient concentrations than *A. monilatum.* Temperature is also a key factor in phytoplankton growth and species succession (Mulholland et al., 2018). Previous studies show *A. monilatum*'s maximum growth rate occurring at 31°C (Juhl, 2005) while other studies have noted *C. furca*'s optimum temperature at 24°C (Baek et al., 2008b). Therefore, *A. monilatum* may be adapted to higher temperatures than *C. furca*. This would explain why a late summer bloom of *A. monilatum* in the York River is followed in early fall by *C. furca* as water temperature cools with the changing seasons.

Given the historic data from the Chesapeake Bay Program (Figure 1.3) and the previous HAB data from 2020 (Figure 1.4), I hypothesize that *C. furca* follows *A. monilatum* in the bloom species succession. I hypothesize that *C. furca* will tolerate lower light levels, lower nutrient

concentrations, and lower temperatures than *A. monilatum*. This would allow *C. furca* to form a bloom in early fall, after an *A. monilatum* bloom had dissipated. To address these hypotheses, I used cultures of *A. monilatum* and *C. furca* isolated from the lower York River Estuary to conduct growth experiments at various light levels, nutrient concentrations, and temperatures. These experiments will help assess the possible influence of important bottom-up controls on the succession of these species. I found that each species showed clear light and temperature preferences but not a preferred N:P ratio. This demonstrates that lower light levels and lower temperatures favor the non-toxic *C. furca* over the toxin-producing *A. monilatum* in the York River.

Methods

Phytoplankton Cultures

Cultures of *A. monilatum* were isolated from the York River in 2020 and *C. furca* was isolated and cultured from samples collected from the lower York River during late summer, 2021. Both cultures were maintained at 20°C, an irradiance level of 32.93 µmol m^{-2} s^{-1}, and on a 12:12 h light:dark (L:D) cycle. *A. monilatum* was grown in L1 media (Guillard & Hargraves, 1993) while *C. furca* was grown in T_1 media (Baek et al., 2006) after preliminary work indicated that T_1 was not a viable growth media for *A. monilatum* and L1 was not a viable growth media for *C. furca*. T_1 media contains higher concentrations of both nitrate and phosphate than used in L1. Both medias contain similar trace metals with a few exceptions. However, the most notable difference is that T_1 contains concentrations of cyanocobalamin (vitamin B_{12}) that are four orders of magnitude higher than those used in L1 (Baek et al., 2006; Guillard & Hargraves, 1993).

Growth Rate Experimental Set-up

The *A. monilatum* growth rate experiments were conducted in 100 mL glass Pyrex bottles with ~100 mL of L1 media in each bottle while the *C. furca* experiments were conducted in 250 mL tissue flasks with ~100 mL of T_1 media in each flask. Different containers were used for the experiments as preliminary work indicated that these were the optimal containers for both species. The starting concentrations of *A. monilatum* and *C. furca* for the experiments were determined based on preliminary growth rate measurements and the carrying capacity of each culture. I wanted to ensure that the starting concentration for each culture was near the beginning of their exponential growth phase. For each experimental bottle, the intended initial cell concentration of *A. monilatum* was 1000 cells mL^{-1} while the intended initial cell concentration of *C. furca* was 100-300 cells mL^{-1}. At the beginning of each experiment, stock cultures of *A. monilatum* and *C. furca* were concentrated by gentle reverse filtration through 20 µm mesh. This reduced the transfer of old culture media into the fresh experimental media. The concentrated cultures were then added to fresh media in the experimental bottles to reach the respective initial cell concentrations. The experiments were conducted in triplicate under five light levels, five nutrient concentrations, and four temperatures. Each factor (light, nutrients, and temperature) was assessed individually with all other factors held constant (Figure 2.1).

To assess light preference, the experimental bottles were covered with different layers of mesh to reduce light levels. Actual light intensity was measured with a LI-COR light meter and quantum sensor and ranged from: 32.93 µmol m^{-2} s^{-1} (uncovered/control), 14.52 µmol m^{-2} s^{-1} (one mesh layer), 7.28 µmol m^{-2} s^{-1} (two layers), 2.84 µmol m^{-2} s^{-1} (three layers), to 1.88 µmol m^{-2} s^{-1} (four layers). To assess nutrient preference, the N:P ratio of L1 and T_1 media was altered for *A. monilatum* and *C. furca*, respectively, by either decreasing or increasing the nitrate concentration. The original L1 media has a 24:1 (882 µM:36.2 µM) nitrate:phosphate (N:P) ratio

(Guillard & Hargraves, 1993), while the original T_1 media has a 10:1 (1000 µM:100 µM) N:P ratio (Baek et al., 2006). The unaltered L1 and T_1 media served as the control for *A. monilatum* and *C. furca*, respectively. For the other treatments the nitrate concentration of each respective media was altered to produce a 6:1, 16:1, 35:1, and 50:1 N:P ratio. For L1 the nitrate concentrations were: 217.2 µM (6:1), 579.2 µM (16:1), 1267 µM: (35:1), and 1810 µM (50:1) while phosphate concentrations remained at 36.2 µM for all treatments. For T_1 the nitrate concentrations were: 600 µM (6:1), 1600 µM (16:1), 3500 µM (35:1), and 5000 µM (50:1) while phosphate concentrations remained at 100 µM for all treatments. To assess temperature preference, four incubators were used set at 20°C (control), 25°C, 27°C, and 30°C. Control conditions reflect the conditions *A. monilatum* and *C. furca* were originally cultured under and acclimated to in the laboratory for many months.

The experiments each ran for ~14 days (290-335 hours). Samples (3 mL) were taken every 3-4 days, fixed with Lugol's solution, and stored in a glass scintillation vial sealed with electrical tape until they were analyzed. Cell counts were conducted using a Sedgewick-Rafter counting chamber under a Zeiss Axio Imager.A2 microscope at 10x magnification. For each sample, the entire chamber was counted or until a minimum of 300 cells was counted. Specific growth rate (µ) of the two species over the length of the experiment was calculated using the following equation:

$$\mu = [\ln(C_2/C_1)/t]$$

where (C_2) and (C_1) are the cell densities in cells mL^{-1} at the final time and initial time and (t) is the total hours that the experiment ran. That value (µ, h^{-1}) was then multiplied by 24 to obtain the growth rate per day (µ, d^{-1}).

Statistical Analyses

A series of one-way ANOVAs were used to determine if the final abundances of *A. monilatum* and *C. furca* in each treatment tested for light, nutrients, and temperature were significantly different ($p < 0.05$). Significant differences ($p < 0.05$) were further evaluated with a post-hoc Tukey test. Means are reported ± standard error (SE) in Results.

A series of one-way ANOVAs were also conducted to test for statistically significant differences ($p < 0.05$) in the overall growth rates of *A. monilatum* and *C. furca* between each treatment tested. Significant differences ($p < 0.05$) were further evaluated with a post-hoc Tukey test.

Results

Effect of Light on Abundance and Growth

There was a significant difference in the final abundance of *A. monilatum* under the five light conditions tested (one-way ANOVA, $p < 0.001$, Figure 2.2a). The highest final abundance (3202 ± 334 cells mL^{-1}) was reached in the treatment with the highest light level and was significantly different than at all other light levels (post-hoc Tukey Test, Figure 2.2a). The lowest final cell abundances (901 ± 87 and 771 ± 70 cells mL^{-1}) were in the two lowest light levels and not significantly different from each other, but were significantly lower than the other light levels (Figure 2.2a). There was also a significant difference in the overall growth rates of *A. monilatum* under the five light conditions tested (one-way ANOVA, $p < 0.001$). The highest growth rate (0.104 d^{-1}) was reached in the treatment with the highest light level but was not significantly different (post-hoc Tukey Test) than the one-layer mesh treatment (0.080 d^{-1}, Table 1.1).

Under the same light conditions, the final abundance of *C. furca* was also significantly different after 14 days (one-way ANOVA, $p < 0.001$, Figure 2.2b). The highest final abundance (78 ± 4 cells mL^{-1}) was reached in the treatment with the highest light level but was not significantly different (post-hoc Tukey Test) than the one- and two-layer mesh treatments (77 ± 5

and 76 ± 1 cells mL^{-1}, respectively, Figure 2.2b). The final abundances in the two treatments with the lowest light (47 ± 10 and 32 ± 4 cells mL^{-1}) were significantly lower than the three higher light treatments, but were not significantly different from each other (Figure 2.2b). There was also a significant difference in the overall growth rates of *C. furca* under the five light conditions tested (one-way ANOVA, $p < 0.05$). The highest growth rate (0.006 d^{-1}) was reached in the two-layer mesh treatment but was not significantly different (post-hoc Tukey Test) than the zero-, one- or three-layer mesh treatments (0.001 d^{-1}, -0.013 d^{-1} and -0.039 d^{-1}, respectively, Table 1.1).

Effect of Nutrient Concentration on Abundance and Growth

Under the five nutrient conditions tested, there was no significant difference in the final abundances of *A. monilatum* (one-way ANOVA, $p = 0.088$, Figure 2.2c) or *C. furca* (one-way ANOVA, $p = 0.388$, Figure 2.2d). There also was no significant difference in the overall growth rates of *A. monilatum* (one-way ANOVA, $p = 0.297$, Table 1.1) or *C. furca* (one-way ANOVA, $p = 0.527$, Table 1.1).

Effect of Temperature on Abundance and Growth

There was a significant difference in the final abundance of *A. monilatum* under the four temperature conditions (one-way ANOVA, $p < 0.001$, Figure 2.2e). The highest temperature tested (30ºC) was significantly different than all other temperatures (post-hoc Tukey Test) and had the highest final cell abundance (3530 ± 224 cells mL^{-1}, Figure 2.2e). The second highest final abundance (2477 ± 270 cells mL^{-1}) occurred under 27ºC which was not significantly different from the final abundance under 25ºC (1868 ± 75 cells mL^{-1}) (Figure 2.2e). The lowest final abundance (1348 ± 99 cells mL^{-1}) occurred under 20ºC which was also not significantly different from the final abundance under 25ºC but was significantly different from 27ºC (Figure

2.2e). Growth rate results under the four temperature conditions followed the final abundance results with significant differences found in the overall growth rates (one-way ANOVA, $p < 0.001$). The highest growth rate (0.102 d^{-1}) was reached under the highest temperature tested (30ºC) and was significantly different than all other temperatures (post-hoc Tukey Test, Table 1.1).

Assessing the effect of the same temperature conditions on *C. furca*, a one-way ANOVA showed a significant difference in the final abundances of *C. furca* ($p < 0.001$, Figure 2.2f). A post-hoc Tukey Test showed that the final abundances under each temperature were significantly different from each other (Figure 2.2f). The highest final abundance (1314 ± 90 cells mL^{-1}) was reached when *C. furca* was grown under 25ºC (Figure 2.2f). This was followed in order of decreasing final abundance by 20ºC (730 ± 15 cells mL^{-1}), 27ºC (338 ± 48 cells mL^{-1}), and lastly 30ºC (88 ± 15 cells mL^{-1}) which had the lowest final abundance (Figure 2.2f). As with *A. monilatum*, growth rate results under the four temperature conditions followed the final abundance results (one-way-ANOVA, $p < 0.001$). The highest growth rate of *C. furca* (0.115 d^{-1}) occurred when *C. furca* was grown under 25ºC and was significantly different than all other temperatures (post-hoc Tukey Test, Table 1.1).

Discussion

The effect of light level, nutrient concentration, and temperature on *A. monilatum* and *C. furca* abundance and growth rate was evaluated in the laboratory for approximately 14 days. The results reveal that *A. monilatum* showed a clear preference for higher light levels and temperatures than *C. furca,* but not a preferred nutrient concentration. This suggests that the influence of light and temperature are likely impacting the succession of these species, with

lower light levels and lower temperatures favoring non-toxic *C. furca* over toxin-producing *A. monilatum* in the York River.

Under the various light levels, *A. monilatum* and *C. furca* demonstrated different preferences in light level, with *C. furca* able to tolerate lower light levels. While *A. monilatum* showed a clear preference for the highest light level and exhibited a significant decrease in abundance in the treatment that had one layer of light-reducing mesh, *C. furca* abundance was not different under the highest three light levels. This differed slightly from the overall growth rate results where *A. monilatum*'s highest growth rate was reached in the treatment with the highest light level but was not significantly different than the one-layer mesh treatment. *C. furca's* overall growth rate was also not significantly different under the highest four light levels. *A. monilatum's* preference for the highest light level suggests that the experimental treatments may not have reached saturating irradiance levels for *A. monilatum*. While previous studies are not available on the response of *A. monilatum* growth to light, these results suggest *A. monilatum* prefers higher light intensities compared to *C. furca* and that *C. furca* can tolerate lower light intensities. Prior research shows *C. furca* experiences light limitation at levels below 1000 µW cm^{-2} (~46 µmol m^{-2} s^{-1}) in culture (Meeson & Sweeney, 1982). This irradiance level differs from what was seen in this experiment where *C. furca* did not show any signs of light limitation until 2.84 µmol m^{-2} s^{-1} (three-layer treatment). Baek et al. (2008b) also explored the effect of light on *C. furca* and reported that *C. furca* reached maximum growth rate at 216 µmol m^{-2} s^{-1}, a light level nearly two orders of magnitude higher than in my results. However, *C. furca* cells in that experiment were acclimated to 180 µmol m^{-2} s^{-1} prior to testing and were subject to much higher light intensities than were tested in this experiment (up to 796 µmol m^{-2} s^{-1}) Therefore, *C. furca* in Baek's experiments also became light saturated at relatively low irradiance levels (Baek et al.,

2008b). It should also be noted that the *C. furca* culture used in this experimental run was not fully stable and starting cell concentrations were low (105 cells mL^{-1}) compared to those of the nutrient and temperature experimental runs (300 cells mL^{-1}). This could explain why the highest growth rates for *C. furca* were ~0 d^{-1} but positive growth was measured in other experimental runs. However, while little to no growth was seen during the 14 days, negative impacts of the lowest light levels were still observed. *C. furca's* ability to tolerate lower light levels than *A. monilatum* is consistent with *C. furca* persisting later in the year than *A. monilatum*.

Under the various nutrient conditions, neither species showed a clear preference for any of the treatments. Previous research has shown *C. furca* reaching high abundances under high N:P ratios, i.e., P-limited conditions, suggesting their growth may depend more on nitrogen concentration than on phosphorus concentration (Baek et al., 2008a). In these previous studies, the highest *C. furca* abundances were observed with N:P ratios of 200:1, much higher than the highest N:P ratio of 50:1 tested in my study (Baek et al., 2008a). Baek et al. (2008a) also found the lowest *C. furca* abundances under the lowest N:P ratio they tested of 16:1, further suggesting *C. furca's* preference for higher nitrate availability. Likewise, studies have shown *A. monilatum* abundance increasing following the addition of nitrate (Juhl, 2005), suggesting that nitrate is their growth-limiting factor. While my experiment found no significant differences in end abundances or overall growth rates between treatments, there was an indication that *A. monilatum* abundance was starting to plateau in the 6:1 treatment (highest N-limitation). If the experiment ran longer, it is possible this would have become a significant result. Given that *C. furca* and *A. monilatum* have been shown to respond negatively to nitrate limitation, it is possible the treatments did not create sufficiently limiting conditions or did not run long enough for limitation to occur. As a result, I consider my nutrient experimental results to be inconclusive.

As with light level, *A. monilatum* and *C. furca* both showed clear temperature preferences. *A. monilatum* showed a clear preference for the highest temperature tested or the 30°C treatment. *A. monilatum* exhibited a significant decrease in abundance and growth rate with only a 3°C temperature drop to the next treatment of 27°C. This is similar to previous research that has noted the highest growth rate of *A. monilatum* at 31°C and little to no growth at 15°C (Juhl, 2005). Conversely, *C. furca* showed a clear aversion to the 30°C treatment, as it resulted in the lowest final abundance and growth rate. *C. furca* preferred the 25°C treatment which is comparable to the 24°C Baek et al. (2008b) reported as *C. furca'*s optimum temperature. Previous research has also shown that *C. furca* has high growth rates between 18-28°C while no growth was shown below 10°C (Baek et al., 2008b). My data varies slightly from the upper part of this range with the lowest *C. furca* growth rates and final abundances found at 27°C and 30°C.

This study provides insight into what environmental factors are controlling the species succession during the late summer within the York River. *A. monilatum* showed a clear preference to high light intensities while *C. furca* was able survive at reduced light levels as well as it did at the highest light level. This could lead *A. monilatum* to bloom in the summer when days are longer and irradiance levels are higher, while *C. furca'*s ability to survive at lower light levels allows it to persist in early fall as daylight hours and irradiance levels decrease. This research also shows that temperature is likely influencing the species succession in the York River. *A. monilatum* showed a distinct preference to the highest temperature (30°C) which supports why *A. monilatum* blooms in late August, when water temperature in Chesapeake Bay is at its peak (Ding & Elmore, 2015). *C. furca* showed a clear preference to 25°C which would align with temperatures seen in the Bay during September (Ding & Elmore, 2015). As the water cools from August to September this would cause conditions to shift from favoring *A. monilatum*

to favoring *C. furca.* This temperature preference also supports what was seen in the historic data as *C. furca* often had an initial peak in July when water temperatures are also closer to 25°C (Figure 1.3; Ding & Elmore, 2015). While nutrient concentration does not appear to be a main component of this bloom succession, only five N:P ratios were tested within this study. It is possible that if more extreme N:P ratios were tested *A. monilatum* or *C. furca* may show a preference.

This research reevaluated the end of the late summer phytoplankton bloom succession in the lower York River Estuary by assessing the presence of *C. furca,* a species that has never before been considered as part of the bloom succession. As a non-toxic species, conditions that favor *C. furca* are important to understand. Given the clear temperature preference of *A. monilatum,* future management should consider encouraging aquaculture farms to establish in tributaries and areas of Chesapeake Bay that are not typically reaching temperatures upwards of 30°C. This could help mitigate the risk of toxic *A. monilatum* blooms impacting their brood as lower temperatures do not favor *A. monilatum* growth. It is also important to convey this information to citizen oyster cultivators, such as members of the Tidewater Oyster Gardeners Association (TOGA), to ensure that they also know what conditions may be best for cultivating. The knowledge this research provides could help minimize revenue loss in commercial aquaculture operations and allow citizen cultivators to farm their oysters effectively. Furthermore, current climate change projections suggest a potential 2-6°C increase in Chesapeake Bay water temperature by the end of the 21st century (Najjar et al., 2010; Muhling et al., 2018). This may lead to temporal and/or spatial shifts in the phytoplankton community and bloom timing as well as potential increased severity of *A. monilatum* blooms. As climate change progresses, understanding the current phytoplankton assemblage in the lower York River and

what environmental factors may be driving their succession will be critical to the success of future management efforts and the overall health of the system.

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Tables

	A. monilatum		*C. furca*	
	Treatment	μ (d^{-1})	Treatment	μ (d^{-1})
Light	**32.93**	**0.104**	**32.93**	**0.001**
	14.52	**0.080**	**14.52**	**-0.013**
	7.27	0.054	**7.27**	**0.006**
	2.84	-0.004	**2.84**	**-0.039**
	1.88	-0.013	1.88	-0.050
Nutrients	6:1	0.110	6:1	0.048
	16:1	0.121	10:1	0.042
	24:1	0.106	16:1	0.046
	35:1	0.125	35:1	0.058
	50:1	0.118	50:1	0.045
Temperature	20	0.037	20	0.083
	25	0.054	**25**	**0.115**
	27	0.070	27	0.006
	30	**0.102**	30	-0.065

Table 1.1: Growth rates (μ, d^{-1}) of *Alexandrium monilatum* and *Ceratium furca* under different light, nutrient, and temperature treatments measured over 14 days. Light treatments are given in μmol m^{-2} s^{-1} with the highest light corresponding with zero mesh layers and subsequent treatments representing one-, two-, three-, and four-layer treatments. Nutrient concentrations are reported as nitrate:phosphate (N:P) ratio and temperature in °C. **Bold** treatments indicate significant results of one-way ANOVA and post-hoc Tukey Test for conditions that resulted in the highest growth rate(s).

Figures

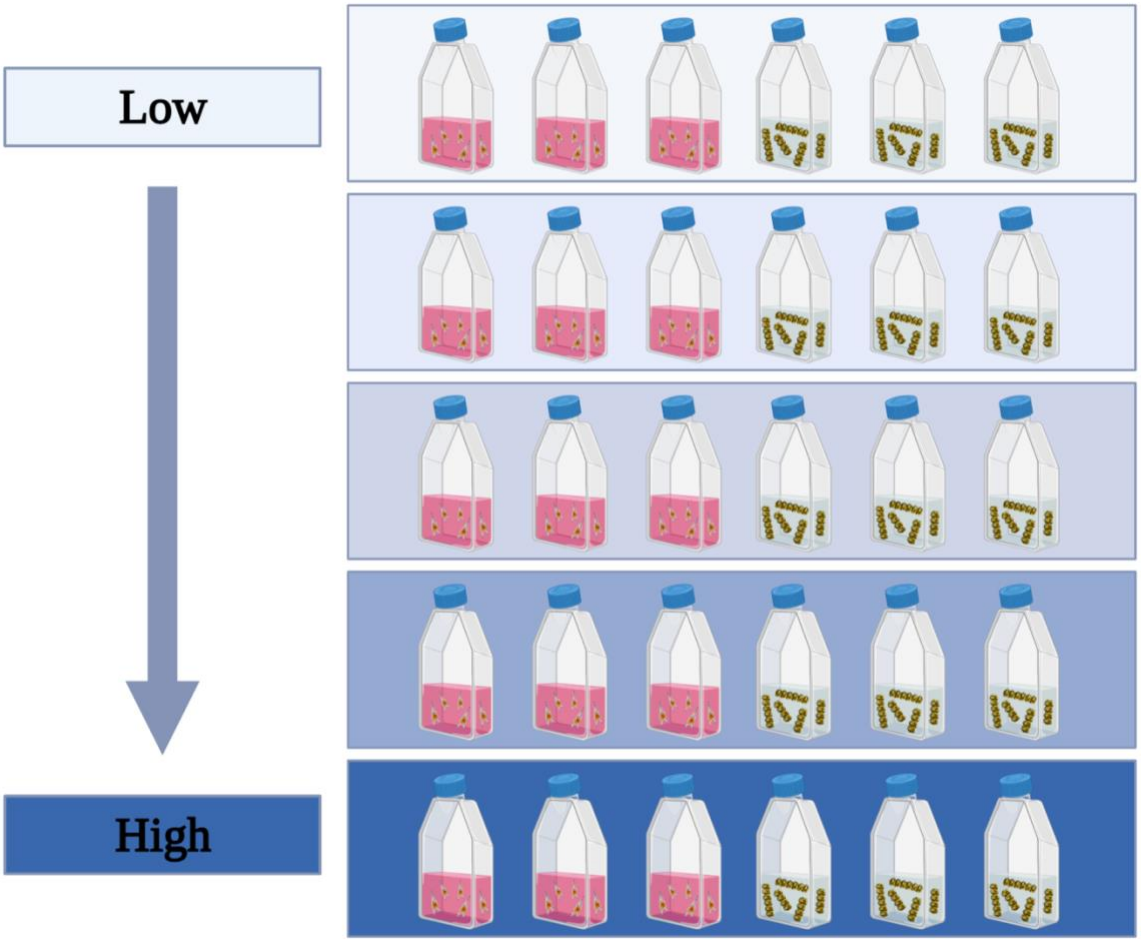

Figure 2.1: Experimental set-up of growth experiments of dinoflagellates *A. monilatum* and *C. furca*. Five levels of light (32.93, 14.52, 7.27, 2.84, and 1.88 µmol m^{-2} s^{-1}), five different nutrient ratios (N:P of 6:1, 16:1, 10:1 or 24:1, 35:1, and 50:1), and four temperatures (20, 25, 27, and 30°C) were tested on triplicate samples of each species.

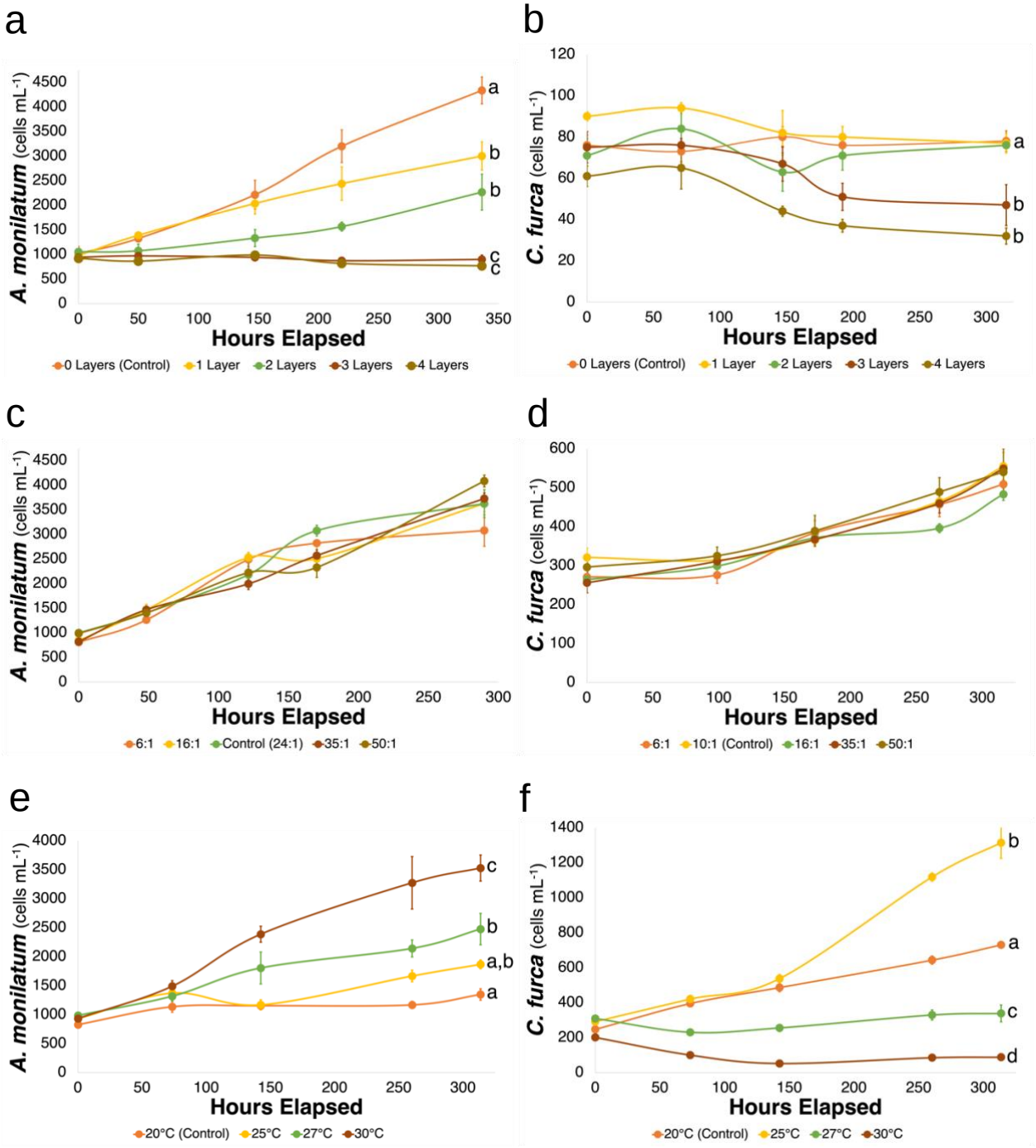

Figure 2.2: Effects of light, nutrients, and temperature on growth of dinoflagellates *A. monilatum* and *C. furca* throughout a 14-day growth experiment. (a)-(b) Effect of light (32.93, 14.52, 7.27, 2.84, and 1.88 µmol m^{-2} s^{-1}) on abundance (cells mL^{-1}) of *A. monilatum* and *C. furca*. (c)-(d) Effect of nutrient concentration (N:P ratio) (6:1, 16:1, 10:1 or 24:1, 35:1, and 50:1) on abundance (cells mL^{-1}) of *A. monilatum* and *C. furca*. (e)-(f) Effect of temperature (20, 25, 27, and 30°C) on abundance (cells mL^{-1}) of *A. monilatum* and *C. furca*. Letters on final abundances indicate significant results of one-way ANOVA and post-hoc Tukey Test. Error bars = standard error (n=3).

Chapter 2

Top-down control of *Acartia tonsa* copepods on harmful *Margalefidinium polykrikoides* dinoflagellate blooms

Introduction

The influence of bottom-up and top-down controls on the formation and persistence of phytoplankton blooms has been widely examined. Phytoplankton blooms occur when favorable environmental conditions (bottom-up controls) support rapid growth and allow for phytoplankton biomass accumulation (Irigoien et al., 2005; Thompson et al., 2008). Alternatively, a phytoplankton bloom can form due to a reduction in grazing pressure (top-down control) (Mitra & Flynn, 2006). Due to environmental conditions such as light, nutrient concentrations, and temperature being relatively easy to quantify, research addressing phytoplankton bloom formation has been bias toward bottom-up controls (Thompson et al., 2008). However, the importance of top-down controls on the formation of phytoplankton blooms can be just as important (Buskey, 2008). There is ample evidence that phytoplankton blooms may be the result of perturbations to trophic coupling resulting in "loopholes" or "windows of opportunity", where reductions in grazing rates provide an opening for a phytoplankton bloom to form (Irigoien et al., 2005; Stoecker et al., 2008). For example, high copepod abundance can release phytoplankton from grazing pressure by consuming and depleting the microzooplankton population (Reaugh et al., 2007; Stoecker et al., 2008). However, Stoecker et al. (2008) describes an alternate scenario where increased abundances of gelatinous zooplankton prey on the copepod population. This causes a trophic cascade which releases microzooplankton from predation pressure, increases grazing on phytoplankton, and inhibits bloom formation (Stoecker et al., 2008). Periods of low grazing rates have also been associated with low temperatures, with Millette et al. (2015) reporting a decline in grazing rates below 1-2ºC associated with the formation of a winter phytoplankton bloom.

Prior studies have also evaluated the influence of top-down controls on the formation and persistence of harmful algal blooms (HABs). Mesozooplankton grazers, such as copepods, have been documented to feed upon a variety of phytoplankton species that are toxic or produce toxin-like effects (Turner, 2006). Some of these HAB species appear to have no adverse effects on their copepod grazers (Colin and Dam 2002; Teegarden et al. 2001; Turner, 2006). However, other studies report adverse effects on copepods, including reduced grazing rates, decline in egg production and hatching success, and increased mortality (Colin & Dam, 2002; Jeong et al. 2004; Teegarden et al., 2001; Turner, 2006). HAB species may also be unpalatable or do not provide proper nutrition to copepods (Teegarden et al., 2001; Teegarden & Cembella, 1996). This can lead to selective feeding by copepods, reducing grazing pressure, or top-down controls, on specific HAB species, and subsequently allowing them to form blooms (Paffenhöfer & Stearns, 1988; Swadling & Marcus, 1994; Teegarden et al., 2001). HABs may therefore persist by acting as their own grazing deterrent, reducing the influence of top-down control by copepods, and supporting bloom proliferation and maintenance.

The dinoflagellate *Margalefidinium polykrikoides* is a prominent HAB species that forms dense blooms in coastal waters around the world (Gobler et al., 2008; Jiang et al., 2010; Mulholland et al., 2009). *M. polykrikoides* blooms cause well-documented ichthyotoxic effects, however the specific toxin(s) have not been categorized (Gobler et al., 2008; Kim et al., 1999; Mulholland et al., 2009). Blooms of *M. polykrikoides* have mainly affected Asia and North America (López-Cortés et al., 2019), reportedly causing millions in annual revenue loss for aquaculture practices in South Korea alone (Park et al., 2013). On the U.S. east coast, blooms of *M. polykrikoides* have been recorded for decades (Fortin et al., 2022; Gobler et al., 2008; Mulholland et al., 2009). However, in recent years the magnitude and distribution of *M.*

polykrikoides blooms has increased, causing near-annual blooms during the late summer in two New York estuaries (Gobler et al., 2008), as well as in the Chesapeake Bay and its tributaries (Fortin et al., 2022; Mulholland et al., 2009). *M. polykrikoides* may also harm zooplankton grazers, potentially allowing this HAB species to escape top-down control (Gobler et al., 2008; Jiang et al., 2009; Mulholland et al., 2009; Shin et al., 2003). Understanding the impact of top-down controls on *M. polykrikoides* blooms will help scientists evaluate how these blooms form and better understand how to manage them.

Prior research indicates two possible effects of zooplankton grazers on *M. polykrikoides* blooms with two different ecological outcomes. A culture-based laboratory experiment by Jiang et al. (2009) found that the copepod *Acartia tonsa* had significantly lower ingestion rates when fed *M. polykrikoides* compared to a non-toxic phytoplankton species. They also found that at high concentrations *M. polykrikoides* was harmful to *A. tonsa* (Jiang et al., 2009). This may result in a "window of opportunity" whereby a decrease in grazing pressure supports the formation of *M. polykrikoides* blooms. However, a subsequent study showed that copepods that were chronically exposed to *M. polykrikoides* exhibited increased resistance to the toxic-like effects of *M. polykrikoides* (Jiang et al., 2011). Colin and Dam (2002) also found a similar increased resistance in *Acartia hudsonica* copepods to a toxin producing *Alexandrium* spp. While enhanced resistance to HAB species could allow copepods to persist throughout HAB blooms, it may also result in greater amounts of toxins transferred through the food web (Jiang et al., 2011). Bioaccumulation of toxins may then pose a serious risk to higher trophic level species (Jiang et al., 2011). However, all of these previous studies were done in the laboratory under controlled conditions using cultures. This study aims to address whether copepods can consume *M.*

polykrikoides without consequence or if they experience deleterious effects during a bloom by conducting experiments using water collected from the field.

In the lower York River, nearly annual summer blooms of *M. polykrikoides* have occurred for over the past 50 years (Fortin, et al., 2022), making the York River an ideal and relatively reliable location to conduct this study. The dominant grazer during summer in this region is the calanoid copepod, *A. tonsa* (Steinberg & Condon, 2009), which I selected to study top-down impacts on *M. polykrikoides* bloom formation. I used a combination of weekly field sampling along with prey removal experiments during the late summer of 2021 and 2022 to assess the role that top-down control from *A. tonsa* has on harmful *M. polykrikoides* blooms. Sampling occurred during two *M. polykrikoides* blooms in the lower York River Estuary, U.S. and grazing experiments were conducted using the water and copepods collected during sampling. I hypothesize that *M. polykrikoides* blooms will negatively impact *A. tonsa* ingestion rates. However, given that *M. polykrikoides* blooms are a chronic occurrence in the York River, it is possible that *A. tonsa* may have developed resistance to the blooms and *M. polykrikoides* blooms may not have an impact on *A. tonsa*. From the 2021 and 2022 data I found evidence that at high abundances, *M. polykrikoides* blooms had a clear negative impact on copepod survival. This would suggest that at high concentrations, *M. polykrikoides* may act as its own grazing deterrent, reducing the influence of top-down control and supporting HAB proliferation and maintenance.

Methods

Pre-bloom Sampling

Sampling occurred July–September in the summers of 2021 and 2022 in the lower York River. Before the bloom was detected, sampling occurred every other week at the end points of

the bloom sampling region (sites 1 and 5 in Figure 3.1). At each site, temperature (ºC) and salinity were recorded using an EXO1 Multiparameter Sonde (Xylem Inc.). Niskin bottle (5 L) casts were used to collect water from ~0.5m below the surface for chlorophyll *a*, inorganic nutrient concentrations, and microscopy. Two vertical plankton tows were used at each station to collect copepods using a 0.5 m diameter plankton ring net fitted with 200 µm mesh and non-filtering cod end. The distance of each tow was recorded to then calculate the total volume of water filtered. One tow was used to collect live copepods for grazing experiments while the other tow was preserved in formalin to enumerate the copepod population.

Triplicate samples of 10-80 mL of water collected using the Niskin bottle were filtered onto 25 mm GF/F glass microfiber filters. The filters were placed in 20 mL glass scintillation vials with 7 mL of 90% acetone and stored for 24-hours in a -20ºC freezer. Fluorescence of each sample was analyzed with a Turner Designs 10-AU fluorometer using the 10% HCL method described by Holm-Hansen et al. (1965) and the measurement was converted to chlorophyll *a* concentration (ug L^{-1}) (UMCES, 2022). For each site, two 15 mL samples of water for nutrient analysis were filtered through 0.45 µm filters into plastic scintillation vials and stored in a -20ºC freezer. The samples were then analyzed by the VIMS Analytical Services Lab for nitrate + nitrite (NO_x) and phosphate (PO_4^{3-}) using the method detection limits (MDLs) of 0.10 µM and 0.03 µM, respectively (EPA Method 353.2 & EPA Method 365.1).

At least 6 L of water from each site was set aside for prey removal experiments (details below). Initial cell counts of *M. polykrikoides* from the experiments were used as *in situ M. polykrikoides* abundances for each sample date. Microscopy samples were fixed with Lugol's solution and stored in a glass scintillation vial sealed with electrical tape until they were analyzed. *M. polykrikoides* abundance was estimated using a Sedgewick-Rafter counting

chamber under a Zeiss Axio Imager.A2 microscope at 10x magnification. For each sample, the entire chamber was counted or until a minimum of 300 *M. polykrikoides* cells was reached. Preserved copepod samples were split using a Folsom Plankton Splitter until a subsample of 200-500 individuals was obtained. The subsample was then counted for adult *A. tonsa* under a Zeiss Stemi 305 stereo microscope, scaled up to the whole sample, and divided by the volume of water filtered by the net to calculate copepod density (individuals L^{-1}).

Bloom Sampling

When a bloom was present, sampling occurred weekly at two sampling sites chosen within the sections in Figure 3.1. Sites were selected based on visual observation of where the bloom concentrations appeared highest at the surface. Since *M. polykrikoides* vertically migrates to the surface during the day, water samples were collected via a 20 L polycarbonate carboy dipped just below the river surface (~10 cm) for chlorophyll *a,* inorganic nutrient concentrations, and microscopy. Temperature and salinity measurements were also collected via a flow-through system or an EXO1 Multiparameter Sonde. Additionally, following the pre-bloom methods, vertical plankton tows were performed at each site to collect copepods for grazing experiments. Also, following the pre-bloom methods, water for nutrient samples was collected and processed. NO_x and phosphorous were analyzed by the Nutrient Cycling Laboratory at VIMS in 2021 using the method detection limits (MDLs) of 0.20 µM and 0.16 µM, respectively (EPA Method 353.2 & EPA Method 365.1) and VIMS Analytical Services Laboratory in 2022 using the MDLs of 0.10 µM and 0.03 µM, respectively.

Prey Removal Experiments

In the laboratory, water and copepods collected from each sampling site were used for experiments to estimate copepod ingestion rates on *M. polykrikoides*. For each site, the collected

bloom water was gently filtered through 210 µm mesh to remove any large zooplankton. The water was then transferred into six clear 1 L polycarbonate bottles using plastic tubing to minimize potential cell lysis. Three of the experimental bottles had 20-30 *A. tonsa* added, while three bottles had no *A. tonsa* added to serve as controls (Figure 3.2). *A. tonsa* copepods used in the experiment were hand-picked from the live vertical plankton tow sample using a wide-bore glass pipette under a Zeiss Stemi 305 stereo microscope. Only actively swimming adult copepods with both of their antennae intact were selected for each experiment. Samples were collected at this time (t_0) from each bottle to evaluate chlorophyll *a* concentrations and *M. polykrikoides* abundances and analyzed using the methods described in the pre-bloom sampling section. The bottles were placed into mesh bags and incubated for 24-hours off the VIMS beach in the York River (37.248333, -76.498889) to maintain *in situ* temperature and light.

After 24-hours, the water was gently filtered through 210 µm mesh to collect and enumerate the live copepods in each bottle. Samples for chlorophyll *a* concentration and *M. polykrikoides* abundance were collected and analyzed as previously described to serve as the final (t_{24}) concentrations and abundances. The t_0 and t_{24} values and number of live copepods were then used to calculate the *A. tonsa* ingestion rate (copepod^{-1} day^{-1}) on chlorophyll *a* and *M. polykrikoides* according to the calculations in Frost (1972). If *A. tonsa* experienced an average mortality exceeding 50% between the three replicates, then that experimental run was omitted from analysis as ingestion rates could not be accurately calculated. Means are reported ± standard error (SE) throughout the results.

Results

Environmental Conditions

Average water temperature in late summer of 2021 (27.9 ± 0.4ºC) was not significantly different than the average water temperature in late summer of 2022 (26.1+ 1.3ºC) (two-sample t-test, p = 0.164, Figure 3.3). Water temperature ranged from 26.0-30.6ºC in 2021 from July 7 through September 21, 2021 (Figure 3.3a) and from 18.1-28.9ºC from July 15 through October 6, 2022 (Figure 3.3b). Salinity was significantly lower in 2021 (20.2 ± 0.3) compared to 2022 (21.7 ± 0.5) (two-sample t-test, p = 0.025, Figure 3.3). Salinity ranged from 18.7-21.9 in 2021 (Figure 3.3a) and 18.4-23.6 in 2022 (Figure 3.3b).

Average phosphate concentrations were significantly higher in 2021 (0.81 ± 0.21 µM) compared to 2022 (0.13 ± 0.03 µM, two-sample t-test, $p < 0.05$, Figure 3.4, while nitrate + nitrite concentrations were not significantly different in 2021 (0.67 ± 0.27 µM) and 2022 (0.20 ± 0.09 µM, two-sample t-test, p = 0.098, Figure 3.4). Phosphate concentrations ranged from 0.07-2.36 µM in 2021 (Figure 3.4a) and 0.05-0.29 µM in 2022 (Figure 3.4b). Nitrate + nitrite concentrations ranged from 0.04-2.55 µM in 2021 (Figure 3.4a) and 0.02-0.46 µM in 2022 (Figure 3.4b).

Plankton Abundance

Using the definition of a bloom as cell abundances >1000 cells mL^{-1} (Mulholland et al., 2018), there was an ~5-week long *M. polykrikoides* bloom in 2021 and an ~7-week long bloom in 2022. The 2021 bloom was initiated around August 10, 2021 when chlorophyll *a* concentrations and *M. polykrikoides* abundances increased to 131.3 ± 5.4 µg L^{-1} and 1130 ± 287 cells mL^{-1}, respectively (Figure 3.5a,c). The peak of the bloom occurred around August 24, 2021 when chlorophyll *a* concentrations reached 806.4 ± 36.0 µg L^{-1} and *M. polykrikoides* abundances reached 7097 ± 1792 cells mL^{-1} (Figure 3.5a,c).

The 2022 bloom was initiated around August 8, 2022 when chlorophyll *a* concentrations and *M. polykrikoides* abundances increased to 176.5 ± 21.6 µg L^{-1} and 2597 ± 133 cells mL^{-1}, respectively (Figure 3.5b,d). While the bloom had no clear peak, the highest values were measured around August 23, 2022 when the chlorophyll *a* concentrations reached 1327.2 ± 56.5 µg L^{-1} and *M. polykrikoides* abundances reached 18 530 ± 1914 cells mL^{-1} (Figure 3.5b,d). The average chlorophyll *a* concentrations and *M. polykrikoides* abundances were higher in 2022 (333.8 ± 125.1 µg L^{-1} and 4344 ± 1804 cells mL^{-1}) than in 2021 (144.4 ± 59.2 µg L^{-1} and 1348 ± 619 cells mL^{-1}) however they were not significantly different from each other between the years (two-sample t-test, p = 0.172, p = 0.125, respectively, Figure 3.5).

A. tonsa abundance was also measured over the course of the blooms. The average *A. tonsa* abundance was higher in 2021 (4 ± 1 individuals L^{-1}) than 2022 (2 ± 1 individuals L^{-1}), however, there was no significant difference between the two years (two-sample t-test, p = 0.208, Figure 3.5e,f). Pre-bloom in 2021, the average copepod abundance was 10 ± 3 individuals L^{-1} and decreased to 2 ± 1 individuals L^{-1} during and post-bloom (Figure 3.5e). In 2022, pre-bloom the average copepod abundance was 4 ± 1 individuals L^{-1} and deceased to 1 ± 1 individuals L^{-1} during the bloom (Figure 3.5f). The average copepod abundance further decreased post-bloom to <1 individual L^{-1} (Figure 3.5f).

Copepod Ingestion Rates

The grazing experiments showed negative ingestion rates by *A. tonsa* on chlorophyll *a* prior to the bloom in 2021 but showed positive ingestion rates prior to the bloom in 2022 (Figure 3.6a,b). Ingestion of chlorophyll *a* and *M. polykrikoides* during the blooms was highly varied, however, there were some experiments where *A. tonsa* was recorded to be positively ingesting *M. polykrikoides* (Figure 3.6). During the bloom in 2021 and 2022, there were several instances

where many or all the copepods died within the 24-hour experimental duration. When this occurred, I was unable to accurately calculate ingestion rates as only live copepods could be assumed to be ingesting prey for the duration of the experiment.

At the peak of the 2021 bloom, all copepods died during two experiments (Figure 3.6a). In 2022, copepods experienced >50% mortality during seven experiments (Figure 3.6b). This resulted in *A. tonsa* ingestion not being calculated for the majority of the 2022 bloom. When evaluating percent *A. tonsa* mortality experienced over 24-hours in prey removal experiments in comparison to *M. polykrikoides* abundances, it was found that above *M. polykrikoides* abundances of 2000 cells mL^{-1}, the majority of *A. tonsa* did not survive (Figure 3.7).

Discussion

Blooms of *M. polykrikoides* formed in the lower York River Estuary during the late summer of 2021 and 2022. While blooms initiated around the same time each year, the bloom in 2022 persisted for longer and was of a greater magnitude compared to 2021. In both years, there was a decrease in *in situ A. tonsa* abundance and 100% mortality of *A. tonsa* in the 24-hour grazing experiments associated with high *M. polykrikoides* abundance. This indicates that *M. polykrikoides* blooms have a negative impact on copepod survival at high enough concentrations (>2000 cells mL^{-1}).

Bloom Dynamics in 2021 & 2022

The 2021 bloom was relatively short and followed a typical bloom pattern of *M. polykrikoides* abundances, gradually increasing to a maximum level before returning to non-bloom conditions (Figure 3.8). In 2022, the bloom was longer, more abundant, and highly variable. *M. polykrikoides* abundances never 'ramped up', rather abundances rapidly increased between two sampling dates, two weeks apart. The bloom also appeared to have two peaks in

abundance. After the first peak in abundance, the bloom seemed to be in decline, only to reemerge late in September (Figure 3.8). The final dissipation of the bloom appeared to be associated with a storm system. The remnants of Hurricane Ian passed through Virginia on September 30 and October 1, resulting in the water temperature of the lower York River dropping over 9°C (Figure 3.3). After the hurricane, *M. polykrikoides* abundance was too low to be reliability detected. It is unclear how much longer the bloom would have persisted if the hurricane had not occurred.

Acartia tonsa Dynamics in 2021 & 2022

In 2021 and 2022, *A. tonsa* abundance declined during the bloom compared to pre-bloom abundances. As the *M. polykrikoides* bloom declined in 2021, *A. tonsa* abundance appeared to stabilize and begin to increase, while in 2022 *A. tonsa* abundance remained low post-bloom (Figure 3.8). This suggests that *M. polykrikoides* blooms can negatively impact the *A. tonsa* population, at high enough concentrations. Furthermore, the severity and length of the bloom could impact the amount of time needed for the *A. tonsa* population to rebound, as seen in 2022. In the prey removal experiments in 2021, chlorophyll *a* concentration increased in the presence of *A. tonsa* (negative ingestion rates) prior to the bloom. These results suggest that *A. tonsa* may have been ingesting microzooplankton before the bloom and releasing the phytoplankton from grazing pressure (Nejstgaard et al., 2001; Reaugh et al., 2007; Stoecker et al., 2008). Once the bloom started, *A. tonsa* ingestion rates on M. *polykrikoides* varied between negative and positive, suggesting that *A. tonsa*, to some extent, may be capable of feeding on the bloom. However, at the peak of the 2021 bloom, *A. tonsa* experienced 100% mortality in the experimental treatments, suggesting that *M. polykrikoides* can reduce survivability at high concentrations. When *M. polykrikoides* began to decline the following week, positive ingestion of *M. polykrikoides* by *A.*

tonsa was again recorded and *A. tonsa* abundances began to rebound. This trend continued through to the post-bloom period. This indicates that the 2021 *M. polykrikoides* bloom had a negative impact on *A. tonsa* when *M. polykrikoides* abundances peaked, but that the *A. tonsa* population was able to stabilize and begin to recover as *M. polykrikoides* quickly declined the following week.

In 2022, the prey removal experiments showed that *A. tonsa* were ingesting chlorophyll *a* prior to the bloom. This suggests that *M. polykrikoides* blooms can form despite grazing pressure from copepods. Between 2021 and 2022, it is unclear how top-down controls influence the initial development of these blooms. During the bloom, similar to 2021, there was positive ingestion of *M. polykrikoides*, further indicating *A. tonsa* was feeding on the bloom at times. As *M. polykrikoides* abundances rapidly increased, *A. tonsa* abundance declined and copepod mortality increased during the prey removal experiments. 100% *A. tonsa* mortality occurred for multiple weeks in 2022. However, for one week in 2022 (9/13/2022), when the *M. polykrikoides* abundances substantially declined, positive ingestion rates were again measured. When *M. polykrikoides* abundances increased the following week, *A. tonsa* experienced 100% mortality again. This suggests that the higher magnitude and longer duration of the 2022 *M. polykrikoides* bloom, compared to 2021, had an increased negative impact on the *A. tonsa* population and they needed a longer time to recover.

Impacts of *M. polykrikoides* Blooms

Based on grazing experiments from 2021 and 2022, I propose that during *M. polykrikoides* blooms when abundances exceed 2000 cells mL^{-1}, *A. tonsa* begins to experience a substantial increase in mortality (Figure 3.7). If *M. polykrikoides* abundances rarely surpass 2000 cells mL^{-1}, then the *A. tonsa* population can quickly recover, as in 2021. However, if *M.*

polykrikoides abundances exceed this threshold for multiple weeks, then the *A. tonsa* population may take several weeks to recover, as in 2022. This impact was evident when looking at the *A. tonsa* population abundances in both years. In 2021 copepod abundances began to rebound as the bloom declined whereas in 2022, the copepod abundance remained low into the post-bloom period and did not show signs of recovery.

The ichthyotoxic effects of *M. polykrikoides* blooms are well documented in places like the coast of South Korea where dense blooms are responsible for $4-60 million in aquaculture revenue loss annually (Park et al., 2013). However, in North America there are fewer studies available on the negative ramifications of *M. polykrikoides* blooms in the field. Particularly, the impact of *M. polykrikoides* on copepods within the environment is lesser known (Gobler et al., 2012; Mulholland et al., 2009). Experiments conducted by Jiang et al. (2009, 2010) show that cultured *A. tonsa* copepods fed diets of cultured *M. polykrikoides* at concentrations of 110 cells mL^{-1} or less had significantly higher egg production rates and naupliar recruitment rates than *A. tonsa* fed a non-toxic phytoplankton. However, when concentrations of *M. polykrikoides* exceeded 330 cells mL^{-1}, the *M. polykrikoides* diet became nutritionally inadequate relative to the non-toxic species. Jiang et al. 2009 and Jiang et al. 2010 did note that at concentrations of 550 cells mL^{-1} *M. polykrikoides* became toxic to *A. tonsa*, but this is one-fourth the concentration of 2000 cells mL^{-1} when *A. tonsa* mortality was noted in our study. The difference in threshold values could be explained by using laboratory cultures versus natural populations of *A. tonsa* and *M. polykrikoides*. Culture based studies, as in Jiang et al. (2009, 2010), could limit the dissipation of potential toxins and allow them to build up over time, thus higher abundances of *M. polykrikoides* would be needed in the field to induce mortality in *A. tonsa* over a 24-hour period.

A previous study suggests that copepod populations can evolve resistance to *M. polykrikoides* with repeat exposure over time (Jiang et al., 2011). However, there is no evidence that *A. tonsa* in the York River were developing resistance to *M. polykrikoides* blooms in 2021 or 2022. However, in the Jiang et al. (2011) study *M. polykrikoides* abundances were kept at relatively low concentrations and it took four generations of copepods to see resistance. The York River bloom concentrations were much higher, exceeding 7000 cells mL^{-1} in both 2021 and 2022. This suggests that while *A. tonsa* may be able to develop resistance to the deleterious effects of *M. polykrikoides* at low *M. polykrikoides* abundance, if *M. polykrikoides* abundances are too high, *A. tonsa* will likely not survive long enough to develop resistance.

Implications for Trophic Dynamics and Future Bloom Management

This research assessed the possible role of top-down control from *A. tonsa* grazing on *M. polykrikoides* blooms. While there is evidence that *A. tonsa* ingested *M. polykrikoides* at low concentrations, at high abundances the *M. polykrikoides* blooms had a negative impact on *A. tonsa* survival. During the 2021 and 2022 blooms, I found that when *M. polykrikoides* abundances exceeded 2000 cells mL^{-1}, *A. tonsa* experienced >50% mortality, and often 100% mortality, during the 24-hour prey removal experiments. Furthermore, *A. tonsa* abundances in the lower York River also declined over the course of the bloom in both years. The time needed for the *A. tonsa* population to recover to pre-bloom levels will likely be dependent on the severity of the *M. polykrikoides* bloom. This suggests that at high concentrations, *M. polykrikoides* may act as its own grazing deterrent, reducing the impact of top-down control and allowing blooms to persist.

M. polykrikoides blooms causing a decline in *A. tonsa* will subsequently impact upper trophic levels due to the reduction of zooplankton prey. Copepods are the main food source for

many species (Abdulhussain et al., 2021; Abdulhussain et al., 2020) and thus play a key role in energy transfer to higher trophic levels. Prior research shows increased mortality of multiple bait fish species exposed to *M. polykrikoides* blooms (Gobler et al., 2008) and my results indicate that these blooms lead to a decline in the mesozooplankton population. This suggests that *M. polykrikoides* blooms, in addition to directly impacting bait fish, may also be reducing the energy transferred up the food web to larger, commercially valuable fish.

As the frequency, duration, and magnitude of HABs are expected to increase in the coming years (Hallegraeff, 1993), developing standardized regional thresholds for threatening *M. polykrikoides* abundances will be critical in mitigating the harmful effects of these blooms. These thresholds can be shared with regional fisheries managers and aquaculture farms to assess when waters may pose a threat to their stock, resulting in the development of an early-warning system. This research provides such a threshold for harmful *M. polykrikoides* in the York River, with further research necessary to fine-tune and implement these thresholds in other regions.

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Figures

Figure 3.1: Sampling locations in the lower York River Estuary, VA. Station markers depict pre- and post-bloom sampling stations. Black lines depict regions in which bloom samples were collected.

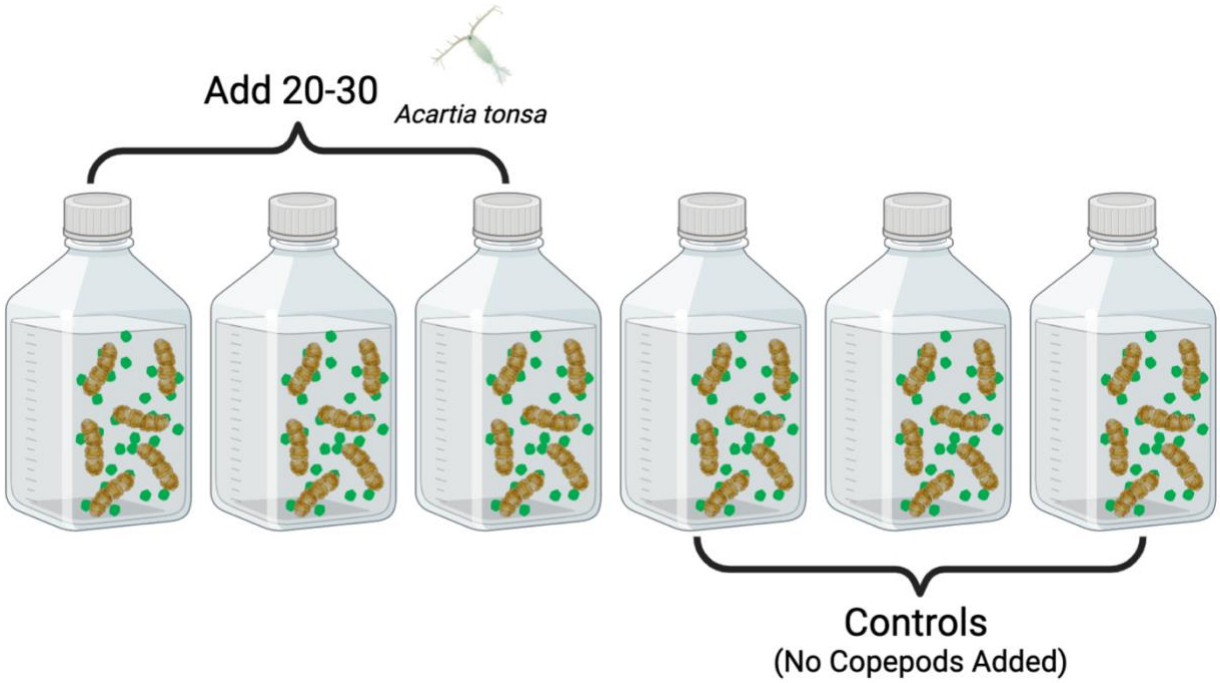

Figure 3.2: Experimental set-up of grazing experiments using water and copepod samples from the lower York River Estuary, VA. For each site, six 1 L bottles were filled with collected water that was filtered through 210 µm mesh to remove any large zooplankton. Three of the experimental bottles had 20-30 copepods added, while three bottles had no copepods added to serve as controls.

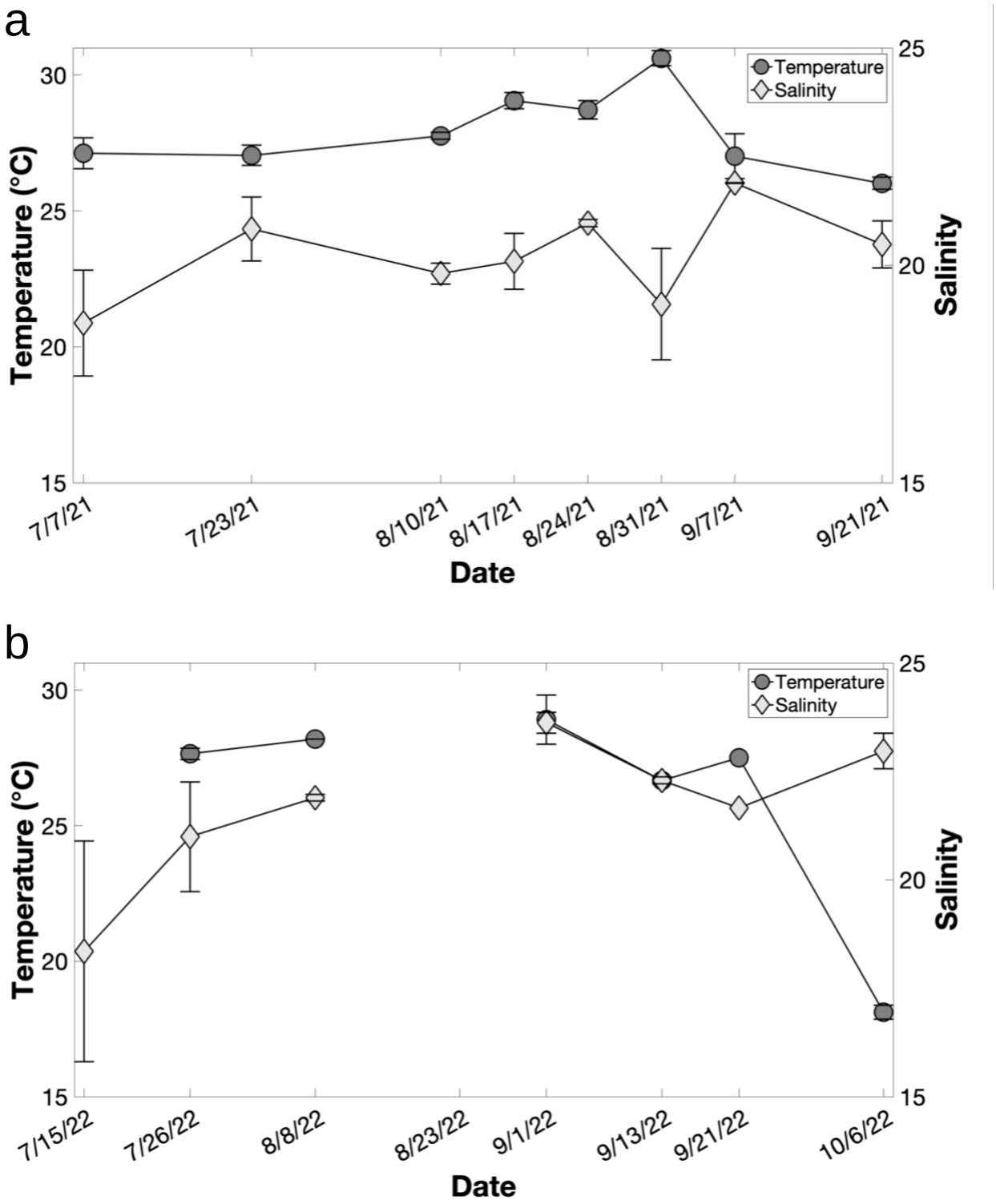

Figure 3.3: Water temperature and salinity in the lower York River Estuary, VA during the late summer of (a) 2021 and (b) 2022. Temperature was not recorded on 7/15/22 and temperature and salinity were not recorded on 8/23/22. Error bars = standard error.

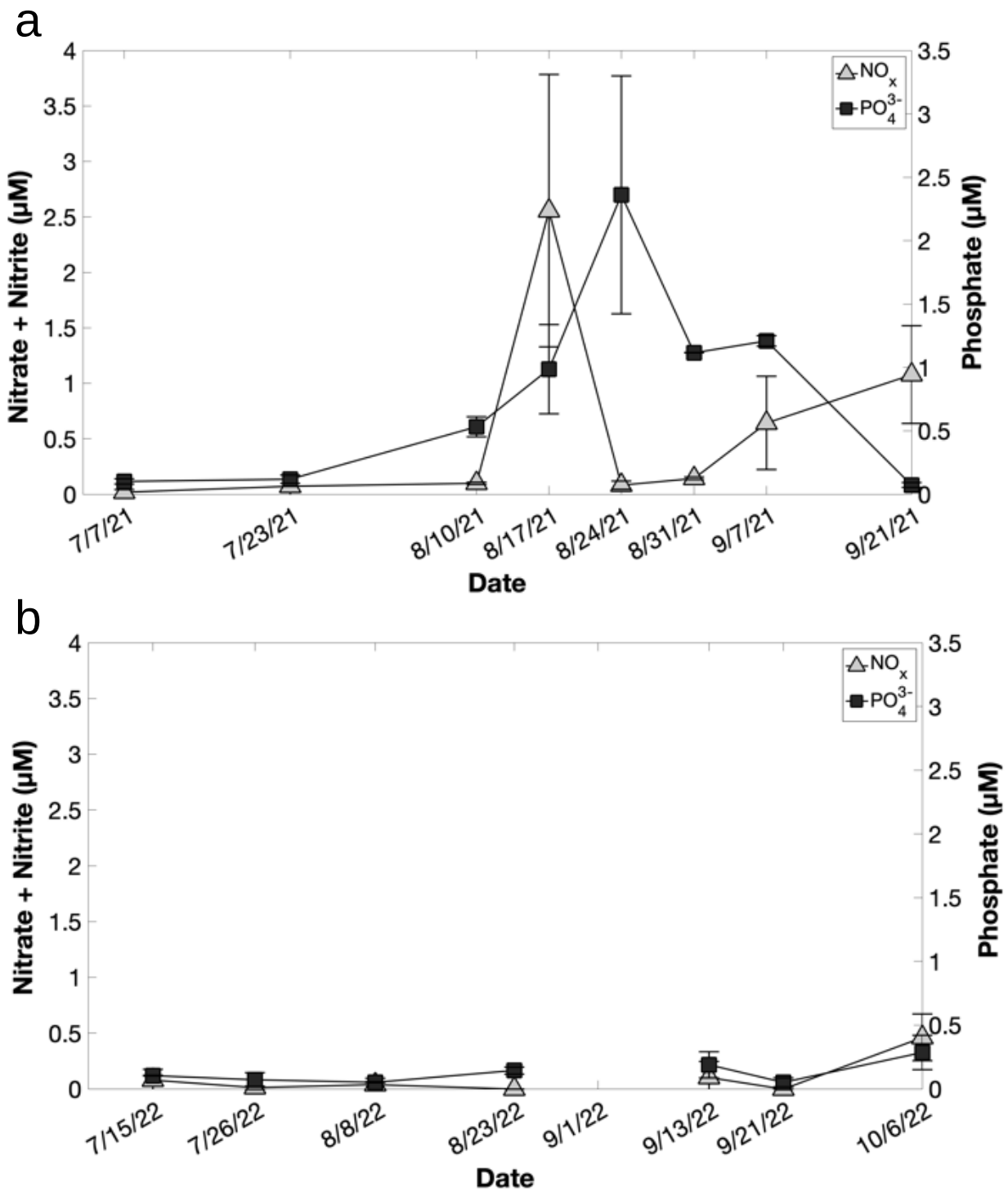

Figure 3.4: Nutrient concentrations (nitrate + nitrite and phosphate, µM) in the lower York River Estuary during the late summer of (a) 2021 and (b) 2022. Nutrient samples were not collected on 9/1/22. Error bars = standard error.

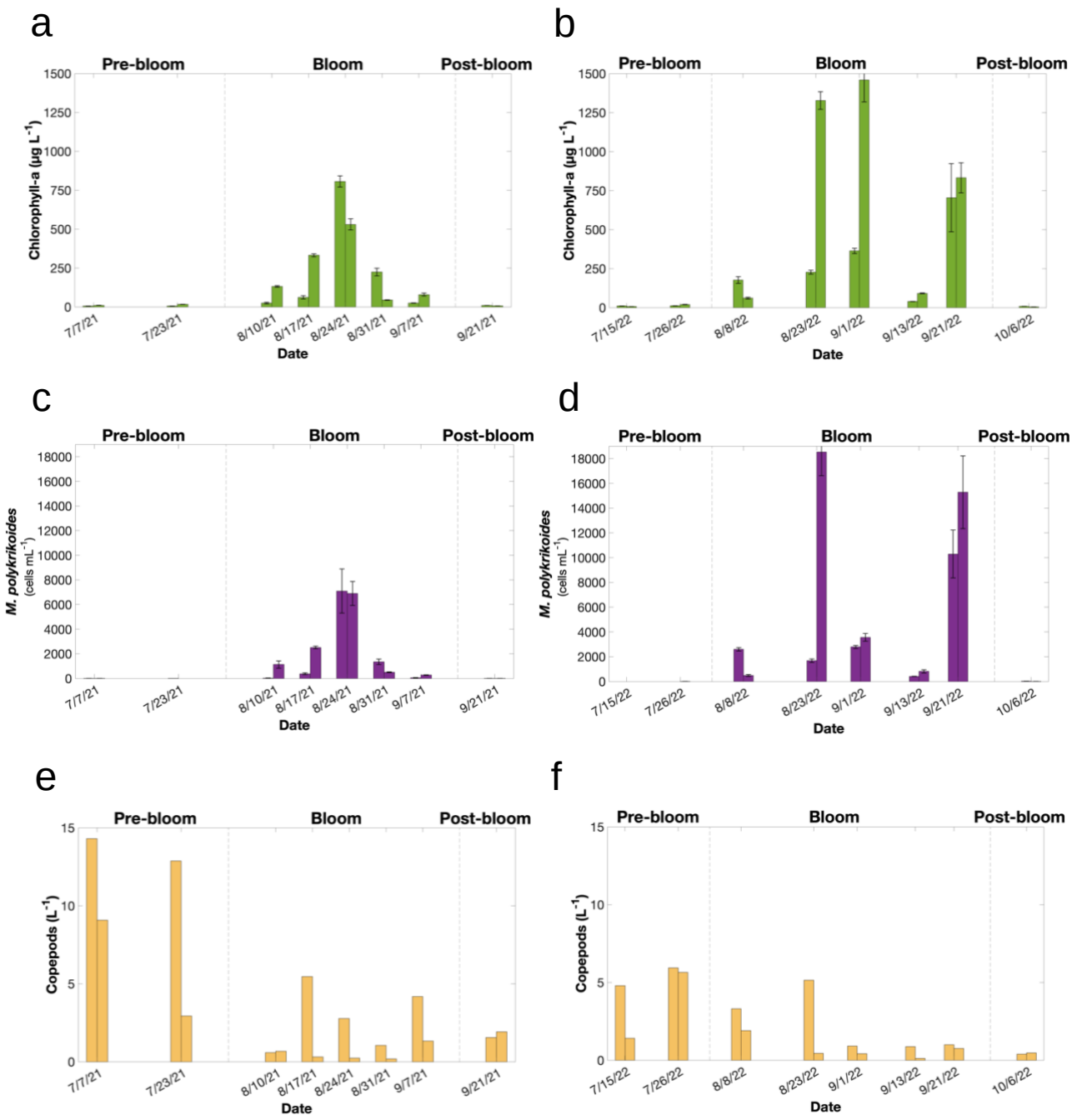

Figure 3.5: Late summer bloom dynamics of 2021 and 2022 in the lower York River Estuary, VA. Chlorophyll-a concentrations (µg L^{-1}) in the lower York River Estuary, VA during the late summer of (a) 2021 and (b) 2022. *Margalefidinium polykrikoides* abundance (cells mL^{-1}) in the lower York River Estuary, VA during the late summer of (c) 2021 and (d) 2022. *Acartia tonsa* copepod abundance (individuals L^{-1}) in the lower York River Estuary, VA during the late summer of (e) 2021 and (f) 2022.

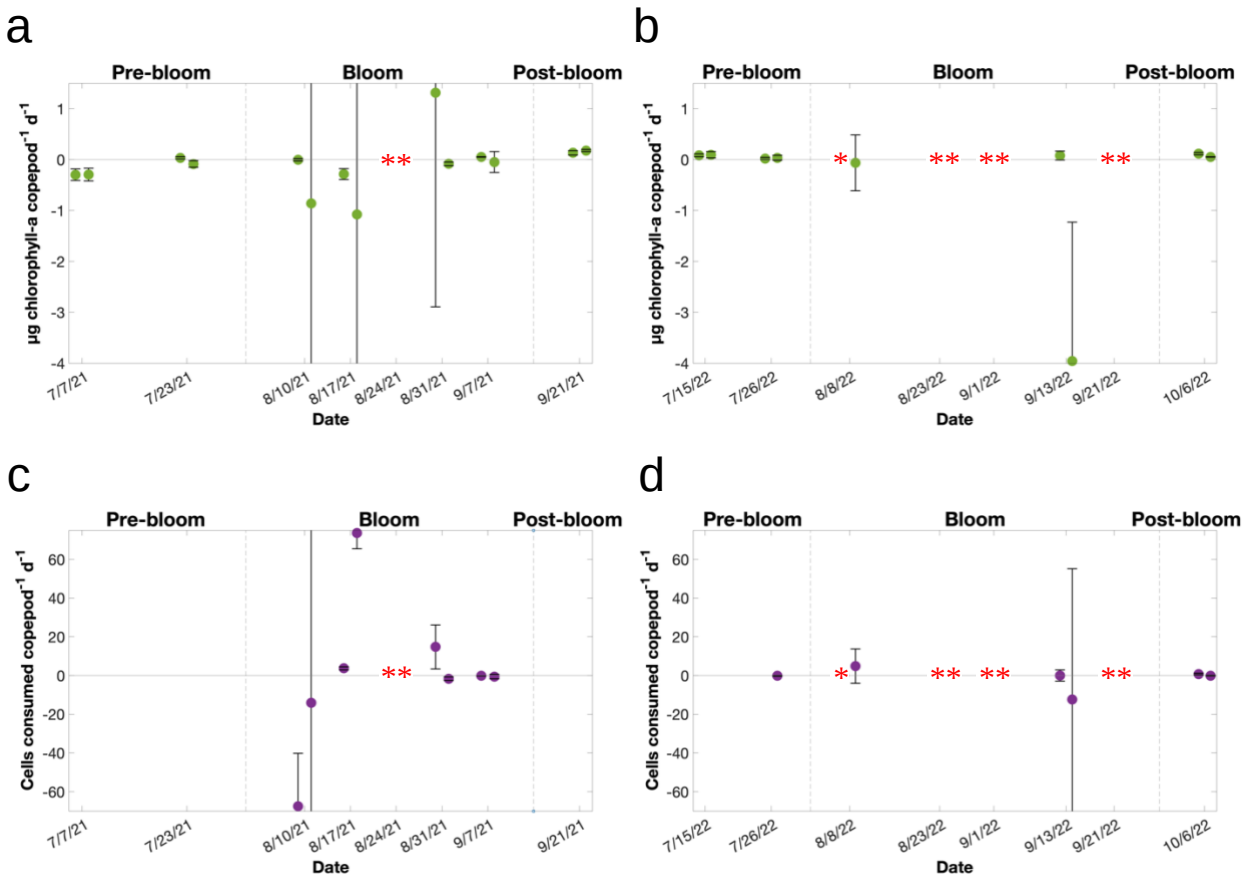

Figure 3.6: *Acartia tonsa* copepod ingestion rates in the lower York River Estuary, VA during the late summer of 2021 and 2022. Ingestion of chlorophyll-a (µg chlorophyll-a copepod^{-1} d^{-1}) in (a) 2021 and (b) 2022. Ingestion of *Margalefidinium polykrikoides* (cells consumed copepod^{-1} d^{-1}) in (c) 2021 and (d) 2022. Red asterisks depict instances when average copepod mortality was greater than 50% and ingestion rates could not be accurately measured.

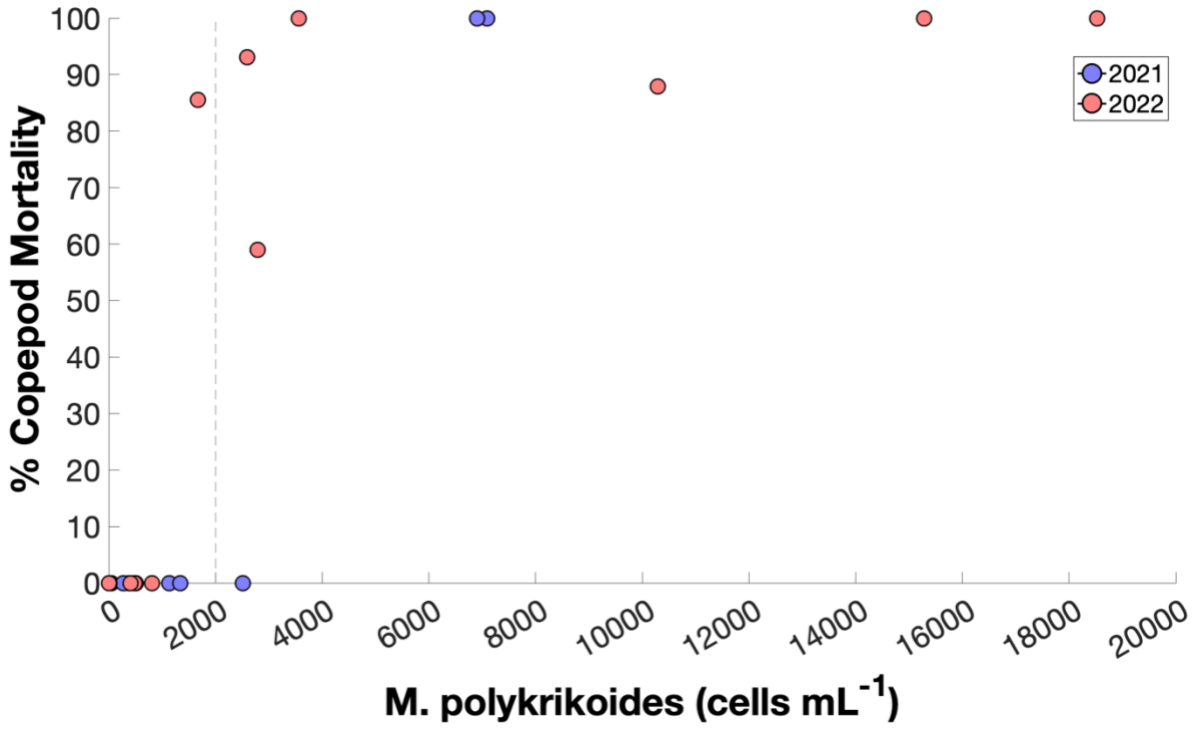

Figure 3.7: Percent copepod mortality in comparison to *Margalefidinium polykrikoides* abundance (cells mL^{-1}) in 2021 and 2022. Copepod mortality was greater than 50% after 24-hours when *M. polykrikoides* exceeded 2000 cells mL^{-1} (dashed line).

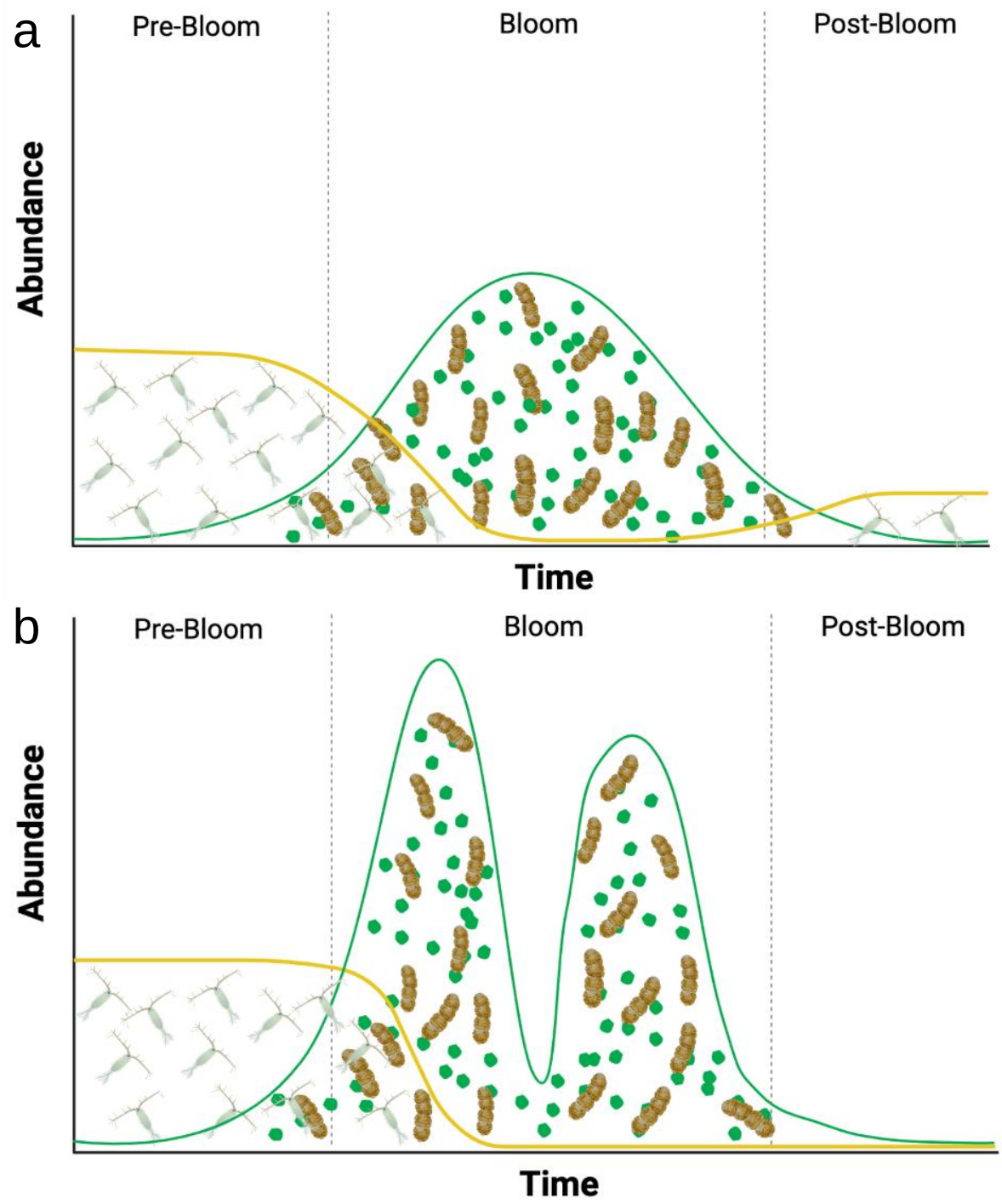

Figure 3.8: Conceptual diagram of the 2021 and 2022 late summer bloom season in the lower York River Estuary, VA. (a) In 2021, *Margalefidinium polykrikoides* abundance (cells mL^{-1}) gradually increased to a maximum level before returning to non-bloom conditions. *Acartia tonsa* abundances declined during the bloom compared to pre-bloom abundances and appeared to stabilize post-bloom. (b) In 2022, the *Margalefidinium polykrikoides* bloom was longer, more abundant, and highly variable. The bloom appeared to have two peaks in abundance. The *Acartia tonsa* abundances declined during the bloom compared to pre-bloom abundances and remained low post-bloom.

CONCLUSIONS

The Chesapeake Bay and its tributaries are a dynamic system that supports a diverse and highly productive phytoplankton community. In recent decades, there has been an increase in the frequency and magnitude of phytoplankton blooms, particularly of potentially harmful dinoflagellate species (Mulholland et al., 2018; Smalley & Coats, 2002). My research aimed to reevaluate the end of the late summer phytoplankton bloom succession in the lower York River Estuary by assessing the presence of *C. furca*, a non-toxic species that was previously not considered part of the bloom succession. The results reveal that *C. furca* preferred lower temperatures than *A. monilatum*, consistent with prior reports of *C. furca* increasing in abundance in July and September. *A. monilatum* preferring higher temperatures and light is consistent with previous bloom occurrences reported in August. While Mulholland et al. (2018) noted a median water temperature of ~27°C for *A. monilatum* blooms, our results suggest a stronger preference for warmer temperatures (i.e., 30°C). However, the discrepancy between my laboratory results and what has been observed in the field, highlights the fact that there are likely multiple factors influencing when a bloom occurs.

This study also aimed to address the influence that top-down control from *A. tonsa* grazing has in the bloom succession of *M. polykrikoides*, *A. monilatum*, and *C. furca*. However, *A. tonsa* grazing could not be measured on *A. monilatum* or *C. furca* as these species did not bloom in the summers of 2021 and 2022. Therefore, this study only evaluated *A. tonsa* grazing on *M. polykrikoides*. *M. polykrikoides* abundances above 2000 cells mL^{-1} had a clear negative impact on *A. tonsa* survival. Not only did *A. tonsa* experience up to 100% mortality at these concentrations, but the *A. tonsa* population within the lower York River also declined. While dense *M. polykrikoides* blooms are largely visible and their magnitude is discernable even to a

relatively untrained eye, establishing concrete thresholds of cell concentrations is critical to effectively managing the negative impacts of these blooms. Having clearly set thresholds of *M. polykrikoides* abundances that are of concern will help other researchers determine regional bloom impact on upper trophic level species. Furthermore, thresholds will help inform aquaculture practices as an early-warning system for harmful blooms.

While this study cannot fully address how top-down control is influencing the late summer bloom succession, it does suggest that at high concentrations, *M. polykrikoides* may act as its own grazing deterrent. This reduces the impact of top-down control from *A. tonsa* and along with favorable environmental conditions, can contribute to bloom formation. The time it takes for *A. tonsa* to recover from the negative impacts of *M. polykrikoides* blooms may also result in a "window of opportunity" where a decrease in grazing pressure supports the formation of other blooms, like *A. monilatum*. *A. monilatum* did not form widespread blooms in 2021 or 2022 suggesting other conditions needed to be met. However, if the environmental conditions became favorable, there also would likely be less grazing pressure after a *M. polykrikoides* bloom. In 2022 the *M. polykrikoides* bloom did not dissipate until the remnants of Hurricane Ian passed through the area. This suggests that a shift in bottom-up controls may be necessary to terminate a *M. polykrikoides* bloom.

This research highlights the importance of studying both bottom-up and top-down controls in order to fully understand species succession and trophic energy transfer. In the lower York River, evidence supports environmental conditions aiding in the transition from *M. polykrikoides* to *A. monilatum* to *C. furca* but grazing to some extent may also open up "windows of opportunity" for blooms to form. Additional field sampling and laboratory experiments are necessary to fully understand the influence of bottom-up and top-down controls

on this succession. Future environmental changes are anticipated to cause temporal and/or spatial shifts in the phytoplankton community and bloom timing. Therefore, better understanding the factors that drive bloom transitions in the lower York River will be critical in managing the health of this ecosystem.

FUTURE RESEARCH

This research emphasized the complexities of the late summer phytoplankton blooms in the York River and provided a baseline for how bottom-up and top-down controls may be influencing the succession. However, limitations in laboratory time as well as bloom samples prevented a full examination of the succession of *M. polykrikoides*, *A. monilatum,* and *C. furca.* I suggest the following future research directions to further understand the late summer phytoplankton blooms in the York River.

1. Evaluate the effect of light, nutrient concentration, and temperature on the abundance and growth rate of *M. polykrikoides.* While my study addressed the effect of these factors on *A. monilatum* and *C. furca* it did not address *M. polykrikoides* which is also a main component in the bloom succession.
2. Evaluate a wider range of light levels, nutrient concentrations, and temperatures on each species. This study found clear evidence of light and temperature preferences for *A. monilatum* and *C. furca,* but it did not find a preference in nutrient concentration for either species. It is possible that if more extreme N:P ratios were tested a preference may be shown. Furthermore, the light levels assessed in this study were much lower than what is found in the natural environment. Testing the effect of higher light intensities would make it easier to compare laboratory and field results.
3. Evaluate the effect of light, nutrient concentration, and temperature together on the abundance and growth rate of each species. Growth rates of *A. monilatum* and *C. furca* found in this study (Table 1.1) were much lower than reported in previous research (Baek et al., 2008a; Baek et al., 2008b; Juhl, 2005). This suggests that the

optimal growth conditions for each species were not met and there were potential confounding variables impacting their growth.

4. Conduct additional field sampling in the lower York River in order to assess top-down control from *A. tonsa* on *A. monilatum* and *C. furca.* Also, consider expanding to other areas of Chesapeake Bay that have experienced harmful *M. polykrikoides* and *A. monilatum* blooms. During the 2021 and 2022 field season encompassed by this study, only *M. polykrikoides* bloomed. Additional sampling years could provide supporting information on top-down control from *A. tonsa* on *M. polykrikoides* as well as the necessary data to assess top-down control on *A. monilatum* and *C. furca.*
5. Work to develop standardized warning thresholds for *M. polykrikoides* and potentially *A. monilatum* abundances. These can then be shared with other researchers as well as aquaculture farms in the region to help assess when waters may be harmful. Additionally, once preferred environmental conditions are more well-established, work with stakeholders to encourage aquaculture in regions that are less likely to be impacted by toxic blooms (i.e., in cooler waters that do not support *A. monilatum* growth).

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APPENDIX

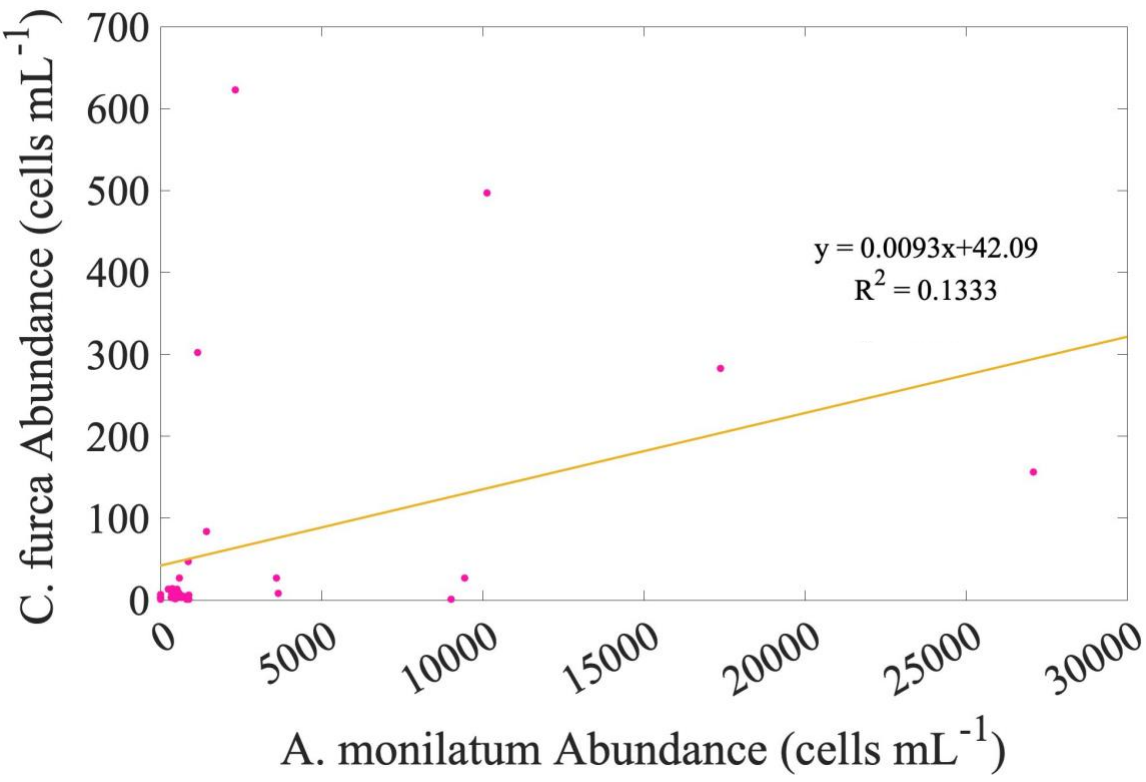

Figure: A.1: Correlation of *A. monilatum* and *C. furca* abundance during a previous HAB. Thirty-eight Lugol's preserved phytoplankton samples taken from the lower York River in 2020 were analyzed. A significant correlation between *A. monilatum* abundance and *C. furca* abundance was not apparent.