

TEMPORAL DYNAMICS OF PLANKTON MORTALITY IN THE SKIDAWAY
RIVER ESTUARY (GA, USA)

by

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(Under the Direction of Elizabeth Harvey)

ABSTRACT

The structure and function of marine microbial food webs is largely determined by the ways in which phytoplankton die (microzooplankton grazing, viral lysis, and parasitic infection), as these mortality processes can differentially influence carbon and nutrient cycles. Temporal shifts in mortality have often been overlooked, hindering our ability to identify drivers of plankton mortality or include loss terms in ecosystem models. Temporal resolution in plankton mortality is warranted for coastal marine regions, where environmental conditions and phytoplankton biomass often fluctuate. To assess mortality dynamics, we performed a series of dilution experiments in the Skidaway River Estuary (GA), measuring phytoplankton growth, microzooplankton grazing, and viral lysis rates at time scales commensurate with tidal (hourly) or seasonal (weekly) periods. Significant variability in grazing rates were detected between daily tides and seasons, with rates driven by changes in plankton community composition rather than environmental factors (e.g., temperature or chlorophyll). Though accounted for in dilution experiments, significant viral lysis rates were rarely observed at all time scales. Given the inability to estimate bulk parasitic infection rates, we applied 18S

amplicon sequencing with network correlation analysis to identify temporal trends of the parasite group, Syndiniales (Alveolata), along with parasite-host interactions within the protist community. Syndiniales were temporally variable, accounting for up to 28% of protist relative abundance in summer-fall, which coincided with increased temperatures in the estuary. Yet, as with microzooplankton grazing rates, Syndiniales abundance and composition were poorly explained by any single environmental factor. Syndiniales sequences represented 20% of all interactions in the network and were positively correlated to a range of plankton groups (e.g., dinoflagellates, ciliates, and diatoms). Parasitic relationships inferred from the network typically involved a single Syndiniales parasite and multiple hosts, implying flexible parasite-host interactions. Our findings of temporal variability in grazing rates and parasite-host relationships further emphasize the heterogenous nature of coastal marine systems, with implications for accurate plankton population monitoring and ecosystem modeling in these locations.

INDEX WORDS: Phytoplankton, Tidal Mixing, Microzooplankton, Dilution experiments, FlowCam, Dinoflagellates, Diatoms, Temperature, Seasonality, Parasitism, Syndiniales, Biotic interactions, Amplicon sequencing, Network analysis

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Microbial eukaryotes (i.e., protists) are central to life in the ocean, fueling marine food webs via fixation of organic matter (Field et al. 1998) and mediating key biogeochemical and global climate cycles (Falkowski and Raven 2007). Protist species are highly diverse, spanning more than five orders of magnitude in size ($1\text{-}10^5\ \mu\text{m}$) and a range of nutritional strategies such as autotrophy, heterotrophy, and mixotrophy (Caron et al. 2011). Protist growth and mortality depend on interactions that occur between species or with the surrounding physical or chemical environment, which can ultimately determine patterns of abundance, diversity, and composition (Rose and Caron 2007; Worden et al. 2015). Over the past decade, much has been learned on the growth of autotrophic protists (phytoplankton), while considerably less is known regarding phytoplankton mortality (Anderson et al. 2002). Yet, identifying and quantifying processes that elicit phytoplankton mortality (e.g., grazing, parasitism, or viral lysis) is important due to the contrasting effects these mortality processes exert on primary production, carbon and nutrient cycling, and the microbial loop (Azam et al. 1983; Worden et al. 2015). The ability to incorporate plankton mortality within food web and ecosystem models hinges on a deeper understanding of mortality-based interactions (e.g., predator-prey or parasite-host), as well as the rates at which these interactions occur.

The most quantitatively significant form of marine phytoplankton mortality is due to grazing by microzooplankton (20-200 μm protists), which consume ~67% of daily

primary production on average across the oceans (Calbet and Landry 2004; Schmoker et al. 2013). Commonly observed microzooplankton taxa, including dinoflagellates and ciliates, often grow at rates that are on par with their phytoplankton prey (Sherr et al. 2003) and grazers can ingest a wide range of prey type (cyanobacteria to chain-forming diatoms), enabling them to rapidly exploit elevated host biomass and control phytoplankton blooms (Sherr and Sherr 2009). Microzooplankton are consumed by larger zooplankton (e.g., copepods and larval plankton), and thus play an important role in the transfer of organic matter to higher trophic levels (Calbet and Saiz 2005).

Despite the known importance of grazing as an agent of phytoplankton mortality, poor temporal resolution of grazing rates has made it difficult to identify abiotic or biotic factors that may drive grazing, which in turn, has limited the predictive power of microzooplankton grazing as a loss term in food web models (Li et al. 2011; Caron and Hutchins 2013). For instance, only a handful of studies have measured microzooplankton grazing rates over an annual cycle at weekly to monthly intervals (Calbet et al. 2008; Lawrence and Menden-Deuer 2012). Limited annual sampling has revealed seasonality in grazing rates, driven in part by surface water temperature, though temperature effects on herbivory remain unclear and other factors such as plankton community composition are likely important (Lawrence and Menden-Deuer 2012). Much less consideration has been given to measuring grazing (and growth) rates on short-term time scales (e.g., hourly-daily), despite these scales being relevant to many coastal marine habitats that experience strong semidiurnal tides and rapid turnover of the phytoplankton community (Cloern and Jassby 2012; Blauw et al. 2018). Additional temporal rate measurements are needed to establish drivers of phytoplankton mortality, which will be useful in predicting shifts in

primary productivity and carbon cycling over a range of temporal scales in the marine environment.

There are other forms of plankton mortality, such as parasitism, that have been explored far less than microzooplankton grazing and have seldom been considered with respect to time. The most ubiquitous and diverse group of protist parasites belong to Syndiniales (marine alveolates; Guillou et al. 2008; de Vargas et al. 2015), which infect and kill a range of other protists (e.g., dinoflagellates, ciliates, and radiolarians) and can terminate or prevent coastal plankton blooms (Coats 1999). The life cycle of Syndiniales alternates between an intracellular (trophont) stage and a free-living (dinospore) stage (Alves-de-Souza et al. 2015). After 2-3 days within the infected host, the parasitic trophont enlarges, initiating host lysis and the release of hundreds of dinospores ($\sim 5 \mu\text{m}$ in size) that can readily infect new hosts (Coats and Park 2002). As with viral lysis, bursting of the host cell following parasitic infection facilitates bacterial growth and the recycling of carbon and nutrients, preventing energy transfer to larger organisms (Jephcott et al. 2016). An accurate representation of carbon flow in microbial food webs will benefit from a deeper understanding of Syndiniales-host population dynamics.

Despite their dominance among protist communities and relatively simple life cycles, it has been challenging to include parasitism within the context of ecosystem or food web models because there is little information available on their temporal patterns and parasite-host infection dynamics. Host biomass is thought to be the primary driver of Syndiniales abundance and infection rates, however, there are other factors (e.g., temperature or nutrients) that may also drive Syndiniales infection dynamics (Coats et al. 1996; Yih and Coats 2000). The specificity of Syndiniales infection also remains

ambiguous, as varying levels of host specificity have been reported depending on the parasite species or strain tested (Chambouvet et al. 2008, 2011). Within the natural environment, Syndiniales sequences have been found to correlate with a range of other protist groups via network analyses (Lima-Mendez et al. 2015) and have been found in virtually all marine habitats (Guillou et al. 2008; de Vargas et al. 2015), which suggests a generalist infection strategy.

The difficulty in characterizing Syndiniales infection dynamics may be related to the enormous diversity within the group, as most parasites have yet to be taxonomically classified at the species level or successfully brought into culture (Guillou et al. 2008). Moreover, unlike rates of microzooplankton grazing, which can be measured *in situ* using dilution-based approaches (Landry and Hassett 1982), there does not exist similar applications for estimating bulk rates of parasitic infection. Dilutions may not be applicable to parasites, given their wide host range (autotrophs to heterotrophs) and infection cycle that spans longer than a typical dilution assay (24 hours). A similar caveat has been described when applying the dilution technique to measure viral lysis rates (Kimmance et al. 2007). Nevertheless, understanding the underlying parasite-host interactions that govern rates of parasitic mortality offers a first step in determining their infection dynamics in the marine environment and will depend on consistent and high-throughput sampling of *in situ* protist communities.

Coastal estuaries are an ideal location to examine phytoplankton mortality dynamics, as they exhibit strong temporal variation in abiotic factors, leading to ephemeral shifts in phytoplankton communities (Cloern and Jassby 2012). The Skidaway River Estuary (GA, USA) represents a temporally complex estuarine system,

experiencing seasonal shifts in environmental conditions and semidiurnal tides (6 hours apart) with a 2-3 m tidal range (Verity and Borkman 2010). Previous time series sampling in the Skidaway River Estuary has revealed temporal variability (hourly to decadal) in phytoplankton abundances and physiochemical variables (Verity et al. 2002 a, b; Verity and Borkman 2010; Bittar et al. 2016), though shifts in phytoplankton mortality rates and protist parasites have not been reported.

Findings on phytoplankton mortality presented in this dissertation are centered around a long-term time series in the Skidaway River, whereby surface water samples were collected weekly over a full calendar year (February 21, 2017 to February 21, 2018). On each of the 41 weekly sampling days, dilution experiments were conducted to measure rates of phytoplankton growth, microzooplankton grazing, and viral lysis. On a subset of days (June 22 to August 17), dilution experiments were conducted at same-day high and low tides to resolve short-term shifts in phytoplankton mortality rates. Lastly, samples were collected concurrently each week for 18S rRNA amplicon profiling, which was used to investigate temporal shifts in protist parasites (Syndiniales) and putative host groups. Environmental variables (e.g., temperature, chlorophyll, and nutrients) were included to establish possible drivers of grazing rates or parasite infection dynamics. Resolving temporal variability in phytoplankton mortality will help to improve our understanding of these loss processes in the ocean and inform future predictions of primary production and biogeochemical cycling.

In **Chapter 2**, microzooplankton grazing rates were measured at same-day high and low tides over an 8-week period in the summer. Hourly variability in grazing rates was observed over the tidal cycle, with rates being consistently higher at low tide

compared to high tide. Differences in rates between tides were not driven by environmental factors (including tide height), instead likely related to observed and pronounced shifts in plankton composition between tides.

In **Chapter 3**, microzooplankton grazing rates were measured over a year-long time series in the estuary (41 days) at weekly intervals. Significant seasonal shifts in microzooplankton grazing rates were observed, with rates less driven by environmental factors compared to changes in their prey composition. Diatoms were especially variable over the year (e.g., *Chaetoceros* and *Guinardia*), with such shifts in the diatom community likely influencing microzooplankton grazing pressure.

In **Chapter 4**, 18S rRNA amplicon sequencing of the V4 region was used to identify Syndiniales relative abundance, composition, and diversity over a subset (33 days) of the year-long time series. Environmental metadata collected for chapter 2 was used in this chapter to identify possible abiotic drivers of Syndiniales populations. Co-occurrence network analysis was also applied, which revealed significant relationships between parasites and putative protist hosts and novel insights into the specificity of such interactions.

REFERENCES

- Alves-de-Souza, C., and others. 2015. Significance of plankton community structure and nutrient availability for the control of dinoflagellate blooms by parasites: a modeling approach. *PLoS ONE* 10:e0127623. doi:10.1371/journal.pone.0127623
- Anderson, D. M., P. M. Glibert, and J. M. Burkholder. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25: 704-726. doi:10.1007/BF02804901
- Azam, F., T. Fenchel, J. G. Field, J. C. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257-263. doi:10.3354/meps010257
- Bittar, T. B., and others. 2016. Seasonal dynamics of dissolved, particulate and microbial components of a tidal saltmarsh-dominated estuary under contrasting levels of freshwater discharge. *Estuarine, Coastal Shelf Sci.* 182: 72-85. doi:10.1016/j.ecss.2016.08.046
- Blauw, A. N., E. Benincà, R. W. Laane, N. Greenwood, and J. Huisman. 2018. Predictability and environmental drivers of chlorophyll fluctuations vary across different time scales and regions of the North Sea. *Prog. Oceanogr.* 161: 1-18. doi:10.1016/j.pocean.2018.01.005
- Calbet, A., and M. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.* 49: 51-57. doi:10.4319/lo.2004.49.1.0051
- Calbet, A., and R. Saiz. 2005. The ciliate-copepod link in marine ecosystems. *Aquat. Microb. Ecol.* 38: 157-167. doi:10.3354/ame038157
- Calbet, A., and others. 2008. Impact of micro-and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments. *Aquat. Microb. Ecol.* 50: 145-156. doi:10.3354/ame01171
- Caron, D. A., P. D. Countway, A. C. Jones, D. Y. Kim, and A. Schnetzer. 2011. Marine protistan diversity. *Annu. Rev. Mar. Sci.* 4: 467-493. doi:10.1146/annurev-marine-120709-142802
- Caron, D. A., and D. A. Hutchins. 2013. The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J. Plankton Res.* 35: 235-252. doi:10.1093/plankt/fbs091
- Chambouvet, A., P. Morin, D. Marie, and L. Guillou. 2008. Control of toxic marine dinoflagellate blooms by serial parasitic killers. *Science* 322: 1254-1257. doi:10.1126/science.1164387

- Chambouvet, A., M. Laabir, M. Sengco, A. Vaquer, and L. Guillou. 2011. Genetic diversity of Amoebophryidae (Syndiniales) during *Alexandrium catenella/tamarense* (Dinophyceae) blooms in the Thau lagoon (Mediterranean Sea, France). *Res. Microbiol.* 162: 959-968. doi:10.1016/j.resmic.2011.03.002
- Cloern, J. E., and A. D. Jassby. 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. *Rev. Geophys.* 50: 1-33. doi:10.1029/2012RG000397
- Coats, D. W., E. J. Adam, C. L. Gallegos, and S. Hedrick. 1996. Parasitism of photosynthetic dinoflagellates in a shallow subestuary of Chesapeake Bay, USA. *Aquat. Microb. Ecol.* 11: 1-9. doi:10.3354/ame011001
- Coats, D. W. 1999. Parasitic life styles of marine dinoflagellates. *J. Eukaryot. Microbiol.* 46: 402-409. doi:10.1111/j.1550-7408.1999.tb04620.x
- Coats, D. W., and M. G. Park. 2002. Parasitism of photosynthetic dinoflagellates by three strains of *Amoebophrya* (Dinophyta): parasite survival, infectivity, generation time, and host specificity. *J. Phycol.* 38: 520-528. doi:10.1046/j.1529-8817.2002.01200.x
- de Vargas, C., and others. 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science* 348: 1261605-1261601. 11. doi:10.1126/science.1261605
- Falkowski, P. G., and J. A. Raven. 2007. The photosynthetic light reactions. *Aquatic photosynthesis*, 2nd edition. New Jersey: Princeton University Press. 81-118
- Field, C., M. Behrenfeld, J. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281: 237-240. doi:10.1126/science.281.5374.237
- Guillou, L., and others. 2008. Widespread occurrence and genetic diversity of marine parasitoids belonging to syndiniales (alveolata). *Environ. Microbiol.* 10: 3349-3365. doi:10.1111/j.1462-2920.2008.01731.x
- Jephcott, T. G., and others. 2016. Ecological impacts of parasitic chytrids, syndiniales and perkinsids on populations of marine photosynthetic dinoflagellates. *Fungal Ecol.* 19: 47-58. doi:10.1016/j.funeco.2015.03.007
- Kimmance, S. A., W. H. Wilson, and S. D. Archer. 2007. Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: Limitations associated with method sensitivity in natural waters. *Aquat. Microb. Ecol.* 49: 207-222. doi:10.3354/ame01136

- Landry, M. R., and R. Hassett. 1982. Estimating the grazing impact of marine microzooplankton. *Mar. Biol.* 67: 283-288. doi:10.1007/BF00397668
- Lawrence, C., and S. Menden-Deuer. 2012. Drivers of protistan grazing pressure: Seasonal signals of plankton community composition and environmental conditions. *Mar. Ecol. Prog. Ser.* 459: 39-52. doi:10.3354/meps09771
- Li, Q. P., P. J. S. Franks, and M. R. Landry. 2011. Microzooplankton grazing dynamics: parameterizing grazing models with dilution experiment data from the California Current Ecosystem. *Mar. Ecol. Prog. Ser.* 438: 59-69. doi:10.3354/meps09320
- Lima-Mendez, G., and others. 2015. Determinants of community structure in the global plankton interactome. *Science* 348: 1262073. doi:10.1126/science.1262073
- Montagnes, D. J., A. Chambouvet, L. Guillou, and A. Fenton. 2008. Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms. *Aquat. Microb. Ecol.* 53: 211-225. doi:10.3354/ame01245
- Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol. Oceanogr.* 53: 886-895. doi:10.4319/lo.2007.52.2.0886
- Schmoker, C., S. Hernandez-Leon, and A. Calbet. 2013. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. *J. Plankton Res.* 35: 691-706. doi:10.1093/plankt/fbt023
- Sherr, E. B., B. F. Sherr, P. A. Wheeler, and K. Thompson. 2003. Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. *Deep. Res. Part I* 50: 557-571. doi:10.1016/S0967-0637(03)00031-1
- Sherr, E. B., and B. F. Sherr. 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. *Aquat. Microb. Ecol.* 57: 253-262. doi:10.3354/ame01358
- Verity, P. G. 2002a. A decade of change in the Skidaway River Estuary. I. Hydrography and Nutrients. *Estuaries Coast* 25: 944-960. doi:10.1007/BF02691343
- Verity, P. G. 2002b. A decade of change in the Skidaway River estuary II. Particulate organic carbon, nitrogen, and chlorophylla. *Estuaries Coast* 25: 961-975. doi:10.1007/BF02691343
- Verity, P. G., and D. G. Borkman. 2010. A decade of change in the Skidaway River Estuary. III. Plankton. *Estuaries Coasts* 33: 513-540. doi:10.1007/s12237-009-9208-2

- Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman, and P. J. Keeling. 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science* 347: 735. doi:10.1126/science.1257594
- Yih, W., and D. W. Coats. 2000. Infection of *Gymnodinium sanguineum* by the dinoflagellate *Amoebophrya* sp.: Effect of nutrient environment on parasite generation time, reproduction, and infectivity. *J. Eukaryot. Microbiol.* 47: 504-510. doi:10.1111/j.1550-7408.2000.tb00082.x

CHAPTER 2

SHORT-TERM ESTIMATES OF PHYTOPLANKTON GROWTH AND MORTALITY

IN A TIDAL ESTUARY¹

¹ Anderson SR, Diou-Cass QP, and Harvey EL. 2018. Limnology and Oceanography 63: 2411-2422. Reprinted with permission of publisher.

ABSTRACT

Rates of phytoplankton growth and mortality are poorly defined over short-time scales (hours to days), despite these scales being relevant over the daily tidal cycle in coastal marine areas. To assess the influence of tidal variability on phytoplankton rates, we performed eight, same-day dilution experiments at high and low tide (6-h intervals) in the Skidaway River Estuary, Georgia, measuring phytoplankton growth, microzooplankton grazing, and virus-induced mortality. Chlorophyll and group-specific biomass (*Synechococcus* spp., picoeukaryotes, and nanoeukaryotes) varied between tides and multidimensional scaling clustering of tidal biomass revealed separation in community composition based on tide. Rates also varied significantly over the tidal cycle, especially microzooplankton grazing, which was higher at low tide in most experiments with rates ranging from 0–3.77 d⁻¹ at low tide to 0–1.51 d⁻¹ at high tide. Virus-induced mortality rates were rarely detected, only being observed in *Synechococcus* spp. in three experiments (0.3–0.8 d⁻¹) and picoeukaryotes twice (~ 0.36 d⁻¹). The differences in grazing and growth rates observed between the two tides were not explained by temperature, salinity, or sunlight (i.e., time of day) and grazing was only weakly explained by initial chlorophyll concentration ($R^2 = 0.36$), highlighting the influence of community composition on rate measurements. These results suggest that within tidally influenced areas, short-term sampling over the tidal cycle is essential to accurately characterize daily phytoplankton dynamics and reliably monitor and predict shifts in primary production and coastal ecosystem health.

INTRODUCTION

Phytoplankton are the dominant primary producers in the ocean, structuring food web communities and mediating the flow of carbon and nutrients (Field et al. 1998). Processes that regulate phytoplankton growth or mortality play a significant role in controlling the fate of populations (e.g. abundance, composition, and diversity) and associated biogeochemical pathways (Strom et al. 2001; Mariani et al. 2013; Brum et al. 2014). Phytoplankton mortality is primarily attributed to grazing by microzooplankton (e.g. < 200 μm protists), which has been estimated to account for 60-70% of daily production loss across a wide range of ocean regimes (Calbet and Landry 2004; Schmoker et al. 2013). However, grazing pressure often varies in time and space, disrupted by changes to the physical or chemical environment (Rose and Caron 2007; Behrenfeld 2010) or availability of prey (Sherr and Sherr 2009), all influencing the fate of primary production and phytoplankton bloom dynamics.

Most microzooplankton grazing studies have been conducted under a spatial context, often sampling along a horizontal or vertical gradient (Ruiz et al. 1998; Murrell et al. 2002; Schmoker et al. 2013). While temporal shifts in rates at a single location have been assessed, most repeated sampling has occurred over weekly to monthly scales, revealing seasonal patterns in rates driven by changing environmental conditions (Kim et al. 2007; Calbet et al. 2008; Lawrence and Menden-Deuer 2012). It remains unclear how rates vary over short temporal scales, on the order of hours to days, experienced over the tidal cycle in dynamic coastal regions like estuaries (Cloern and Jassby 2010). Further, most estimates of microzooplankton grazing in coastal ecosystems are based on studies performed in estuaries (~40%; Schmoker et al. 2013), with little reference to potential

grazing variation over the tidal cycle, emphasizing the need for such short-term rate estimates to better characterize these dynamic systems.

Within estuaries, phytoplankton biomass is susceptible to rapid turnover, mainly through the presence of strong tidal forcing (Cloern and Jassby 2010). Tides induce horizontal and vertical mixing, which may redistribute phytoplankton and their grazers and ultimately influence community structure and phytoplankton population dynamics (i.e. growth and grazing rates; Lewitus et al. 1998; Domingues et al. 2010). Despite several observations of tidal variability in estuarine phytoplankton biomass, abundance, and taxonomic composition (Jouenne et al. 2005; Wetz et al. 2006; Domingues et al. 2010; Blauw et al. 2012), the impact of tides on microzooplankton grazing and other important phytoplankton mortality processes (e.g. viral lysis; Tsai et al. 2015) has not been well investigated, with only one account of grazing rate variability over the tidal cycle (Wetz et al. 2006). Additional measurements of phytoplankton rates over short-term scales are critical to provide accurate predictions of primary production and biogeochemical cycling under non-steady state conditions, which commonly define coastal marine environments.

To better resolve the influence of tidal dynamics on phytoplankton growth and grazing rates, we performed a series of eight, same-day dilution experiments (22 June to 17 August 2017) at high and low tide in the Skidaway River Estuary (GA). The estuary is tidally dominated, shallow (4-6 m), and highly productive (Verity and Borkman 2010). The hydrography and biochemistry of the estuary have been characterized through multiple long-term datasets, revealing significant tidal variability in chlorophyll concentration and phytoplankton abundance (Verity et al. 2002a, b; Verity and Borkman

2010; Bittar et al. 2016). However, biological rates of the phytoplankton community within the estuary are poorly resolved, especially over the tidal cycle. By conducting repeated dilution experiments at high and low tide, we quantified short-term rates of mortality (grazing and viral lysis) and phytoplankton growth using measurements of bulk chlorophyll and, via flow cytometry, group-specific phytoplankton abundances. Establishing such rate measurements are necessary to accurately define the fate of coastal phytoplankton and predict primary production and carbon transfer under realistic time scales that reflect the ephemeral nature of estuarine and other coastal systems.

METHODS

Water sampling

Eight dilution experiments were performed weekly from 22 June to 17 August 2017 at both high and low tide using surface water (1 m) from the Skidaway River Estuary, which is accessible by dock via the Skidaway Institute of Oceanography. Sampling always occurred at consecutive high and low tides on the same day. Tidal height was obtained from NOAA tide predictions at the Isle of Hope, Skidaway River station (station ID: TEC3411). Daily tides in the estuary ranged from 2-3 m with a semidiurnal cycle of 6 h (Verity and Borkman 2010; Table 1). Sampling time varied depending on tide schedule, though sampling typically occurred in the morning (near or after sunrise) and afternoon (see Table 1). Surface water temperature, salinity, and dissolved oxygen (DO) concentrations were measured at each tide using a YSI probe (YSI 600 QS).

Modified dilution experiments

The widely-used, modified dilution approach (Landry and Hassett 1982; Evans et al. 2003) was employed to measure concurrent rates of phytoplankton growth, grazing, and virus-induced mortality at semidiurnal tides in the estuary. Traditionally, the dilution experiment involves setting up a series of dilution treatments to create a gradient in encounter rate between grazers and prey. We followed a slightly modified version of this method, using one low dilution level (20%) and an undiluted treatment (Landry et al. 2008; Morison and Menden-Deuer 2015, 2017). The simplified two-point approach has been shown to produce accurate rates compared to the traditional dilution gradient (Worden and Binder 2003; Strom and Fredrickson 2008), is reliable to systematic biases of nonlinearity in grazing responses (Chen 2015; Morison and Menden-Deuer 2017), and, in our case, supported higher sampling frequency.

Due to time constraints, grazer-free and virus-free diluent were always prepared on the day prior to the experiment day. On the appropriate tide, surface seawater (12-14 L) was collected from the estuary via a 5 L Niskin bottle and screened through a 200 μ m mesh to avoid retention of larger mesozooplankton. This whole seawater (WSW) was immediately transferred to the lab for diluent preparation, whereby the entire volume was gravity-filtered through a 0.45 μ m filter (PALL Acropak™ Supor® membrane capsule) into a clean carboy, creating grazer-free diluent. Half of the grazer-free diluent (~6 L) was then filtered through a 30 kDa filter via a tangential flow filtration system (TFF; Millipore), creating virus-free diluent. Diluents were kept overnight at 18°C in the dark and were acclimated to room temperature prior to dilution experiments.

The next day (T0), fresh WSW was collected at each tide and added to both grazer- and virus-free diluents at a proportion of 20% whole seawater. The 20% dilutions and 100% WSW were siphoned into triplicate, 1.2 L bottles and amended with nutrients (10 μ M nitrate + 1.1 μ M phosphate; based on nutrient concentrations for this location found in Bittar et al. 2016). To control for nutrient limitation, additional triplicate bottles of 100% WSW were incubated without nutrients. Bottles (12 per tide) were incubated for 24 h in a clear, 300 L tank fixed on a nearby dock. The tank was kept at ambient temperature using flow-through water from the estuary and mean light intensity in the surface estuary was replicated in the tank using a Hobo data logger and screen mesh. Natural movements of the dock kept the water in the incubating bottles homogeneously mixed throughout the incubation period.

Phytoplankton abundance and biomass estimates

To measure phytoplankton abundance and biomass, samples for chlorophyll a, flow cytometry, and FlowCam imaging analysis were taken from diluted treatments and WSW stock at T0 and from individual bottles after 24 h (T24).

Daily phytoplankton growth and mortality rates were determined from changes in total extracted chlorophyll a in the bottles over 24 h. Triplicate 50 to 100 mL samples were filtered onto 25 mm 0.7 μ m GF/F filters, extracted in the dark for 12-24 h in 91% ethanol, and measured on a Turner AU10 fluorometer (Jespersen and Christoffersen 1987; Graff and Ryneerson 2011). Ethanol blanks were included, and all samples were corrected for phaeophytin.

To assess group-specific growth and mortality rates, flow cytometry was used. Triplicate 5 mL samples were preserved for flow cytometry with 0.5% glutaraldehyde

(final concentration), incubated at 4°C for 10 min and frozen (-80°C) until analysis (within 2-3 weeks; Kemp et al. 1993). To calculate phytoplankton group abundances, 200 µl aliquots of fixed sample were added to a 96-well plate and run on a Guava flow cytometer (Millipore). Diluent water (0.45 µm and TFF) were run as blanks and instrument-specific beads were used to calibrate the cytometer. Samples were analyzed at low flow rate (0.24 µl s⁻¹) for 3 min. Three major phytoplankton groups were distinguishable based on plots of forward scatter vs. orange (phycoerythrin-containing, *Synechococcus* spp.) or red (pico- and nanoeukaryotes) fluorescence signals (Worden and Binder 2003).

Abundances of each phytoplankton group measured via flow cytometry were converted to carbon biomass (µg C L⁻¹) using conservative literature conversion factors. Biomass was converted assuming 255 fg C cell⁻¹ for *Synechococcus* spp. (Buitenhuis et al. 2012), 1500 fg C cell⁻¹ for picoeukaryotes (Zubkov et al. 1998), and 2763 fg C cell⁻¹ for nanoeukaryotes, assuming an average cell diameter of 6 µm (Tarran et al. 2001; Pan et al. 2007).

An automated FlowCam (Fluid Imaging Technologies, Yarmouth, ME, USA) was used to assess the relative abundance of the larger plankton assemblage (20-200 µm), as it is a more rapid approach and yields estimates comparable to traditional microscopy (Alvarez et al. 2014). At T0 and T24 of each experiment, 100-200 ml samples of 100% WSW were collected, fixed in buffered formalin (final concentration 1%), and kept at 4°C until analysis (within 2-3 weeks; Poulton 2016). Samples were pre-filtered through 200 µm mesh and analyzed (5-10 ml) on Autoimage mode using a 300 µm flow cell and a flow rate of 2 ml min⁻¹ (Poulton 2016). For each experiment, one replicate per tide was

analyzed, resulting in 2000-7000 images captured per sample (number of images similar between same-day tides). Based on cell morphology, diatoms and microzooplankton grazers were grouped to finest possible taxonomic resolution (often genus level). Group-specific counts were converted to abundance using the volume analyzed and a particle capture efficiency of 39.7%, which accounts for cells not seen within the flow cell (Poulton 2016). Further, average cell width and length were measured for each defined group. Nano-sized grazers (e.g. $\leq 20 \mu\text{m}$, herbivorous nanoflagellates) were not detected with our flow cell. Chain-forming diatoms were counted and sized as one cell.

Diatom biovolume was estimated using cellular length and width measurements from the FlowCam, along with the closest geometric formula for solitary (prolate spheroid) and chain-forming (cone, 1/2 sphere + cone) groups (Hillebrand et al. 1999). Biovolumes of microzooplankton were estimated assuming a prolate spheroid (Hillebrand et al. 1999). Carbon biomass ($\mu\text{g C L}^{-1}$) was estimated for diatoms and grazers using volume to carbon conversion equations (Menden-Deuer and Lessard 2000). Final biomass estimates were pooled for diatoms (solitary + chain-forming) and the two microzooplankton types, heterotrophic dinoflagellates and ciliates, were considered separately. The mixotrophic dinoflagellate, *Akashiwo sanguinea*, was well represented and often dominant during the sampling period and was grouped separately.

Estimation of growth and mortality rates

Apparent growth rate (k) was calculated from daily changes in both chlorophyll and population abundances from triplicate treatments using the equation:

$$(1) k = 1/t * \ln (P_t/P_0),$$

where t was the incubation time (units of day), and P_0 and P_t represent either the concentration of chlorophyll or the abundance of specific groups at T0 or T24 of the experiment, respectively. Grazing (g) was calculated using the equation:

$$(2) \ g = (k_d - k_1) / (1 - x),$$

where k_d and k_1 are apparent growth in diluted treatments or WSW, respectively, and x is the corresponding fraction of WSW from the dilution (Landry et al. 1984). Grazing was always determined from k in the nutrient-amended WSW. Estimates of negative grazing were measured, especially for *Synechococcus* spp. at high tide (three of eight experiments), and in these instances, negative grazing rates were set to zero (Strom and Fredrickson 2008). The in situ instantaneous growth rates (μ_0) were determined from the equation:

$$(3) \ \mu_0 = g + k_1$$

To account for in situ nutrient limitation (often observed in the estuary), k_1 was taken from non-amended WSW. Nutrient-amended growth rates (μ_n) were also estimated using equation 3, with k_1 from nutrient-amended WSW. Accumulation rates (r) represented the net phytoplankton growth over 24 h and were estimated from $\mu_0 - g$; when grazing was zero, accumulation rates were assumed to be equivalent to μ_0 or $\mu_0 - v$, with v as viral-induced mortality. Estimates of g from the virus-free series represented the total rate of mortality and estimates of virus-induced mortality rates (v) were obtained from the difference in rates from the parallel dilution series (Evans et al. 2003).

Statistical analyses

Initially, paired t -tests were used to test if apparent growth rates in the diluted (k_d) and non-diluted (k_1) treatments were significantly different ($p < 0.05$). When apparent

growth rates between the two dilution levels were not significantly different, grazing rate was set to zero for future analysis. Paired *t*-tests were used to test the significant difference ($p < 0.05$) between rates of grazing, growth (μ_0 and μ_n), and net accumulation measured at same-day low and high tides. Linear regression analyses were performed using GraphPad Prism 7 software to quantify relationships between environmental variables and rates of growth and grazing.

The composition of phytoplankton and microzooplankton were compared between high and low tide using the multivariate statistics package PRIMER Version 7 (Clarke & Gorley 2015). Carbon biomass values for flow cytometry (*Synechococcus* spp., pico- and nanoeukaryotes) and FlowCam (diatoms, heterotrophic dinoflagellates, ciliates, and *A. sanguinea*) groups were used, as well as initial chlorophyll concentrations. Biomass data were initially square root transformed to evenly scale the contribution among groups and reduce the influence of dominant taxa, and a Bray-Curtis similarity matrix was generated. Non-metric multidimensional scaling (MDS) was then applied to visualize the relationship between communities at high and low tide on each experiment day; each symbol on the MDS plot represents the total plankton community (microzooplankton + phytoplankton) per sample. Samples closer together on the MDS plot indicate similarity between plankton communities. To test for significant differences between high and low tide samples, we used an analysis of similarity (ANOSIM) test on the MDS resemblance matrix that computes a global *R* statistic (range -1 to 1); values of *R* approaching zero indicate less similarity between tides (Clarke and Warwick 2001).

RESULTS

Estuarine conditions and community composition

Surface temperature, salinity, and dissolved oxygen varied in the estuary over the sampling period at high and low tide (Table 1). However, when averaged across the entire sampling period, salinity was the only significantly different factor between tides ($p < 0.001$), with, on average, higher salinity measured at high tide (30.26 ± 1.38) compared to low tide (27.81 ± 1.28 ; Table 1).

Initial chlorophyll concentration ($< 200 \mu\text{m}$) varied on weekly time scales, ranging from $1.94 - 6.38 \mu\text{g L}^{-1}$ at high tide and $3.72 - 20.1 \mu\text{g L}^{-1}$ at low tide (Table 1; Fig. 1a). Chlorophyll was 47 - 82% higher at low tide compared to high tide in five of eight experiments ($p < 0.05$; Fig. 1a). Community composition varied over the sampling period, with high carbon biomass measured at both tides for *Akashiwo sanguinea* (low = $33.36 - 1161.17 \mu\text{g C L}^{-1}$; high = $13.21 - 69.78 \mu\text{g C L}^{-1}$) and picoeukaryotes (low = $36.37 - 105.37 \mu\text{g C L}^{-1}$; high = $29.15 - 83.36 \mu\text{g C L}^{-1}$; Fig. 1b). Diatoms contributed most to high tide carbon biomass on Aug 3 and 10 (123.4 and $262.69 \mu\text{g C L}^{-1}$), though in general, carbon biomass of $< 20 \mu\text{m}$ pico- and nanoeukaryotes were higher than diatoms, irrespective of tide (Fig. 1b). Though observed at each tide, heterotrophic dinoflagellates ($\leq 44.03 \mu\text{g C L}^{-1}$) and ciliates ($\leq 22.27 \mu\text{g C L}^{-1}$) were smaller components of biomass. Commonly identified diatoms included solitary groups like *Coscinodiscus*, *Ditylum*, and *Nitzschia*, along with chain-forming groups *Skeletonema* and *Chaetoceros*. Microzooplankton largely consisted of *Gymnodinium*-like cells, though *Protoperidinium*, and unidentified aloricated and loricated ciliates were also found.

Community composition also varied between same-day high and low tides. For instance, *A. sanguinea* carbon biomass was higher at low tide versus high tide in all experiments, with especially high biomass differences (186 - 188%) in three experiments (Jun 28, July 6, Aug 3) (Fig. 1b). There was no clear trend in tidal diatom biomass, though on days where diatoms dominated high tide (Aug 3, 10), measurements of same-day, low tide diatom biomass were 84 and 142% lower, respectively (Fig. 1b). Biomass of pico- and nanoeukaryotes was often higher at low tide (15 - 37%) compared to same-day biomass at high tide; *Synechococcus* spp. carbon biomass was lower than picoeukaryotes for both tides ($2.06 - 13.41 \mu\text{g C L}^{-1}$), though biomass was higher at high tide (7 - 116%) in all experiments (Fig. 1b).

Differences in total community composition (microzooplankton + phytoplankton) were observed between high and low tide using a multivariate statistical approach (Fig. 1c). Community composition at low tide was significantly different from high tide over all sampling points, though differences were poorly supported (ANOSIM $R = 0.22$; $p = 0.01$). There was clustering between same-day high and low tide samples in two experiments (Jul 27 and Aug 10), though most high tide samples were distinctly clustered together, separated from more-scattered low tide samples (Fig. 1c). Low tide samples on Jun 28 and Jul 6, which had the highest biomass estimates of *A. sanguinea*, were clustered farthest away and least similar to other high and low tide samples (Fig. 1b, c).

Community-based growth and mortality estimates

Variation in chlorophyll-based grazing and growth rates was detected over the sampling period at both high ($g = 0.57 - 1.27 \text{ d}^{-1}$; $\mu_0 = 0.5 - 1.61 \text{ d}^{-1}$) and low tides ($g = 0 - 2.28 \text{ d}^{-1}$; $\mu_0 = -0.13 - 2.07 \text{ d}^{-1}$; Table 2). Grazing rates were significantly higher at low

versus high tide in five of the eight experiments ($p \leq 0.002$; Fig. 2a), whereas growth rates were significantly higher at high tide in four experiments ($p \leq 0.02$; Fig. 2b). Net chlorophyll accumulation rates were significantly lower at low tide compared to high in seven of eight tidal experiments ($p \leq 0.01$; Table 2). Nutrient-amended growth rates exceeded instantaneous growth for high and low tide in all experiments, though significant differences in nutrient-amended growth between high and low tide were only detected in four experiments ($p \leq 0.02$; Table 2). There were no significant viral-induced mortality rates detected from bulk chlorophyll for either tide.

Differences in grazing rates between high and low tides were not explained by predicted tidal range in the estuary ($R^2 = 0.03$; $p = 0.68$). Average grazing rates at high and low tide were not related to temperature ($R^2 = 0.05$; $p = 0.42$) or salinity ($R^2 = 0.24$; $p = 0.05$). There was a weak, positive relationship between tidal grazing rates and initial chlorophyll a concentration ($R^2 = 0.36$; $p = 0.01$). Growth rates at high and low tide were not explained by any of these environmental parameters ($R^2 \leq 0.2$; $p \geq 0.08$). No apparent relationship was measured between grazing (Fig. S1a) or growth rates (Fig. S1b) at high and low tide versus sampling time of day in the estuary.

Group-specific growth and mortality estimates

Synechococcus spp. grazing and growth varied over the sampling period at high ($g = 0 - 1.51 \text{ d}^{-1}$; $\mu_0 = -0.5 - 1.76 \text{ d}^{-1}$) and low tides ($g = 0 - 3.77 \text{ d}^{-1}$; $\mu_0 = 0.98 - 2.86 \text{ d}^{-1}$; Table S1). All but one experiment at high tide yielded grazing rates equal to zero, which resulted in significantly higher *Synechococcus* spp. grazing rates at low tide versus high tide in seven of eight experiments ($p \leq 0.03$; Fig. 3a; Table S1). Growth rates were significantly higher at low tide compared to high tide in four experiments ($p \leq 0.02$; Fig.

3b). Higher grazing at low tide led to significantly lower net accumulation of *Synechococcus* spp. at low tide versus high tide in six of eight experiments ($p \leq 0.02$; Table S1). Estimates of nutrient-amended growth were often well-aligned with instantaneous growth for both high and low tide (Table S1). Viral-induced mortality of *Synechococcus* spp. was measured in three experiments at high tide (0.29 - 0.57 d⁻¹) and one at low tide (0.8 d⁻¹; Fig. 3a).

Measurements of picoeukaryote grazing and growth also varied over the sampling period at high ($g = 0 - 1.51$ d⁻¹; $\mu_0 = 0.9 - 2.43$ d⁻¹) and low tides ($g = 0 - 2.61$ d⁻¹; $\mu_0 = 0.36 - 3.47$ d⁻¹; Table S2). Picoeukaryote grazing rates were significantly higher at low versus high tide in seven of eight experiments ($p \leq 0.01$; Fig. 3c; Table S2). Growth rates were significantly different between high and low tide in five experiments, though neither tide had consistently higher growth relative to the other ($p \leq 0.01$; Fig. 3d). As with *Synechococcus* spp., higher grazing at low tide resulted in lower rates of picoeukaryote accumulation for five of eight experiments ($p \leq 0.001$; Table S2). Nutrient-amended and instantaneous growth rates for picoeukaryotes were similar (Table S2). Viral-induced mortality of picoeukaryotes was measured in one experiment at high tide (0.36 d⁻¹) and one at low tide (0.37 d⁻¹; Fig. 3c).

Nano-eukaryote growth and grazing rates significantly varied between high and low tide in six of eight experiments, though no trends in tidal rates were apparent ($p \leq 0.02$; Table S3; Fig. 3e, f). Nano-eukaryote accumulation rates were positive irrespective of tide and significantly different between high and low tide in five experiments ($p \leq 0.01$; Table S3). Nutrient-amended growth was on par with instantaneous growth for nano-eukaryotes and significantly differed between high and low tide in four experiments

($p \leq 0.01$; Table S3). Viral-induced mortality rates were not detected for nanoeukaryotes at either tide.

DISCUSSION

Across a range of global estuaries, primary production has been shown to vary temporally over seasonal, weekly, and even hourly scales (Cloern and Jassby 2012; Blauw et al. 2018). However, in these ecosystems, rates of phytoplankton growth and mortality over these same time scales are scarce, particularly at hourly resolution commensurate with the tidal cycle. We performed repeated dilution experiments in the Skidaway River Estuary at daily low and high tides and found significant tidal variability in phytoplankton biomass, community composition, phytoplankton growth and grazing rates, and net phytoplankton accumulation rates. Failure to consider the variability in rates of the phytoplankton community between tides has potential implications for our ability to predict estuarine production and adequately sample these dynamic environments.

Phytoplankton community variability

Over the course of sampling (~2 months), total chlorophyll concentration ($< 200 \mu\text{m}$) and group-specific phytoplankton carbon biomass estimates fluctuated from week to week at each respective tide, supporting previous findings of weekly phytoplankton variation in estuaries, including the Skidaway River Estuary (Verity et al. 2002b; Blauw et al. 2012, 2018; Bittar et al. 2016). Previous long-term sampling efforts in the Skidaway River Estuary have revealed typically higher baseline levels and weekly variability in chlorophyll concentration and phytoplankton abundance ($< 20 \mu\text{m}$) in summer relative to other seasons (Bittar et al. 2016). Though weekly-biweekly tidal features (i.e. spring-neap

tides) are important in some estuaries (Blauw et al. 2012, 2018), we did not find a relationship between biomass or phytoplankton rates and predicted tide height in the estuary and trends in short-term biomass variability between same-day tides were more pronounced.

Variability in chlorophyll and group-specific phytoplankton biomass over the daily tidal cycle has been observed in other estuaries (Trigueros and Orive 2000; Jouenne et al. 2005; Wetz et al. 2006; Domingues et al. 2010). Chlorophyll concentrations measured in our study were often higher at low tide, which may have been attributed to the seaward advection of upstream water that is nutrient-rich and more productive (Trigueros and Orive 2000; Wetz et al. 2006). However, there is no definitive relationship between tidal phase and chlorophyll biomass maxima, as multiple studies have observed higher productivity at either tide (Lehman 2000; Aubry and Acri 2004; Domingues et al. 2010). Previous work in the Skidaway River Estuary has reported 10-15% higher chlorophyll concentration at low compared to high tide, which supports the general trend in chlorophyll variability we observed (Verity et al. 2002b). Group-specific carbon biomass for *Akashiwo sanguinea*, picoeukaryotes, and nanoeukaryotes varied considerably in our study and were often higher at low tide, resulting in differential clustering of tidal communities. Related studies have observed variation in diatom biomass between high and low tides (Jouenne et al. 2005; Wetz et al. 2006), though consistent trends in biomass between tides remain unclear.

The factors driving the observed shifts in chlorophyll and biomass between tides may be hard to generalize given the dynamic nature of estuarine systems, where a myriad of processes drive variability in primary production over a range of spatiotemporal scales

(Cloern and Jassby 2010, 2012). Changing abiotic processes can play a prominent role in structuring planktonic communities in estuaries, for example, patterns in phytoplankton production and composition in estuaries over the tidal cycle have been shown to be largely driven by tidally-induced mixing (Domingues et al. 2010; Blauw et al. 2012, 2018). Horizontally, tides transport phytoplankton and grazer populations that originate in oceanic or upstream waters (Lucas et al. 1999; Trigueros and Orive 2000), and vertical mixing re-suspends populations that may have settled during the slack tide (Lauria et al. 1999). Within the Skidaway River Estuary, the semidiurnal tidal cycle (2-3 m) is the dominant feature, as there is little freshwater discharge into the estuary, except following heavy local rainfall, which was not observed during our sampling (Verity and Borkman 2010). Moreover, over the 6-h tidal period examined here, other bottom-up factors such as wind speed, turbidity, and sunlight likely play a minor role in driving phytoplankton productivity compared to physical tidal mixing (Cloern 1987; Blauw et al. 2012, 2018).

Beyond abiotic processes, biotic factors such as microzooplankton grazing, benthic grazing (e.g. bivalves) and viral lysis can also structure production in estuaries (Cloern et al. 2014; Tsai et al. 2015). High rates of microzooplankton grazing have previously been estimated in estuaries (Wetz et al. 2006; Lawrence and Menden-Deuer 2012), and as evidenced in our study, grazing rates can vary significantly over the tidal cycle, influencing short-term chlorophyll and group-specific phytoplankton biomass. Partitioning the impact of abiotic versus biotic factors in controlling phytoplankton biomass represents a challenge in highly dynamics estuaries, especially as microzooplankton grazers are themselves subject to the above-mentioned abiotic factors. For instance, several studies have demonstrated that salinity tolerance can structure

microzooplankton grazer communities, likely influencing grazing interactions (Lehrter et al. 1999; Strom et al. 2013). Therefore, while tidal mixing may dominate grazer and prey distributions over the tidal cycle, the interactions between grazers and prey in this system are likely cyclical, whereby microzooplankton grazing influences phytoplankton community composition, which in turn, drives future grazer-prey interactions and rate dynamics.

Phytoplankton rate variability

Higher microzooplankton grazing rates were frequently measured for total chlorophyll, *Synechococcus* spp., and picoeukaryotes at low compared to high tide, whereas differences in instantaneous growth were not significant for most samples. Due to high grazing, estimates of net phytoplankton accumulation were lower at low tide. While the range in grazing estimates ($0 - 3.77 \text{ d}^{-1}$) measured in this study were similar to rates observed in other estuarine environments (Wetz et al. 2006; Kim et al. 2007; Lawrence and Menden-Deuer 2012), there is no study, to our knowledge, that has directly compared microzooplankton grazing rates between high and low tide. A study by Wetz et al. (2006) measured grazing rates over a tidal cycle but did not report direct comparisons between tides. However, they did partly attribute chlorophyll loss between tides (47 - 51%) to microzooplankton grazing (Wetz et al. 2006). Significant variability in grazing between high and low tides demonstrates the need to examine these rate dynamics over short time scales (e.g. hourly), particularly given the strong impact of microzooplankton grazing on controlling phytoplankton biomass accumulation and potential bloom formation (Sherr and Sherr 2009).

Most temporal studies of microzooplankton grazing have occurred seasonally, and differences in observed grazing rates were partly attributed to shifts in the environment (e.g. temperature; Kim et al. 2007; Calbet et al. 2008; Lawrence and Menden-Deuer 2012). However, this was not the case over time periods of the tidal cycle, as there were no discernible trends between grazing rates (or growth rates) and environmental variables like temperature, salinity, or time of day (i.e. light level). A weak positive relationship ($R^2 = 0.36$) was found between grazing rates and initial chlorophyll concentration. However, chlorophyll is likely a poor predictor of grazing rates in general, because grazing is often species-specific with grazers exhibiting different degrees of prey palatability (Irigoien et al. 2005; Lawrence and Menden-Deuer 2012). Nutrients have been shown to fluctuate slightly between tides due to horizontal tidal transport (Wetz et al. 2006), which may affect the health of tidal phytoplankton populations and lead to overestimates of phytoplankton loss from grazing. However, nutrient-limitation was controlled for in our experiments, and cases of limitation ($\mu_n > \mu_0$) were more common for total chlorophyll and consistent between same-day high and low tides. Thus, over hourly time scales, environmental parameters may not play as large a role in driving growth and microzooplankton rates as they do over longer, seasonal scales (Kim et al. 2007; Lawrence and Menden-Deuer 2012). Instead, differences in microzooplankton grazing rates measured at same-day high and low tides are likely driven by tidal variability in the community composition of grazers and prey.

As stated above, phytoplankton community structure likely impacts grazing interactions and may have contributed to differences in grazing rates we observed between tides in our study (Strom and Strom 1996; Ruiz et al. 1998). For example, low

tide communities were dominated by the mixotroph *A. sanguinea*, which is considered an important grazer of smaller eukaryotes and cyanobacteria and has regularly been observed in the Skidaway River Estuary (Jeong et al. 2005; Verity and Borkman 2010). *A. sanguinea* can rapidly ingest *Synechococcus* spp. (63 cells grazer⁻¹ mL⁻¹), and so its consistently higher biomass at low tide in our study may have explained higher grazing rates of *Synechococcus* spp. and similar-sized picoeukaryotes at low tide relative to high tide on most days (Jeong et al. 2005). Grazing rate estimates may also depend on specific grazer-prey interactions, as microzooplankton grazers can be highly selective and preferentially ingest prey based on cell type and size, cellular composition, or nutritional quality (Tillmann 2004; Montagnes et al. 2008) and may even exhibit selectivity between distinct phytoplankton strains (Apple et al. 2011). Though not detected within the context of our dilution experiments, such selectivity in grazing may have contributed to grazer-prey mismatches and variability in rate estimates. Therefore, short-term variability in grazing rates we measured over the tidal cycle were likely attributed to measurable differences in community composition (e.g. biomass variability and MDS clustering), and the potential dynamics of grazer-prey pairings and grazer feeding selectivity.

Other sources of mortality, such as viral lysis, can contribute greatly to phytoplankton loss in estuaries (Ortmann et al. 2011; Tsai et al. 2015), though in our study, virus-induced mortality for *Synechococcus* spp. and picoeukaryotes were only detected in a handful of tidal experiments. Virus communities and infection dynamics have been observed to change spatially in estuaries along a salinity gradient (Lu et al. 2001; Tsai et al. 2015), though over the tidal cycle, there was no clear trend in virus-induced mortality rates. Viruses exhibit considerable host specificity, so it is possible the

viral community present at high or low tide was not specific to the phytoplankton groups considered (Kimmance et al. 2007). The length of incubation in the dilution experiment may also contribute to inconsistent lysis measurements, as it may not cover the full lytic infection cycle of the virus (Evans et al. 2003; Jacquet et al. 2005). For instance, viruses with a lytic cycle extending beyond a 24 h period would not be captured in our incubations. In addition to viruses, benthic grazing has also been argued to be an important source of phytoplankton mortality in estuaries (Murrell and Hollibaugh 1998; Cloern et al. 2014). However, benthic grazing is not measurable within the context of the dilution experiment and so was not considered in our study. Finally, mesozooplankton (e.g. > 200 μm copepods or larval zooplankton) can contribute to phytoplankton grazing, though typically exert a lower grazing impact on primary production compared to microzooplankton (Sherr and Sherr 2007; Schmoker et al. 2013).

Implications of tidal variability

The variability in phytoplankton community composition and rates we observed over the tidal cycle supports growing evidence that coastal phytoplankton do not behave like passive tracers over short-term (hourly) periods, but rather are subject to fluctuation over these scales, largely driven by tidal effects (Blauw 2012, 2018). Other studies have suggested that short-term sampling and strict attention to the tidal phase is needed to accurately monitor primary production in estuaries (Wetz et al. 2006; Domingues et al. 2010; Blauw et al. 2012, 2018). The same applies for biological rates, which varied widely between tides in our study, particularly estimates of microzooplankton grazing. Therefore, in estuarine environments it may be necessary to measure rates over the entire

tidal cycle, if the goal is to use such rates to correctly report daily shifts in net phytoplankton accumulation and primary productivity.

To accurately capture rates of production and loss in dynamic coastal ecosystems, sampling efforts in these areas should be implemented at frequencies that reflect tidal variability. Measurements that neglect the different phases of the tidal cycle, and only represent one daily time point, may significantly misrepresent estimates of productivity or net phytoplankton accumulation and could ultimately result in unreliable predictions of phytoplankton abundance and biogeochemical cycling within estuarine systems (Wetz et al. 2006; Blauw et al. 2012). There are instances whereby sampling at same-day tides may not be reasonable, such as in long-term monitoring programs that may sample at weekly to monthly frequencies and have limited resources. In these cases, it may be necessary to make consistent measurements at the same phase of the tidal cycle to alleviate sampling biases and accurately compare rates over time (Blauw et al. 2012, 2018). Overall, our findings highlight the importance of short-term sampling in capturing the natural variability within tidally-dominated environments and have important implications for reliably monitoring and predicting trends in coastal phytoplankton communities.

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REFERENCES

- Apple, J. K., S. L. Strom, B. Palenik, and B. Brahamsha. 2011. Variability in protist grazing and growth on different marine *Synechococcus* isolates. *Appl. Environ. Microbiol.* 77: 3074-3084. doi:10.1128/AEM.02241-10
- Aubry, F. B., and F. Aciri. 2004. Phytoplankton seasonality and exchange at the inlets of the Lagoon of Venice (July 2001–June 2002). *Journal of Marine Systems* 51: 65-76. doi:10.1016/j.jmarsys.2004.05.008
- Álvarez, E., M. Moyano, Á. López-Urrutia, E. Nogueira, and R. Scharek. 2013. Routine determination of plankton community composition and size structure: a comparison between FlowCAM and light microscopy. *J. Plankton Res.* 36: 170-184. doi:10.1093/plankt/fbt069
- Behrenfeld, M. J. 2010. Abandoning Sverdrup's critical depth hypothesis on phytoplankton blooms. *Ecology* 91: 977-989. doi:10.1890/09-1207.1
- Bittar, T. B., and others. 2016. Seasonal dynamics of dissolved, particulate and microbial components of a tidal saltmarsh-dominated estuary under contrasting levels of freshwater discharge. *Estuarine, Coastal Shelf Sci.* 182: 72-85. doi:10.1016/j.ecss.2016.08.046
- Blauw, A. N., E. Benincà, R. W. P. M. Laane, N. Greenwood, and J. Huisman. 2012. Dancing with the Tides: Fluctuations of Coastal Phytoplankton Orchestrated by Different Oscillatory Modes of the Tidal Cycle. *PLoS ONE* 7: e49319. doi:10.1371/journal.pone.0049319
- Blauw, A. N., E. Benincà, R. W. Laane, N. Greenwood, and J. Huisman. 2018. Predictability and environmental drivers of chlorophyll fluctuations vary across different time scales and regions of the North Sea. *Prog. Oceanogr.* 161: 1-18. doi:10.1016/j.pocean.2018.01.005
- Brum, J. R., J. J. Morris, M. Décima, and M. R. Stukel. 2014. Mortality in the oceans: Causes and consequences, in *Eco-DAS IX Symposium Proceedings*, *Limnol. and Oceanogr.* 16-48. doi:10.4319/ecodas.2014.978-0-9845591-3-8.16
- Buitenhuis, E. T., and others. 2012. Picophytoplankton biomass distribution in the global ocean. *Earth Syst. Sci. Data* 4: 37-46. doi:10.5194/essd-4-37-2012
- Calbet, A., and M. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.* 49: 51-57. doi:10.4319/lo.2004.49.1.0051

- Calbet, A., and others. 2008. Impact of micro-and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments. *Aquat. Microb. Ecol.* 50: 145-156. doi:10.3354/ame01171
- Chen, B. 2015. Assessing the accuracy of the “two-point” dilution technique. *Limnol. Oceanogr. Methods* 13: 521-526. doi:10.1002/lom3.10044
- Clarke, K. R., and R. M. Warwick. 2001. Change in marine communities: An approach to statistical analysis and interpretation, 2nd ed, 172p. PRIMER-E.
- Clarke, K. R., and R. N. Gorley. 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth.
- Cloern, J. E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Cont. Shelf Res.* 7: 1367-1381. doi:10.1016/0278-4343(87)90042-2
- Cloern, J., and A. Jassby. 2010. Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems. *Estuaries Coast.* 33: 230-241. doi:10.1007/s12237-009-9195-3
- Cloern, J. E., and A. D. Jassby. 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. *Rev. Geophys.* 50: 1-33. doi:10.1029/2012RG000397
- Cloern, J. E., S. Q. Foster, and A. E. Kleckner. 2014. Phytoplankton primary production in the world’s estuarine-coastal ecosystems. *Biogeosciences* 11: 2477-2501. doi:10.5194/bg-11-2477-2014
- Domingues, R. B., T. P. Anselmo, A. B. Barbosa, U. Sommer, and H. M. Galvão. 2010. Tidal variability of phytoplankton and environmental drivers in the freshwater reaches of the Guadiana estuary (SW Iberia). *Int. Rev. Hydrobiol.* 95: 352-369. doi:10.1002/iroh.201011230
- Evans, C., S. D. Archer, S. Jacquet, and W. H. Wilson. 2003. Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population. *Aquat. Microb. Ecol.* 30: 207-219. doi:10.3354/ame030207
- Field, C., M. Behrenfeld, J. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281: 237-240. doi:10.1126/science.281.5374.237
- Graff, J. R., and T. A. Ryneerson. 2011. Extraction method influences the recovery of phytoplankton pigments from natural assemblages. *Limnol. Oceanogr. Methods* 9: 129-139. doi:10.4319/lom.2011.9.129

- Hillebrand, H., C. Durselen, D. Kirschtel, U. Pollinger, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35: 403-424. doi:10.1046/j.1529-8817.1999.3520403.x
- Irigoin, X., K. J. Flynn, and R. P. Harris. 2005. Phytoplankton blooms: A 'loophole' in microzooplankton grazing impact? *J. Plankton Res.* 27: 313-321. doi:10.1093/plankt/fbi011
- Jacquet, S., I. Domaizon, S. Personnic, A. S. P. Ram, M. Hedal, S. Duhamel, and T. Sime-Ngando. 2005. Estimates of protozoan- and viral-mediated mortality of bacterioplankton in Lake Bourget (France). *Freshw. Biol.* 50: 627-645. doi:10.1111/j.1365-2427.2005.01349.x
- Jeong, H. J., and others. 2005. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquat. Microb. Ecol.* 41: 131-143. doi:10.3354/ame041131
- Jespersen, A. M., and K. Christoffersen. 1987. Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. *Arch. Hydrobiol.* 109: 445-454.
- Jouenne, F., S. Lefebvre, B. Véron, and Y. Lagadeuc. 2005. Biological and physiochemical factors controlling short-term variability in phytoplankton primary production and photosynthetic parameters in a macrotidal ecosystem (eastern English Channel). *Estuar. Coast. Shelf. Sci.* 65: 421-439. doi:10.1016/j.ecss.2005.05.023
- Kemp, P., B. F. Sherr, E. B. Sherr, and J. J. Cole. 1994. *Handbook of methods in aquatic microbial ecology*. Lewis Publishing.
- Kim, S., M. G. Park, C. Moon, K. Shin, and M. Chang. 2007. Seasonal variations in phytoplankton growth and microzooplankton grazing in a temperate coastal embayment, Korea. *Estuarine, Coastal Shelf Sci.* 71: 159-169. doi:10.1016/j.ecss.2006.07.011
- Kimmance, S. A., W. H. Wilson, and S. D. Archer. 2007. Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: Limitations associated with method sensitivity in natural waters. *Aquat. Microb. Ecol.* 49: 207-222. doi:10.3354/ame01136
- Landry, M. R., and R. Hassett. 1982. Estimating the grazing impact of marine microzooplankton. *Mar. Biol.* 67: 283-288. doi:10.1007/BF00397668
- Landry, M. R., L. W. Hass, and V. L. Fagerness. 1984. Dynamics of microbial plankton communities: Experiments in Kaneohe Bay, Hawaii. *Mar. Ecol. Prog. Ser.* 16: 127-133. doi:10.3354/meps016127

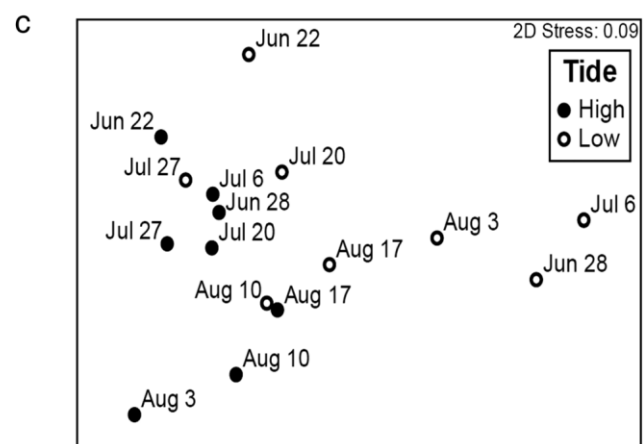
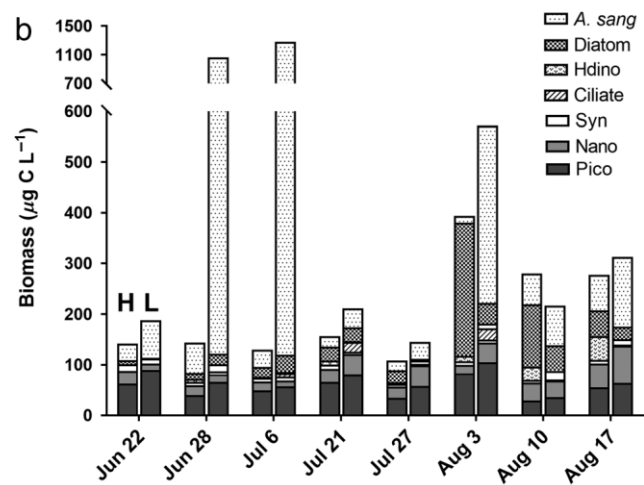
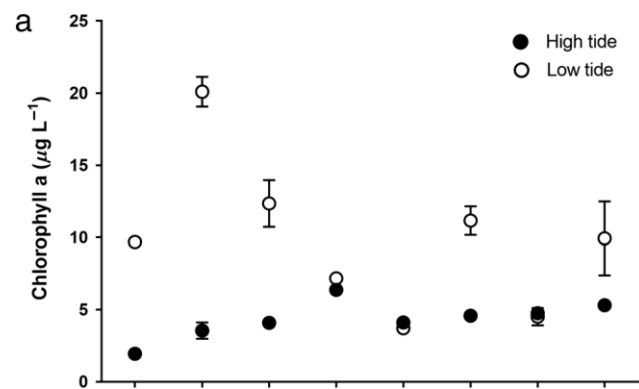
- Lauria, M. L., D. A. Purdie, and J. Sharples. 1999. Contrasting phytoplankton distributions controlled by tidal turbulence in an estuary. *J. Mar. Syst.* 21: 189-197. doi:10.1016/S0924-7963(99)00013-5
- Lawrence, C., and S. Menden-Deuer. 2012. Drivers of protistan grazing pressure: Seasonal signals of plankton community composition and environmental conditions. *Mar. Ecol. Prog. Ser.* 459: 39-52. doi:10.3354/meps09771
- Lehman, P. W. 2000. The influence of climate on phytoplankton community biomass in San Francisco Bay Estuary. *Limnol. Oceanogr.* 45: 580-590. doi:10.4319/lo.2000.45.3.0580
- Lehrter, J. C., J. R. Pennock, and G. B. McManus. 1999. Microzooplankton grazing and nitrogen excretion across a surface estuarine-coastal interface. *Estuaries* 22: 113-125. doi:10.2307/1352932
- Lewitus, A. J., E. T. Koepfler, and J. T. Morris. 1998. Seasonal variation in the regulation of phytoplankton by nitrogen and grazing in a salt-marsh estuary. *Limnol. Oceanogr.* 43: 636-646. doi:10.4319/lo.1998.43.4.0636
- Lucas, L. V., J. R. Koseff, S. G. Monismith, J. E. Cloern, and J. K. Thompson. 1999. Processes governing phytoplankton blooms in estuaries. II. The role of horizontal transport. *Mar. Ecol. Prog. Ser.* 187: 17-30. doi:10.3354/meps187017
- Lu, J., F. Chen, and R. E. Hodson. 2001. Distribution, isolation, host specificity, and diversity of cyanophages infecting marine *Synechococcus* spp. in river estuaries. *Appl. Environ. Microbiol.* 67:3285-3290. doi:10.1128/AEM.67.7.3285-3290.2001
- Mariani, P., K. H. Andersen, A. W. Visser, A. D. Barton, and T. Kiørboe. 2013. Control of plankton seasonal succession by adaptive grazing. *Limnol. Oceanogr.* 58: 173-184. doi:10.4319/lo.2013.58.1.0173
- Menden-Deuer, S., and E. J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.* 45: 569-579. doi:10.4319/lo.2000.45.3.0569
- Montagnes, D. J., and others. 2008. Selective feeding behaviour of key free-living protists: avenues for continued study. *Aquat. Microb. Ecol.* 53: 83-98. doi:10.3354/ame01229
- Morison, F., and S. Menden-Deuer. 2015. Early spring phytoplankton dynamics in the subpolar North Atlantic: The influence of protistan herbivory. *Limnol. Oceanogr.* 60: 1298-1313. doi:10.1002/lno.10099

- Morison, F., and S. Menden-Deuer. 2017. Doing more with less? Balancing sampling resolution and effort in measurements of protistan growth and grazing-rate. *Limnol. Oceanogr.: Methods* 15: 794-809. doi:10.1002/lom3.10200
- Murrell, M. C., and J. T. Hollibaugh. 1998. Microzooplankton grazing in northern San Francisco Bay measured by the dilution method. *Mar. Ecol. Prog. Ser.* 15: 53-63. doi:10.3354/ame015053
- Murrell, M. C., R. S. Stanley, E. M. Lores, G. T. DiDonato, and D. A. Flemer. 2002. Linkage between microzooplankton grazing and phytoplankton growth in a Gulf of Mexico estuary. *Estuaries* 25: 19-29. doi:10.1007/BF02696046
- Ortmann, A. C., R. C. Metzger, J. D. Liefer, and L. Novoveska. 2011. Grazing and viral lysis vary for different components of the microbial community across an estuarine gradient. *Aquat. Microb. Ecol.* 65: 143-157. doi:10.3354/ame01544
- Pan, L. A., J. Zhang, and L. H. Zhang. 2007. Picophytoplankton, nanophytoplankton, heterotrophic bacteria and viruses in the Changjiang Estuary and adjacent coastal waters. *J. Plankton Res.* 29: 187-197. doi:10.1093/plankt/fbm006
- Poulton, N. J. 2016. FlowCam: quantification and classification of phytoplankton by imaging flow cytometry. *Imaging Flow Cytometry: Methods and Protocols* 237-247. doi:10.1007/978-1-4939-3302-0_17
- Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol. Oceanogr.* 53: 886-895. doi:10.4319/lo.2007.52.2.0886
- Ruiz, A., J. Franco, and F. Villate. 1998. Microzooplankton grazing in the Estuary of Mundaka, Spain, and its impact on phytoplankton distribution along the salinity gradient. *Aquat. Microb. Ecol.* 14: 281-288. doi:3354/ame014281
- Schmoker, C., S. Hernandez-Leon, and A. Calbet. 2013. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. *J. Plankton Res.* 35: 691-706. doi:10.1093/plankt/fbt023
- Sherr, E. B., and B. F. Sherr. 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar. Ecol. Prog. Ser.* 352: 187-197. doi:10.3354/meps07161
- Sherr, E. B., and B. F. Sherr. 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. *Aquat. Microb. Ecol.* 57: 253-262. doi:10.3354/ame01358

- Strom, S. L., and M. W. Strom. 1996. Microplankton growth, grazing, and community composition in the northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 130: 229-240. doi:10.3354/meps130229
- Strom, S. L., M. A. Brainard, J. L. Holmes, and M. B. Olson. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. *Mar. Biol.* 138: 355-368. doi:10.1007/s002270000461
- Strom, S. L., and K. Fredrickson. 2008. Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep-Sea Res. Part 2 Top. Stud. Oceanogr.* 55: 1761-1774. doi:10.1016/j.dsr2.2008.04.008
- Strom, S. L., E. L. Harvey, K. A. Fredrickson, and S. Menden-Deuer. 2013. Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashiwo* (Raphidophyceae). *J. Phycol.* 49: 20-31. doi:10.1111/jpy.12013
- Tarran, G. A., M. V. Zubkov, M. V. Sleigh, P. H. Burkill, and M. Yallop. 2001. 3 Microbial community structure and standing stocks in the NE Atlantic in June and 4 July of 1996. *Deep Sea Res. II* 48: 963-985. doi:10.1016/S0967-0645(00)00104-1
- Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. *J. Eukaryot. Microbiol.* 51: 156-168. doi:10.1111/j.1550-7408.2004.tb00540.x
- Trigueros, J. M., and E. Orive. 2000. Tidally driven distribution of phytoplankton blooms in a shallow, macrotidal estuary. *J. Plankton Res.* 22: 969-986. doi:10.1093/plankt/22.5.969
- Tsai, A. Y., G. C. Gong, Y. W. Huang, and C. F. Chao. 2015. Estimates of bacterioplankton and *Synechococcus* spp. mortality from nanoflagellate grazing and viral lysis in the subtropical Danshui River estuary. *Estuar. Coast. Shelf. Sci.* 153: 54-61. doi:10.1016/j.ecss.2014.11.032
- Verity, P. G. 2002a. A decade of change in the Skidaway River Estuary. I. Hydrography and Nutrients. *Estuaries Coast* 25: 944-960. doi:10.1007/BF02691343
- Verity, P. G. 2002b. A decade of change in the Skidaway River estuary II. Particulate organic carbon, nitrogen, and chlorophyll a. *Estuaries Coast* 25: 961-975. doi:10.1007/BF02691343
- Verity, P. G., and D. G. Borkman. 2010. A decade of change in the Skidaway River Estuary. III. Plankton. *Estuaries Coasts* 33: 513-540. doi:10.1007/s12237-009-9208-2

- Wetz, M. S., K. C. Hayes, A. J. Lewitus, J. L. Wolny, and D. L. White. 2006. Variability in phytoplankton pigment biomass and taxonomic composition over tidal cycles in a salt marsh estuary. *Mar. Ecol. Prog. Ser.* 320: 109-120. doi:10.3354/meps320109
- Worden, A., and B. Binder. 2003. Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquat. Microb. Ecol.* 30: 159-174. doi:10.3354/ame030159
- Zubkov, M. V., M. A. Sleigh, G. A. Tarran, P. H. Burkhill, and R. J. G. Leakey. 1998. Picoplanktonic community structure on an Atlantic transect from 50 degrees N to 50 degrees S. *Deep-Sea Res. Pt. I* 45: 1339-1355. doi:10.1016/S0967-0637(98)00015-6

Figure 2.1: Time zero (T0) chlorophyll concentration ($\mu\text{g L}^{-1}$) at high (black) and low (white) tide (**a**). Error bars represent one standard deviation of the mean. Carbon biomass ($\mu\text{g C L}^{-1}$) of flow cytometry (*Synechococcus* spp. = Syn; Nanoeukaryotes = Nano; Picoeukaryotes = Pico) and FlowCam (*Akashiwo sanguinea* = *A. sang*; Diatom; Heterotrophic dinoflagellates = Hdino; Ciliate) groups at high (H) and low (L) tides (**b**). High and low tide biomass are plotted next to each other for each experiment (labeled on Jun 22). Multidimensional scaling (MDS) analysis of total community composition at high (black) and low (white) tide experiments (**c**) using square root transformed carbon biomass from (**a**) and (**b**). Distances between samples on MDS are relative, thus axes are without absolute scale and unlabeled. Stress = 0.09, indicating good ordination between samples.



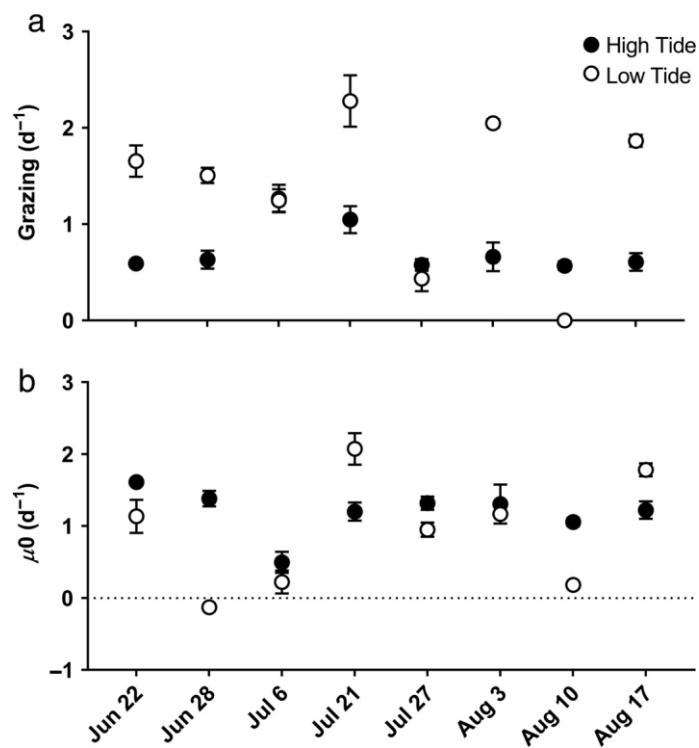


Figure 2.2: Microzooplankton grazing rates (g ; **a**) and instantaneous growth rates (μ_0 ; **b**) for total chlorophyll ($< 200 \mu m$) at high (black) and low (white) tides over the sampling period. Error bars represent one standard deviation from the mean; small error contained within symbol.

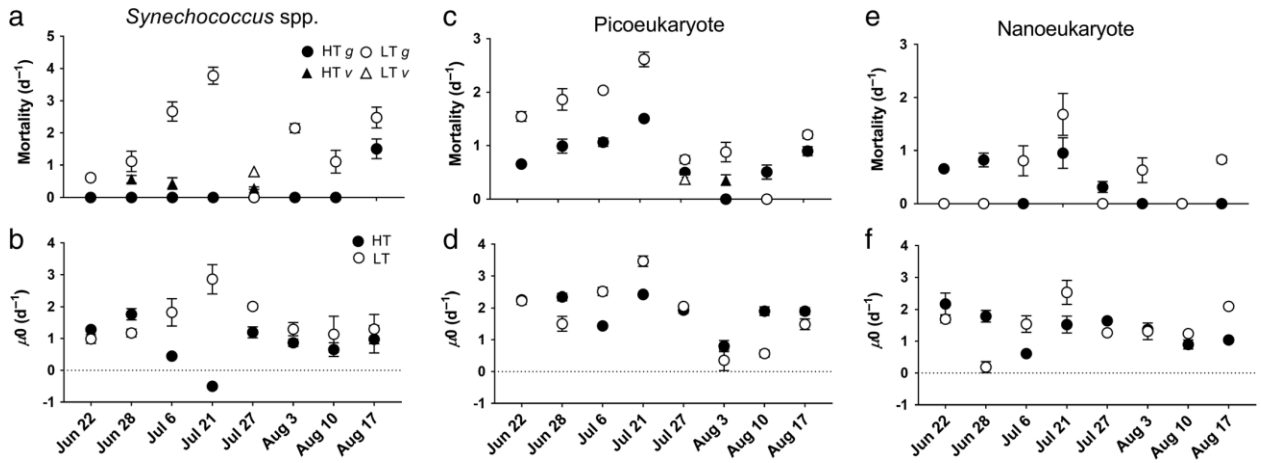


Figure 2.3: Rates of mortality (g = microzooplankton grazing; v = viral lysis) and instantaneous growth (μ_0) for *Synechococcus* spp. (**a**, **b**), picoeukaryotes (**c**, **d**) and nanoeukaryotes (**e**, **f**) at high (HT; black) and low (LT; white) tides over the sampling period. Error bars represent one standard deviation from the mean; small error contained within samples. Viral lysis rates were not detected for nanoeukaryotes.

Table 2.1: Chlorophyll a concentration ($\mu\text{g L}^{-1}$), temperature (T; $^{\circ}\text{C}$), salinity (S), dissolved oxygen (DO; mg L^{-1}), tidal range (TR; meters), and sampling time of day (TOD; eastern standard time) for each low (L) and high tide (H) experiment over the sampling period. Tidal range estimated from NOAA tide predictions (see Methods section). Chlorophyll measured in triplicate (± 1 SD).

Date	Tide	Chl a	T	S	DO	TR	TOD
22 Jun	L	9.69 (0.41)	29.31	28.92	3.75	2.73	13:45
	H	1.94 (0.06)	28.93	31.48	6.19		07:30
28 Jun	L	20.1 (1.03)	28.55	29.59	5.51	2.69	07:05
	H	3.56 (0.57)	28.7	31.85	5.49		13:30
06 Jul	L	12.35 (1.63)	30.64	27.07	9.66	2.02	13:30
	H	4.09 (0.3)	30.01	28.12	9.44		08:03
21 Jul	L	7.18 (0.17)	30.55	27.5	5.94	2.54	12:40
	H	6.38 (0.15)	30.23	30.15	6.22		06:39
27 Jul	L	3.72 (0.18)	29.91	28.9	5.33	2.8	06:10
	H	4.11 (0.19)	29.74	32.14	5.43		12:15
03 Aug	L	11.17 (0.99)	29.25	28.04	8.05	2.09	11:30
	H	4.59 (0.31)	29.13	29.94	7.75		15:13
10 Aug	L	4.51 (0.59)	29.63	27.34	6.66	2.31	05:18
	H	4.76 (0.18)	29.22	29.73	6.5		10:42
17 Aug	L	9.94 (2.56)	31.22	25.45	6.18	2.67	10:24
	H	5.32 (0.16)	31.23	29.28	6.82		16:10

Table 2.2: Daily chlorophyll-based rates of instantaneous growth (μ_0), nutrient-amended growth (μ_n), grazing (g), and net phytoplankton accumulation (r) at low (L) and high tide (H) experiments. Standard deviation (± 1) of triplicate rates shown. Rates which were significantly different ($p < 0.05$) between same-day high and low tides are in bold.

Date	Tide	μ_0	μ_n	g	r
22 Jun	L	1.14 (0.23)	2.09 (0.05)	1.66 (0.16)	-0.52 (0.1)
	H	1.61 (0.03)	2.02 (0.02)	0.59 (0.04)	1.02 (0.1)
28 Jun	L	-0.13 (0.03)	1.01 (0.09)	1.51 (0.08)	-1.63 (0.11)
	H	1.38 (0.11)	2.3 (0.06)	0.63 (0.09)	0.75 (0.03)
06 Jul	L	0.22 (0.16)	1.21 (0.04)	1.25 (0.12)	-1.02 (0.08)
	H	0.5 (0.15)	1.99 (0.04)	1.27 (0.15)	-0.77 (0.04)
21 Jul	L	2.07 (0.22)	2.81 (0.12)	2.28 (0.27)	-0.21 (0.06)
	H	1.2 (0.13)	1.95 (0.06)	1.05 (0.14)	0.15 (0.04)
27 Jul	L	0.95 (0.1)	1.68 (0.21)	0.44 (0.13)	0.59 (0.13)
	H	1.32 (0.09)	1.96 (0.05)	0.58 (0.06)	0.74 (0.06)
03 Aug	L	1.17 (0.03)	2.23 (0.1)	2.05 (0.04)	-0.88 (0.07)
	H	1.31 (0.27)	2.32 (0.03)	0.66 (0.15)	0.64 (0.12)
10 Aug	L	0.18 (0.04)	1.08 (0.16)	0	0.18 (0.04)
	H	1.06 (0.06)	1.76 (0.03)	0.57 (0.04)	0.49 (0.01)
17 Aug	L	1.78 (0.09)	2.42 (0.08)	1.87 (0.06)	-0.09 (0.04)
	H	1.22 (0.12)	2.2 (0.06)	0.61 (0.09)	0.61 (0.03)

CHAPTER 3

SEASONAL VARIABILITY AND DRIVERS OF MICROZOOPLANKTON GRAZING AND PHYTOPLANKTON GROWTH IN A SUBTROPICAL ESTUARY¹

¹Anderson SR, and Harvey EL. 2019. *Frontiers in Marine Science* 6: 174. Reprinted with permission of publisher.

ABSTRACT

Rates of microzooplankton grazing and phytoplankton growth are seldom measured with respect to time, yet such estimates may better reflect temporal variability in coastal phytoplankton communities and offer insight into mechanisms that control populations. To assess seasonal patterns in rates, we performed 41, weekly dilution experiments over a full year in the Skidaway River Estuary (GA), measuring rates of phytoplankton growth, microzooplankton grazing, and viral lysis based on total chlorophyll and group-specific abundances (*Synechococcus* spp., picoeukaryotes, and nanoeukaryotes). Seasonal variability in microzooplankton grazing ($0-2.11\text{ d}^{-1}$) and phytoplankton growth rates ($-0.3-2.43\text{ d}^{-1}$) was observed, with highest values typically recorded in summer and lowest in winter. Grazing pressure was strongest in winter-spring, as phytoplankton accumulation rates were often negative ($-0.16-0.28\text{ d}^{-1}$). Rates varied similarly over seasons for chlorophyll, pico-, and nanoeukaryotes, while rates on *Synechococcus* spp. were rarely significant in dilutions and did not follow seasonal trends. Few experiments (7%) yielded significant rates of viral lysis. While temperature was an important predictor of phytoplankton rates via PLS analysis, temperature exhibited stronger linearity with growth rates ($R^2 = 0.46-0.56$) compared to grazing rates ($R^2 = 0.11-0.27$), which were more likely driven by observed seasonal shifts in plankton community composition (e.g. fall diatom blooms). Establishing temporal rate measurements is critical to identify factors that drive phytoplankton growth and mortality and accurately predict shifts in phytoplankton population dynamics and food web processes within marine systems.

INTRODUCTION

The fate of phytoplankton populations largely depends on the balance between growth and mortality, which can determine the productivity of a marine system, as well as the availability and cycling of carbon and nutrients (Steinberg and Landry, 2017). The bulk of phytoplankton production in the global oceans is consumed daily by microzooplankton (60-70%), which represent a diverse assemblage of 20-200 μm herbivorous grazers (Sherr and Sherr, 2007; Schmoker et al., 2013). Microzooplankton are important in marine food webs, as they can ingest a wide range of autotrophic prey (cyanobacteria to chain-forming diatoms), grow at rates on par with their prey, and contribute to the transfer of organic biomass to higher trophic levels (Sherr et al., 2003; Calbet and Saiz, 2005; Steinberg and Landry, 2017). Despite the well-understood ecological impact of microzooplankton grazing (Calbet and Landry, 2004), limited temporal resolution in grazing rates has made it difficult to identify drivers of grazing pressure. Yet, knowledge of what drives trends in microzooplankton grazing is essential for accurate model predictions of phytoplankton population dynamics (Li et al., 2011) and related shifts in trophic activity and biogeochemical cycling.

Compared to an extensive collection of spatial grazing rate data (> 100 studies from polar to tropical regions; Schmoker et al., 2013), only a handful of studies have consistently measured microzooplankton grazing rates over an annual cycle at monthly (Calbet et al., 2008; Gutiérrez-Rodríguez et al., 2011) to weekly intervals (Kim et al., 2007; Lawrence and Menden-Deuer, 2012). Such efforts have revealed seasonality in microzooplankton grazing (and phytoplankton growth), typically associated with shifts in plankton community composition, though temperature has also emerged as a potential

driver of grazing rates (Rose and Caron, 2007; Calbet et al., 2008; Lawrence and Menden-Deuer, 2012; Zhou et al., 2015). Evidence supports temperature as a reliable predictor of phytoplankton growth (Eppley, 1972; Bissinger et al., 2008), while the relationship between temperature and microzooplankton herbivory remains uncertain. For instance, the average grazing impact at temperatures $< 5^{\circ}\text{C}$ is often lower than the global average (i.e., $< 67\%$; Caron et al., 2000; Calbet et al., 2011), yet grazing rates of 0.5 d^{-1} have been recorded under these conditions. This suggests that microzooplankton can remove primary production even at near-freezing temperatures (Lawrence and Menden-Deuer, 2012; Sherr et al., 2013; Menden-Deuer et al., 2018). Likewise, temperatures $> 15^{\circ}\text{C}$ may not always translate in higher grazing rates, due in part to complex feeding interactions (e.g., grazer-prey selectivity) that may mask temperature effects (Kimmance et al., 2006; Chen et al., 2012). To better address the complexities in what drives microzooplankton grazing, it is therefore necessary to employ temporal sampling strategies and rate measurements that encompass a wide range of environmental conditions.

Given the strong temporal variability in environmental and biological processes in coastal environments, like estuaries, these systems are ideal for examining drivers of microzooplankton grazing (Cloern and Jassby, 2010; Blauw et al., 2012; Lawrence and Menden-Deuer, 2012). Seasonal changes in primary production, phytoplankton biomass, and community composition, including episodic bloom events (e.g., chain-forming diatoms), are commonplace in estuaries (Cloern et al., 2014; Carstensen et al., 2015; Cloern, 2018). Further, the magnitude or duration of a seasonal phytoplankton bloom can depend on shifting abiotic (e.g., temperature and nutrients) or biotic processes (e.g.,

grazing and viral lysis; Cloern et al., 2014; Tsai et al., 2015). Ultimately, temporal patterns in production can have globally relevant consequences for carbon cycling, as estuaries act to mediate land-sea fluxes of dissolved inorganic and organic matter (DIM and DOM; Bittar et al., 2016). Despite the importance of microzooplankton in consuming estuarine production (Calbet and Landry, 2004), trends in grazing, including potential biochemical drivers of grazing pressure, have been poorly resolved over seasonal cycles. Incorporation of microzooplankton grazing rates on these time scales will help to resolve estimates of annual productivity, phytoplankton biomass accumulation, and compositional shifts that occur within coastal areas.

To explore temporal rate dynamics, we performed 41, weekly to bi-weekly dilution experiments over an annual cycle in the Skidaway River Estuary (Georgia, United States), measuring rates of phytoplankton growth, microzooplankton grazing, and viral lysis for bulk chlorophyll and specific phytoplankton groups (*Synechococcus* spp., pico-, and nanoeukaryotes). Recently, these methods were used to measure phytoplankton rates over the tidal cycle in the estuary (see Anderson et al., 2018), though seasonal patterns in rates remain unclear. Previous long-term sampling in the estuary has revealed significant seasonal and interannual variability in many surface-layer properties like temperature, nutrients, dissolved and particulate organic carbon (DOC and POC), chlorophyll, and plankton biomass (Verity, 2002a, 2002b; Verity and Borkman, 2010; Bittar et al., 2016). Such environmental and biological measurements were included in our weekly sampling to establish possible drivers of growth and grazing rates over the year. Determining drivers of population rates, especially microzooplankton grazing, will

allow for more accurate predictions of primary productivity, trophic transfer, and biogeochemical cycling within marine systems and inform future climate scenarios.

METHODS

Water sampling

Over a full year (February 21, 2017 to February 21, 2018), 41 weekly dilution experiments were conducted using surface water (1 m) from the Skidaway River Estuary, which is a well-mixed and highly productive subtropical system accessible by dock at the Skidaway Institute of Oceanography (31°99'N, 81°02'W). Dilutions were always performed at high tide, which allowed for temporal comparison between dates and minimized strong tidal effects previously observed in the estuary (see Anderson et al., 2018). Tide height predictions were taken from a nearby station (NOAA ID: TEC3411); semidiurnal tides in the estuary consistently ranged between 2-3 m and sampling dates rarely coincided with spring or neap tides. Sampling varied due to tide schedule, either occurring at the first high tide in the morning (near or after sunrise) or the subsequent high tide in the afternoon (see Supplementary Table 1). Solar radiation data was collected at a nearby land-based site on Skidaway Island (University of Georgia Weather Network). Surface water temperature, salinity, and dissolved oxygen were measured using a YSI probe (YSI 600 QS).

On each experimental day, single water samples (60 ml) were filtered through a 0.2 μm filter into acid-washed bottles and stored at -20°C for nutrient analysis. Dissolved nutrients (NO_3 , NH_4 , PO_4 , and SiO_4) were measured following standard colorimetric protocols at the UGA Stable Isotope Laboratory using a Technicon AutoAnalyzer unit (SEAL Analytical). Particulate organic carbon (POC) and nitrogen (PON) were also

measured each week by filtering single surface water samples (100 ml) onto pre-combusted 0.7 μm GF/F filters (stored at -20°C). Prior to analysis, filters were dried at 60°C for 24 h and packed into tin cups (Costech); combusted GF/F filters were used as blanks (Bittar et al., 2016). Concentrations of POC and PON were obtained using a Thermo Flash elemental analyzer (Bittar et al., 2016). Samples for nutrients and particulate organic matter were not taken for the first 2-3 weeks of sampling (see Supplementary Table 2).

Modified dilution method

Phytoplankton growth, microzooplankton grazing, and viral-induced mortality rates were measured using a modified dilution technique (Landry and Hassett, 1982; Evans et al., 2003). We employed a two-point modification of the method using whole seawater (WSW) and a diluted fraction of 20% WSW (Worden and Binder, 2003; Landry et al., 2008; Lawrence and Menden-Deuer, 2012). The use of an abbreviated dilution approach has been shown to yield rate estimates that are accurate to a traditional gradient (Worden and Binder, 2003; Strom and Fredrickson, 2008) and reliable to nonlinearity in grazing (Chen, 2015; Morison and Menden-Deuer, 2017).

Diluent preparation was similar across the 41 sampling days. Due to time constraints, grazer-free and virus-free diluents were always prepared a day earlier than the start of each experiment. At high tide, 12-14 l of WSW was collected from the estuary and screened through 200- μm Nitex mesh to limit retention of larger mesozooplankton grazers (e.g., copepods). The entire volume of WSW was then gravity filtered through a 0.45 μm filter (PALL Acropak™ Supor® membrane capsule) which represented grazer-free diluent. Half of the grazer-free diluent (~6 l) was then passed through a tangential

flow filtration unit (TFF; Millipore) with a 30-kilodalton filter size, creating virus-free diluent. Diluent carboys were kept overnight at 18°C in the dark to mitigate bacteria or viral growth (verified by flow cytometry) and were acclimated to an appropriate temperature prior to dilution experiments.

The following day (time zero = T₀), fresh WSW was collected at high tide as described above and combined with grazer- and virus-free diluents at a proportion of 20% WSW. The 20% dilutions and 100% WSW treatments were gently siphoned into triplicate 1.2-l bottles and amended with nutrients (10 μ M NO₃ + 1.1 μ M PO₄; Bittar et al., 2016). Additional triplicate 100% WSW bottles were prepared without nutrients to check for effects of nutrient limitation. On each sampling day, 12 total bottles were incubated for 24 h in a clear, 300-l tank situated on a nearby dock. The tank was maintained at ambient water temperature using flow-through from the estuary. A HOBO data logger and screen mesh were used to replicate *in situ* light intensity. Natural movement of the dock kept the bottles in the tank well-mixed over the incubation period.

Estimation of phytoplankton growth and mortality

Extracted chlorophyll and group-specific phytoplankton abundances (via flow cytometry) were measured at the start of each experiment (T₀) and after 24 h (T₂₄) and used to quantify daily phytoplankton growth and mortality rates. For chlorophyll, triplicate 50-100 ml samples were filtered onto 25 mm 0.7 μ m GF/F filters, extracted in the dark at room temperature for 12–24 h in 91% ethanol, and measured on a Turner AU10 fluorometer (Jespersen and Christoffersen, 1987; Graff and Ryneearson, 2011). Triplicate 5 ml samples were preserved for flow cytometry with 0.5% glutaraldehyde (final concentration), frozen at -80°C, and later analyzed on a Guava flow cytometer

(Millipore) to measure phytoplankton abundances. Three major phytoplankton groups, (*Synechococcus* spp., picoeukaryotes, and nanoeukaryotes) were distinguished based on plots of forward scatter vs. orange or red fluorescence. Apparent growth rates (k ; d^{-1}) were calculated using the equation: $k = 1 / t \times \ln (P_t / P_0)$, where t is the incubation time (d^{-1}) and P_0 and P_t represent initial or final concentrations of chlorophyll or phytoplankton abundance in triplicate bottles.

Given the application of the two-point method with a low dilution level (20% WSW), microzooplankton grazing rates (g ; d^{-1}) were calculated with the equation $g = (k_d - k_1) / (1 - x)$, where k_d and k_1 are the apparent growth in the 20% or 100% WSW treatments and x is the exact factor of dilution (Landry et al., 1984; Morison and Menden-Deuer, 2017). Accordingly, the instantaneous phytoplankton growth rate was calculated as $\mu = g + k_1$ (Landry et al., 2008). Values of μ represent k_1 in bottles without nutrient additions to control for effects of nutrient limitation, though rates were also calculated using k_1 from nutrient-amended bottles (μ_n ; d^{-1}). To assess phytoplankton nutrient limitation, paired t -tests ($p < 0.05$) were performed between μ and μ_n and ratios of μ/μ_n were calculated, with lower ratios indicative of stronger nutrient limitation (Landry et al., 1995; Kim et al., 2007). Net phytoplankton accumulation rate (r ; d^{-1}), which describes the relationship between μ and g , was inferred from the above equations and is equal to the value of k_1 .

For cases where no grazing was measured, which occurred when apparent growth was not significantly different between the two dilution levels (t -test, $p < 0.05$) or when the growth in the 100% WSW was higher (e.g., positive slope), grazing rates were recorded as zero (Strom and Fredrickson, 2008; Anderson et al., 2018; Menden-Deuer et

al., 2018). Negative grazing rates were observed for *Synechococcus* spp. (49% of experiments), but not for total chlorophyll. Insignificant grazing rates that were set to zero were still included in all downstream statistical analysis. When grazing was zero, the accumulation rates were assumed to be equal to μ , unless viral-induced mortality rates (v ; d^{-1}) were measured and accounted for in accumulation. Rates from the virus-free treatment represented total mortality and viral-induced mortality rates were estimated as the difference between total mortality and microzooplankton grazing rates (Evans et al., 2003).

Differences in average phytoplankton rates (μ , g , r , and μ/μ_n) between seasons were examined for all phytoplankton groups using a one-way ANOVA with post-hoc Bonferroni analysis ($p < 0.05$). Seasons corresponded to the astronomical Northern Hemisphere calendar year and began on the following days: winter = December 21; spring = March 20; summer = June 21; fall = September 23. To assess the relative importance of environmental and biological factors in predicting temporal growth and grazing rates, a partial least-squares (PLS) regression was applied using the R package *pls* (Wehrens and Mevik, 2007). Briefly, PLS is a dimensional analysis approach (similar to PCA) that determines the linear relationship between a set of predictor variables (x) and a single response variable (y), and in our case, was chosen to mitigate collinearity of the predictor variables (Godhe and McQuoid, 2003; Eriksson et al., 2006). Two separate models were run using the predictor variables and either chlorophyll-based growth or grazing rates. All variables were log-transformed prior to analysis and thereafter standardized by centering and scaling to unit variance (Eriksson et al., 2006). After initial validation, a 3-component model was used for grazing and growth as the response

variable, which captured 50 and 59% of the variance in the predictor variables. Variable influence on the projection (VIP) was determined for each predictor variable, with VIP values > 1 considered more important to the PLS model (Eriksson et al., 2006). Linear regression analyses (Model 1) were performed using GraphPad Prism 7 software to further quantify relationships between rates and specific predictors found to be important in respective PLS models.

Plankton biomass and composition

The relative abundance and biomass of the 20-200 μm plankton assemblage was measured via an automated FlowCam (Fluid Imaging Technologies, Scarborough, Maine). Samples were not taken on three days (March 16, May 16, and August 30), resulting in biomass data for 38 of 41 experiments. At T0 and T24, 100-200 ml samples of 100% WSW were collected, fixed in buffered formalin (final concentration 1%), and stored at 4°C (Poulton, 2016). Fixed samples were prefiltered through 200 μm mesh and analyzed (duplicate 10 ml runs) on Autoimage mode using a 300- μm flow cell and a flow rate of 2 ml min⁻¹ (Poulton, 2016). Duplicate runs were pooled for each experiment, resulting in ~27,000 images captured per sample on average. Based on previously curated library sets, diatoms and microzooplankton grazers were grouped to finest possible taxonomic resolution (often genus level). Group-specific counts were converted to abundance using the volume analyzed and the efficiency of particle capture (39.7%), which accounts for cells outside the flow cell (Poulton, 2016). Chain-forming diatoms were counted and sized as one cell.

Biovolume was estimated using cellular length and width measurements from the FlowCam, along with the closest geometric formula for diatom and microzooplankton

groups (Hillebrand et al., 1999). Biovolume was converted to carbon biomass ($\mu\text{g C L}^{-1}$) using conversion equations (Menden-Deuer and Lessard, 2000). Abundances of the < 20 μm phytoplankton groups counted via flow cytometry (*Synechococcus* spp., pico-, and nanoeukaryotes) were also converted to carbon biomass using conservative literature conversion factors (see Anderson et al., 2018 and references therein).

To evaluate shifts in community composition over the year, the multivariate statistics package PRIMER Version 7 (Clarke and Gorley, 2015) was used. Initially, carbon biomass from FlowCam (genus level diatoms and microzooplankton) and flow cytometry groups were log-transformed to reduce bias of highly abundant taxa; total chlorophyll concentration was also included. A Bray-Curtis similarity matrix was generated using transformed biomass and nonmetric multidimensional scaling (NMDS) was performed to visualize the relationship between plankton communities on different days throughout the year. To capture week-to-week variability, samples were classified by date, though seasonal shifts in composition were examined using the above-mentioned seasonal delineations (see section “Estimation of Phytoplankton Growth and Mortality”). Significant differences in total composition between seasons was tested using analysis of similarity (ANOSIM), which computes a global R statistic (range from 1 to -1), with values of R that approach 1 indicative of high similarity within and dissimilarity between seasons (Clarke and Warwick, 2001). Similarity of percentages (SIMPER) was also performed to examine how much (percentage) each plankton group contributed to the average sample similarity or dissimilarity between seasons (Clarke and Warwick, 2001).

RESULTS

Environmental conditions in the estuary

Annual variability was observed in surface temperature (6.15-31.23°C), salinity (22.68-31.14), and solar radiation (0.86-22.34 MJ m⁻²), all reaching highest values in late spring and summer (Figure 1A and Supplementary Table 1). Concentrations of dissolved NO₃ (0.01-1.77 µM) and SiO₄ (18.38-139.24 µM) were highest in the summer, while NH₄ (0-4.99 µM) peaked in fall-winter and PO₄ (0.36-0.82 µM) remained stable over the year (Figure 1B and Supplementary Table 2). Measurements of N:P ratio (1.18-10.77; often < 5), dissolved oxygen (4.2-21.67 mg L⁻¹), POC (32.21-154.27 µM C L⁻¹), and PON (5.93-21.08 µM N L⁻¹) fluctuated on weekly scales over the year and did not exhibit seasonal signals (Supplementary Tables 1, 2). Total chlorophyll *a* concentration (< 200 µg L⁻¹) exhibited a narrow range over the year (1.32-6.39 µg L⁻¹), though weekly variability in chlorophyll was strong in spring-summer compared to fall-winter (Figure 1C and Supplementary Table 1).

Phytoplankton and microzooplankton biomass and composition

Biomass estimates for phytoplankton and microzooplankton varied over an annual cycle in the estuary (Figure 2A). Total biomass in winter-spring was often < 400 µg C L⁻¹, while biomass in late summer-fall was marked by high weekly variability and reached a maximum of ~1700 µg C L⁻¹ (Figure 2A). Biomass of combined < 20 µm phytoplankton was stable over the year, ranging from 17.99-126.02 µg C L⁻¹, whereas diatom biomass greatly varied (5.04-697.25 µg C L⁻¹) and the presence of chain-forming taxa (e.g., *Chaetoceros* and *Skeletonema*) accounted for a high proportion of total biomass on days in late summer-fall (Figures 2A,B). Commonly observed

microzooplankton included the mixotrophic dinoflagellate *Akashiwo sanguinea* (1.26-579.76 $\mu\text{g C L}^{-1}$) and heterotrophic dinoflagellates (0-431.2 $\mu\text{g C L}^{-1}$), both of which contributed most to total biomass on days in spring and fall (Figure 2A). Commonly identified diatoms included other chain-forming groups like *Thalassiosira* and *Guinardia*, as well as solitary groups such as *Coscinodiscus*, *Ditylum*, *Nitzschia*, and unidentified benthic diatoms. Heterotrophic dinoflagellates were represented by *Gyrodinium* and *Gymnodinium*-like cells and *Protoperidinium*; identified ciliates consisted of aloricated and loricated types.

Seasonal shifts in plankton composition were revealed via NMDS analysis of biomass estimates (Figure 2C). Significant differences in community composition were identified between all seasons (ANOSIM $R = 0.48$, $p < 0.01$, Supplementary Table 3). However, fall-winter and spring-fall communities were most different from each other ($R = 0.64, 0.61$, $p < 0.01$, Supplementary Table 3), with most dissimilarity due to changes in *Chaetoceros* biomass between seasons (SIMPER 29.66% and 33.47%, Supplementary Table 4). Seasonal shifts in composition were less pronounced between winter-spring and spring-summer, likely due to the importance of smaller scale (e.g., weekly) variability in composition during these transitions (Figure 2C and Supplementary Table 3). Changes in *A. sanguinea* biomass contributed most to the differences in composition between winter-spring (20.09%) and spring-summer (21.75%, Supplementary Table 4).

Phytoplankton rate estimates

Similar annual trends were observed for phytoplankton growth and microzooplankton grazing rates based on bulk chlorophyll ($\mu = -0.3$ -1.61 d^{-1} ; $g = 0$ -2.11 d^{-1} , Figure 3A), picoeukaryotes ($\mu = -0.01$ -2.43 d^{-1} ; $g = 0$ -1.72 d^{-1} , Figure 3B), and

nanoeukaryotes ($\mu = -0.3$ - 2.18 d^{-1} ; $g = 0$ - 1.72 d^{-1} , Figure 3C). Grazing rates were significantly higher than zero for total chlorophyll in 66% experiments (Supplementary Table 5), while significant rates were measured in 61% and 32% of experiments for pico- and nanoeukaryotes, respectively (Supplementary Tables 6, 7). Grazing rates on chlorophyll and picoeukaryotes were similar in spring ($0.64, 0.59 \text{ d}^{-1}$) and summer ($\sim 0.69 \text{ d}^{-1}$; ANOVA, $p > 0.05$), though grazing rates in summer were significantly higher than fall ($0.08, 0.18 \text{ d}^{-1}$) or winter ($0.37, 0.23 \text{ d}^{-1}$; ANOVA, $p < 0.01$, Figures 3A,B). Grazing rates on nanoeukaryotes were significantly different between winter (0.07 d^{-1}) and spring (0.4 d^{-1} ; ANOVA, $p = 0.01$, Figure 3C). Phytoplankton growth rates for all groups were significantly higher in summer (1.22 - 1.78 d^{-1}) relative to any other season (ANOVA, $p < 0.002$, Figures 3A-C.). Viral-induced mortality rates were observed twice over the year for chlorophyll (0.17 and 0.52 d^{-1} , Supplementary Table 5), in three experiments for picoeukaryotes (0.28 - 0.52 d^{-1} , Supplementary Table 6), and in four experiments for nanoeukaryotes (0.13 - 0.96 d^{-1} , Supplementary Table 7). Unlike other phytoplankton groups, grazing rates on *Synechococcus* spp. were rarely significant (12%), and growth rates were mostly negative through the year (Supplementary Figure 1 and Supplementary Table 8). Viral lysis rates of *Synechococcus* spp. were detected in four experiments (0.29 - 0.57 d^{-1} , Supplementary Table 8).

Phytoplankton accumulation rates (μ - g) ranged from -0.99 to 1.61 d^{-1} and exhibited remarkable similarity over an annual cycle for chlorophyll, picoeukaryotes, and nanoeukaryotes (Figures 3D-F and Supplementary Tables 5-7). For all phytoplankton groups, net accumulation was significantly higher in summer-fall (0.48 - 1.09 d^{-1}) compared to winter-spring (-0.16 - 0.28 d^{-1} ; ANOVA, $p < 0.003$), with negative

accumulation common in the spring (Figures 3D-F). Phytoplankton growth rates in nutrient-amended treatments (μ_n) were significantly higher than in non-amended treatments (μ) in 68% of experiments for chlorophyll and in 41% and 32% for pico- and nanoeukaryotes, respectively (*t-test*, $p < 0.05$, Supplementary Tables 5-7). Growth ratios (μ/μ_n) had a similar annual range across all phytoplankton groups (-0.47-1.45; Figure 4), except for three unusually negative or positive ratios (e.g., -31.55) that occurred at random in pico- and nanoeukaryotes. Due to strong weekly variability in spring and winter there were no significant differences in μ/μ_n among seasons for any phytoplankton group (ANOVA, $p > 0.05$, Figure 4). However, μ/μ_n frequently approached a 1:1 ratio in summer-fall, whereas ratios decreased in winter-spring (Figure 4).

Environmental and biological drivers of rates

Partial least-squares regression was used to reveal potential drivers of rates. Among all predictor variables tested, surface water temperature (VIP = 1.75) and dissolved nutrients (VIP = 1.01-1.79) were most important in explaining chlorophyll-based growth rates and were positively correlated to growth (Supplementary Table 9). A significant linear relationship was observed between temperature and growth rates for all phytoplankton groups ($R^2 = 0.46-0.56$, $p < 0.001$, Figure 5A and Supplementary Table 10), which confirmed PLS model predictions. Single linear relationships between dissolved nutrients and growth rates spanned a wide range of variation and were typically weaker compared to temperature ($R^2 = 0.04-0.56$, $p < 0.05$, Supplementary Table 10). PLS analysis with grazing rates were also well-explained by temperature (VIP = 1.2), though additional predictor variables were important such as dissolved nutrients (SiO_4 ,

PO₄, NH₄, VIP = 1.01-1.43), solar radiation (VIP = 1.3) and POC (VIP = 1.12, Supplementary Table 9). Unlike growth rates, temperature was not linearly related to microzooplankton grazing rates across phytoplankton groups ($R^2 = 0.11-0.27$, $p < 0.05$, Figure 5B). Additional predictor variables exhibited poor and often insignificant ($p > 0.05$) linear relationships with microzooplankton grazing rates ($R^2 = 0.01-0.27$, Supplementary Table 11), likely related to estimates of zero grazing in late summer-fall.

DISCUSSION

Microzooplankton are ecologically important in marine food webs, as their grazing activity can elicit shifts in phytoplankton populations (e.g., biomass and diversity) and influence trophic transfer and carbon cycling (Schmoker et al., 2013; Steinberg and Landry, 2017). Few studies have considered changes in grazing rates over time at a fixed location (Schmoker et al., 2013), despite such resolution being relevant in coastal regions which often experience temporal variability in environmental factors (e.g., temperature and nutrients) and phytoplankton populations (Cloern and Jassby, 2010; Blauw et al., 2012; Anderson et al., 2018). Moreover, recurrent estimates of grazing over a range of biochemical conditions may offer valuable insight into mechanisms that control microzooplankton grazing in natural communities (Strom et al., 2007; Chen et al., 2012). Assessing temporal grazing rates will not only help to predict shifts in phytoplankton (and microzooplankton) populations but will also elucidate possible environmental or biological drivers of rates that may benefit population modeling under future climate scenarios (Caron and Hutchins, 2013).

Seasonal variability in rates

Phytoplankton growth and microzooplankton grazing rates varied seasonally in the estuary and were highest in the summer, which is consistent with seasonal trends found in temperate and high latitude waters (Kim et al., 2007; Calbet et al., 2008; Gutiérrez-Rodríguez et al., 2011; Lawrence and Menden-Deuer, 2012). The magnitude of grazing rates presented here ($0\text{--}2.11\text{ d}^{-1}$) are similar to previous estimates from the region (southeast United States; Murrell et al., 2002; Wetz et al., 2006; Sitta et al., 2018) and support the role of microzooplankton as important consumers in subtropical systems. Seasonal differences were also observed in the ratio of growth to grazing, resulting in either negative or positive accumulation of plankton biomass. For instance, microzooplankton grazing exerted more control on winter-spring primary production, as evidenced by low (and often negative) accumulation rates (i.e., $g \geq \mu$). Increased grazing pressure during this time may have been facilitated by poor phytoplankton growth conditions, as temperatures were low and phytoplankton groups were often nutrient-limited ($\mu_n > \mu$; Landry et al., 1995; Chen et al., 2009). Phytoplankton communities were likely limited by NO_3 , as other nutrients (PO_4 and SiO_4) were either stable over the year or in high enough concentrations to support growth (Verity and Borkman, 2010; Bittar et al., 2016). Furthermore, cases of nitrogen limitation are common in the Skidaway River Estuary (Verity, 2002a) and many other southeastern United States estuaries (Howarth and Marino, 2006; Sitta et al., 2018), which supports the nitrate-limiting system we observed. During the summer, environmental conditions improved (e.g., increased temperature, sunlight, and nutrients), which likely contributed to estimates of positive accumulation rates. Seasonal differences in growth and grazing rates observed in our

study emphasize the importance of temporal rate dynamics in predicting plankton biomass accumulation, trophic transfer, and carbon cycling in coastal estuaries.

Despite differences in overall magnitude, trends in phytoplankton rates were remarkably similar between those estimated via bulk chlorophyll concentration and those estimated from abundances of pico- and nanoeukaryotes. The exception was grazing on nanoeukaryotes, which was often zero throughout the year (68%), though annual trends in accumulation mirrored other groups. Interestingly, group-specific growth of and grazing rates on *Synechococcus* spp. did not resemble chlorophyll or other group-specific rate estimates. Grazing on *Synechococcus* spp. has been measured in estuaries (Chen et al., 2009; Tsai et al., 2015), though significant grazing rates were hardly measured over the year in our study. Positive slopes were often detected among diluted and non-diluted treatments for *Synechococcus* spp. (i.e., negative grazing rates), which suggests that specific grazer-prey dilution assumptions were violated or growth conditions (e.g., nutrient regeneration) in the non-diluted fraction were enhanced due to grazing activity (Modigh and Franze, 2009). Similar seasonal patterns in rates between phytoplankton groups indicates there may be overarching environmental factors which universally constrain phytoplankton growth and mortality within natural communities. However, identifying what controls phytoplankton rates has been challenging in the field, especially microzooplankton grazing, which may not only depend on environmental factors like temperature, but also on the composition (or quality) of prey (Rose and Caron, 2007; Lawrence and Menden-Deuer, 2012). Weekly sampling over an annual cycle allowed us to examine possible environmental or biological drivers of phytoplankton growth and grazing within a seasonally dynamic estuary (e.g., broad temperature and nutrient range),

which may inform the response of coastal phytoplankton communities to future climate shifts.

Drivers of observed rates

Grazing activity is driven by complex grazer-prey interactions, which can be guided by grazer selectivity of prey sources (e.g., based on size or nutrition) and result in mismatches between resident grazers and prey (Irigoien et al., 2005; Poulin and Franks, 2010). In these instances, bulk proxies like chlorophyll concentration or total grazer biomass may not accurately reflect microzooplankton grazing rates (Strom et al., 2001; Olson and Strom, 2002; Lawrence and Menden-Deuer, 2012; Menden-Deuer et al., 2018). This was likely the case here, where grazing was poorly explained by prey or grazer biomass. Moreover, our results contradict density-dependent prey ingestion kinetics often seen when culturing single grazer-prey pairings (Holling, 1959; Frost, 1972; Jeong et al., 2010), as evidenced by poor relationships between nano- and picoeukaryote-specific grazing rates and their abundances (data not shown). It may be that in a natural setting, microzooplankton grazing rates are poorly explained by the amount of plankton biomass, because grazer assemblages *in situ* are mixed with species-specific feeding activities and physiological demands. To determine important drivers of growth and grazing rates over the annual cycle, we applied several statistical techniques using collected environmental and biological data (PLS analysis), as well as plankton biomass measurements (multivariate clustering). These approaches highlighted several factors (likely compounding) that were important in driving the observed seasonal differences in phytoplankton rates, including plankton community composition, temperature, and other mortality processes.

Multivariate clustering of group-specific biomass revealed seasonal shifts in plankton community composition over the year, with a winter-spring community of $< 20 \mu\text{m}$ phytoplankton that transitioned to a diatom-dominated community in late summer-fall. While fall diatom blooms have been recorded in the Skidaway River Estuary (Verity and Borkman, 2010), typical seasonality in coastal systems is characterized by winter-spring diatom blooms (Carstensen et al., 2015; Cloern, 2018), which was not seen here. Interestingly, the lowest microzooplankton grazing rates (often zero) were measured in the fall across all phytoplankton groups, which may have been influenced by the transition to weekly diatom blooms (mainly *Chaetoceros* and *Guinardia*). Diatom blooms captured during this time may have been facilitated by upstream or downstream transport of communities that had previously bloomed under more favorable conditions. Though SiO_4 ($> 50 \mu\text{M}$) was not limited, total chlorophyll in the fall was low ($< 3 \mu\text{g L}^{-1}$), which suggests diatom communities were likely stressed by other environmental factors (e.g., low NO_3 and/or sunlight). Diatoms have been shown to release polyunsaturated aldehydes in response to physiological (Ribalet et al., 2007) or mechanical stress (e.g., grazing or lysis; Ribalet et al., 2014), which can inhibit microzooplankton feeding and the growth of other phytoplankton species (Franzè et al., 2018). These inhibitory factors may explain low phytoplankton mortality in the fall compared to other seasons. We cannot rule out the effect of additional factors (e.g., trophic cascades, prey switching, or mixotrophic activity) that may have also contributed to low microzooplankton grazing at this time (Calbet et al., 2008; Strom and Fredrickson, 2008).

Temperature emerged as a strong predictor of phytoplankton growth rates (via PLS) and was strongly related to phytoplankton growth on its own, which supports

extensive theoretical (Eppley, 1972) and empirical evidence of this relationship (Rose and Caron, 2007; Bissinger et al., 2008; Kremer et al., 2017). Along with temperature, dissolved nutrients were important covariables in driving phytoplankton growth rates, though individual nutrient concentrations poorly explained growth rates over the year. Temperature is also thought to affect microzooplankton grazing (Rose and Caron, 2007). Despite temperature being identified as an important predictor of grazing in the PLS analysis, linear relationships between temperature and grazing were poor over a wide temperature gradient in the estuary (6-31°C). Others have noted, based on field measurements, that the relationship between temperature and grazing may not behave linearly (Chen et al., 2012; Lawrence and Menden-Deuer, 2012; Liu et al., 2018) and grazing rates $> 0.5 \text{ d}^{-1}$ are frequently recorded at near-freezing temperatures (Sherr et al., 2013; Menden-Deuer et al., 2018). Often in our study, grazing was zero at higher temperatures (20-31°C), which contradicts expected acceleration of heterotrophic processes (e.g., ingestion and respiration) with temperature (Brown et al., 2004; Chen et al., 2012). Rose and Caron (2007) predicted higher capacity for microzooplankton growth compared to phytoplankton growth rates at temperatures $> 15^\circ\text{C}$, which has been confirmed in field-based temperature incubations with natural assemblages (Chen and Laws, 2017; Liu et al., 2018). It is important to note these experiments exposed the same community to rapid shifts in temperature, whereas our study measured fluctuations in plankton composition over a wide temperature gradient that likely masked temperature sensitivity of grazing. Though other environmental factors (e.g., nutrients, sunlight, and POC) were relevant to grazing rates in our study, these relationships were not supported by single linear regressions. The lack of temperature effects (or any other parameter) on

grazing we observed indicates the importance of community composition and complex grazer-prey interactions in driving rates within marine environments.

Additional loss processes were likely important in controlling primary production in the estuary, as average accumulation rates over the year remained positive (0.28-0.58 d⁻¹). Viral lysis represents an important loss process for phytoplankton in coastal ecosystems, particularly during periods of high, monospecific prey biomass (e.g., phytoplankton blooms; Tsai et al., 2015). Nevertheless, in our dilution experiments, viral lysis was rarely measured over an annual cycle among phytoplankton groups, which may be attributed to infection dynamics. For instance, viruses are known to exert host specificity, and so it is possible the viral community present during our dilutions was not specific or in dense enough concentrations to affect population rates (Kimmance et al., 2007). The incubation period used in our dilutions may have been too short to capture viral infection, as often the viral lytic cycle can exceed 24 h (Evans et al., 2003; Jacquet et al., 2005). Lastly, recent meta-analysis revealed that over half of viral-induced mortality rates measured via the modified dilution approach were minimal (-0.1-0.1 d⁻¹), which suggests that typical dilution assays may lack the sensitivity needed to detect viral mortality (Staniewski and Short, 2018). Other sources may have contributed to phytoplankton mortality in the estuary, such as consumption by mesozooplankton or benthic invertebrates (e.g., bivalves), though both processes were not measurable via dilution incubations and typically account for less consumption of primary production compared to microzooplankton (Cloern et al., 2014; Steinberg and Landry, 2017).

Concluding remarks

Our findings of seasonal variability in growth and grazing contributes to the growing knowledge on the importance of time in structuring phytoplankton rate dynamics, particularly in complex coastal regions (Lawrence and Menden-Deuer, 2012; Anderson et al., 2018). Over seasonal scales in the Skidaway River Estuary, phytoplankton growth was universally controlled by temperature and to a lesser extent by concentrations of major dissolved nutrients. Though several environmental factors were important in predicting grazing rates, there was no clear linear relationship between temperature (or any other single factor) and microzooplankton grazing. While temperature may affect grazing under controlled conditions (e.g., single grazer-prey; Rose and Caron, 2007), we demonstrate that temperature fails to explain temporal variability in grazing over wide biochemical gradients in the field. Such empirical evidence supports modeling efforts that have found it difficult to predict microzooplankton grazing rates (and accumulation rates) based solely on remotely sensed variables like temperature and chlorophyll (Chen et al., 2013, 2012; Schmoker et al., 2013). Poor correlations between grazing rates and any single factor is likely related to the fact that grazing is heavily dependent on plankton community composition, a factor not easily incorporated into model scenarios. Certainly, additional temporal rate studies, which include measures of community composition, will help to reveal biological and environmental drivers of grazing and inform future phytoplankton population models (Li et al., 2011; Caron and Hutchins, 2013). Ultimately, our ability to accurately predict the fate of primary production and of related food web processes will rely on continued

measurements of microzooplankton grazing and phytoplankton growth rates over time and across marine ecosystems.

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REFERENCES

- Anderson, S.R., Diou-cass, Q.P., Harvey, E.L., 2018. Short-term estimates of phytoplankton growth and mortality in a tidal estuary. *Limnol. Oceanogr.* 63, 2411–2422. <https://doi.org/10.1002/lno.10948>
- Bissinger, J.E., Montagnes, D.J.S., Sharples, J., Atkinson, D., 2008. Predicting marine phytoplankton maximum growth rates from temperature : Improving on the Eppley curve using quantile regression. *Limnol. Oceanogr.* 53, 487–493. <https://doi.org/10.4319/lo.2008.53.2.0487>
- Bittar, T.B., Berger, S.A., Birsa, L.M., Walters, T.L., Thompson, M.E., Spencer, R.G.M., Mann, E.L., Stubbins, A., Frischer, M.E., Brandes, J.A., 2016. Estuarine, Coastal and Shelf Science Seasonal dynamics of dissolved, particulate and microbial components of a tidal saltmarsh-dominated estuary under contrasting levels of freshwater discharge. *Estuar. Coast. Shelf Sci.* 182, 72–85. <https://doi.org/10.1016/j.ecss.2016.08.046>
- Blauw, A.N., Beninca, E., Laane, R.W.P.M., Greenwood, N., Huisman, J., 2012. Dancing with the Tides: Fluctuations of Coastal Phytoplankton Orchestrated by Different Oscillatory Modes of the Tidal Cycle. *PLoS One* 7, e49319. <https://doi.org/10.1371/journal.pone.0049319>
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward A Metabolic Theory of Ecology. *Ecology* 85, 1771–1789. <https://doi.org/10.1890/03-9000>
- Calbet, A., Landry, M.R., 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.* 49, 51–57. <https://doi.org/10.4319/lo.2004.49.1.0051>
- Calbet, A., Riisgaard, K., Saiz, E., Zamora, S., Stedmon, C., Nielsen, T.G., 2011. Phytoplankton growth and microzooplankton grazing along a sub-Arctic fjord (Godthåbsfjord , west Greenland). *Mar. Ecol. Prog. Ser.* 442, 11–22. <https://doi.org/10.3354/meps09343>
- Calbet, A., Saiz, E., 2005. The ciliate-copepod link in marine ecosystems. *Aquat. Microb. Ecol.* 38, 157–167. <https://doi.org/10.3354/ame038157>
- Calbet, A., Trepát, I., Almeda, R., Salo, V., Saiz, E., Movilla, J.I., Alcaraz, M., Yebra, L., Simo, R., 2008. Impact of micro- and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments. *Aquat. Microb. Ecol.* 50, 145–156. <https://doi.org/10.3354/ame01171>
- Caron, D.A., Dennett, M.R., Lonsdale, D.J., Moran, D.M., Shalapyonok, L., 2000. Microzooplankton herbivory in the Ross Sea, Antarctica 47, 3249–3272. [https://doi.org/10.1016/S0967-0645\(00\)00067-9](https://doi.org/10.1016/S0967-0645(00)00067-9)

- Caron, D.A., Hutchins, D.A., 2013. The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J. Plankton Res.* 35, 235–252. <https://doi.org/10.1093/plankt/fbs091>
- Carstensen, J., Klais, R., Cloern, J.E., 2015. Phytoplankton blooms in estuarine and coastal waters: Seasonal patterns and key species. *Estuar. Coast. Shelf Sci.* 162, 98–109. <https://doi.org/10.1016/j.ecss.2015.05.005>
- Chen, B., 2015. Assessing the accuracy of the “two-point” dilution technique. *Limnol. Oceanogr. Methods* 13, 521–526. <https://doi.org/10.1002/lom3.10044>
- Chen, B., Landry, M.R., Huang, B., Liu, H., 2012. Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? *Limnol. Oceanogr.* 57, 519–526. <https://doi.org/10.4319/lo.2012.57.2.0519>
- Chen, B., Laws, E.A., 2017. Is there a difference of temperature sensitivity between marine phytoplankton and heterotrophs ? *Limnol. Oceanogr.* 62, 806–817. <https://doi.org/10.1002/lno.10462>
- Chen, B., Liu, H., Landry, M.R., Chen, M., Sun, J., Shek, L., Chen, X., Harrison, P.J., 2009. Estuarine nutrient loading affects phytoplankton growth and microzooplankton grazing at two contrasting sites in Hong Kong coastal waters. *Mar. Ecol. Prog. Ser.* 379, 77–90. <https://doi.org/10.3354/meps07888>
- Chen, B., Zheng, L., Huang, B., Song, S., Liu, H., 2013. Seasonal and spatial comparisons of phytoplankton growth and mortality rates due to microzooplankton grazing in the northern South China Sea. *Biogeosciences* 10, 2775–2785. <https://doi.org/10.5194/bg-10-2775-2013>
- Clarke, K.R., Gorley, R.N., 2015. Getting started with PRIMER v7 PRIMER-E:
- Clarke, K.R., Warwick, R.M., 2001. A further biodiversity index applicable to species lists : variation in taxonomic distinctness. *Mar. Ecol. Prog. Ser.* 216, 265–278. <https://doi.org/10.3354/meps216265>
- Cloern, J.E., 2018. Why large cells dominate estuarine phytoplankton. *Limnol. Oceanogr.* 63, 392–409. <https://doi.org/10.1002/lno.10749>
- Cloern, J.E., Foster, S.Q., Kleckner, A.E., 2014. Phytoplankton primary production in the world’s estuarine-coastal ecosystems. *Biogeosciences* 11, 2477–2501. <https://doi.org/10.5194/bg-11-2477-2014>
- Cloern, J.E., Jassby, A.D., 2010. Patterns and Scales of Phytoplankton Variability in Estuarine – Coastal Ecosystems. *Estuaries and Coasts* 33, 230–241. <https://doi.org/10.1007/s12237-009-9195-3>
- Eppley, R.W., 1972. Temperature and Phytoplankton Growth in the Sea. *Fish. Bull.* 70, 1063–1085.

- Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wilkström, C., Wold, S., 2006. Multi- and Megavariable Data Analysis: Part I: Basic Principles and Applications. Umea: Umetrics AB.
- Evans, C., Archer, S.D., Jacquet, S., Wilson, W.H., 2003. Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population. *Aquat. Microb. Ecol.* 30, 207–219. <https://doi.org/10.3354/ame030207>
- Franzè, G., Pierson, J.J., Stoecker, D.K., Lavrentyev, P.J., 2018. Diatom-produced allelochemicals trigger trophic cascades in the planktonic food web. *Limnol. Oceanogr.* 63, 1093–1108. <https://doi.org/10.1002/lno.10756>
- Frost, B.W., 1972. Effects of Size and Concentration of Food Particles on the Feeding Behavior of the Marine Planktonic Copepod *Calanus Pacificus*. *Limnol. Oceanogr.* 17, 805–815.
- Godhe, A., McQuoid, M.R., 2003. Influence of benthic and pelagic environmental factors on the distribution of dinoflagellate cysts in surface sediments along the Swedish west coast. *Aquat. Microb. Ecol.* 32, 185–201. <https://doi.org/10.3354/ame032185>
- Graff, J.R., Ryneerson, T.A., 2011. Extraction method influences the recovery of phytoplankton pigments from natural assemblages. *Limnol. Oceanogr. Methods* 9, 129–139. <https://doi.org/10.4319/lom.2011.9.129>
- Gutiérrez-Rodríguez, A., Latasa, M., Scharek, R., Massana, R., Vila, G., Gasol, J.M., 2011. Growth and grazing rate dynamics of major phytoplankton groups in an oligotrophic coastal site. *Estuar. Coast. Shelf Sci.* 95, 77–87. <https://doi.org/10.1016/j.ecss.2011.08.008>
- Hillebrand, H., Durselen, C.-D., Kirschtel, D., Pollinger, U., Zohary, T., 1999. BIOVOLUME CALCULATION FOR PELAGIC AND BENTHIC MICROALGAE. *J. Phycol.* 35, 403–424. <https://doi.org/10.1046/j.1529-8817.1999.3520403.x>
- Holling, C.S., 1959. Some Characteristics of Simple Types of Predation and Parasitism. *Can. Entomol.* 91, 385–398.
- Howarth, R.W., Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems : Evolving views over three decades. *Limnol. Oceanogr.* 51, 364–376. https://doi.org/10.4319/lo.2006.51.1_part_2.0364
- Irigoin, X., Flynn, K.J., Harris, R.P., 2005. Phytoplankton blooms : a ‘ loophole ’ in microzooplankton grazing impact ? *J. Plankton Res.* 27, 313–321. <https://doi.org/10.1093/plankt/fbi011>
- Jacquet, S., Domaizon, I., Personnic, S., 2005. Estimates of protozoan- and viral-

- mediated mortality of bacterioplankton in Lake Bourget (France). *Freshw. Biol.* 50, 627–645. <https://doi.org/10.1111/j.1365-2427.2005.01349.x>
- Jeong, H.J., Yoo, Y. Du, Kim, J.S., Seong, K.A., Kang, N.S., Kim, T.H., 2010. Growth, Feeding and Ecological Roles of the Mixotrophic and Heterotrophic Dinoflagellates in Marine Planktonic Food Webs. *Ocean Sci. J.* 45, 65–91. <https://doi.org/10.1007/s12601-010-0007-2>
- Kim, S., Gil, M., Moon, C., Shin, K., Chang, M., 2007. Seasonal variations in phytoplankton growth and microzooplankton grazing in a temperate coastal embayment, Korea. *Estuar. Coast. Shelf Sci.* 71, 159–169. <https://doi.org/10.1016/j.ecss.2006.07.011>
- Kimmance, S.A., Atkinson, D., Montagnes, D.J.S., 2006. Do temperature – food interactions matter? Responses of production and its components in the model heterotrophic flagellate *Oxyrrhis marina*. *Aquat. Microb. Ecol.* 42, 63–73. <https://doi.org/10.3354/ame042063>
- Kimmance, S.A., Wilson, W.H., Archer, S.D., 2007. Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: Limitations associated with method sensitivity in natural waters. *Aquat. Microb. Ecol.* 49, 207–222. <https://doi.org/10.3354/ame01136>
- Kremer, C.T., Thomas, M.K., Litchman, E., 2017. Temperature- and size-scaling of phytoplankton population growth rates : Reconciling the Eppley curve and the metabolic theory of ecology. *Limnol. Oceanogr.* 62, 1658–1670. <https://doi.org/10.1002/lno.10523>
- Landry, M.R., Brown, S.L., Rii, Y.M., Selph, K.E., Bidigare, R.R., Yang, E.J., Simmons, M.P., 2008. Depth-stratified phytoplankton dynamics in Cyclone Opal, a subtropical mesoscale eddy. *Deep. Res. Part II* 55, 1348–1359. <https://doi.org/10.1016/j.dsr2.2008.02.001>
- Landry, M.R., Haas, L.W., Fagerness, V.L., 1984. Dynamics of microbial plankton communities : experiments in Kaneohe Bay , Hawaii. *Mar. Ecol. Prog. Ser.* 16, 127–133.
- Landry, M.R., Hassett, R.P., 1982. Estimating the grazing impact of marine micro-zooplankton. *Mar. Biol.* 67, 283–288. <https://doi.org/10.1007/BF00397668>
- Landry, M.R., Kirshtein, J., Constantinou, J., 1995. A refined dilution technique for measuring the community grazing impact of microzooplankton , with experimental tests in the central equatorial Pacific. *Mar. Ecol. Prog. Ser.* 120, 53–63. <https://doi.org/10.3354/meps120053>
- Lawrence, C., Menden-Deuer, S., 2012. Drivers of protistan grazing pressure: seasonal signals of plankton community composition and environmental conditions. *Mar. Ecol. Prog. Ser.* 459, 39–52. <https://doi.org/10.3354/meps09771>

- Li, Q.P., Franks, P.J.S., Landry, M.R., 2011. Microzooplankton grazing dynamics : parameterizing grazing models with dilution experiment data from the California Current Ecosystem. *Mar. Ecol. Prog. Ser.* 438, 59–69. <https://doi.org/10.3354/meps09320>
- Liu, K., Chen, B., Zhang, S., Sato, M., Shi, Z., Liu, H., 2018. Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton. *Limnol. Oceanogr.* 1–17. <https://doi.org/10.1002/lno.11101>
- Menden-Deuer, S., Lawrence, C., Franzè, G., 2018. Herbivorous protist growth and grazing rates at in situ and artificially elevated temperatures during an Arctic phytoplankton spring bloom. *PeerJ* 6, e5264. <https://doi.org/10.7717/peerj.5264>
- Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.* 45, 569–579. <https://doi.org/10.4319/lo.2000.45.3.0569>
- Mevik, B.-H., Wehrens, R. 2007. The pls package: principal component and partial least squares regression in R. *J. Stat. Softw.* 18. Available online at: http://econpapers.repec.org/article/jssjstsof/18_3Ai02.htm
- Modigh, M., Franzè, G., 2009. Changes in phytoplankton and microzooplankton populations during grazing experiments at a Mediterranean coastal site. *J. Plankton Res.* 31, 853–864. <https://doi.org/10.1093/plankt/fbp035>
- Morison, F., Menden-Deuer, S., 2017. Doing more with less ? Balancing sampling resolution and effort in measurements of protistan growth and grazing-rates. *Limnol. Oceanogr. Methods* 15, 794–809. <https://doi.org/10.1002/lom3.10200>
- Murrell, M., 2002. Linkage Between Microzooplankton Grazing and Phytoplankton Growth in a Gulf of Mexico Estuary. *Estuaries* 25, 19–29.
- Olson, M.B., Strom, S.L., 2002. Phytoplankton growth , microzooplankton herbivory and community structure in the southeast Bering Sea : insight into the formation and temporal persistence of an *Emiliania huxleyi* bloom. *Deep. Res. Part II* 49, 5969–5990. [https://doi.org/10.1016/S0967-0645\(02\)00329-6](https://doi.org/10.1016/S0967-0645(02)00329-6)
- Poulin, F.J., Franks, P.J.S., 2010. Size-structured planktonic ecosystems : constraints , controls and assembly instructions. *J. Plankton Res.* 32, 1121–1130. <https://doi.org/10.1093/plankt/fbp145>
- Poulton, N., 2016. FlowCam: quantification and classification of phytoplankton by imaging flow cytometry. In *Imaging Flow Cytometry*.
- Ribalet, F., Bastianini, M., Vidoudez, C., Acri, F., Berges, J., Ianora, A., Miralto, A., Pohnert, G., Romano, G., Wichard, T., Casotti, R., 2014. Phytoplankton Cell Lysis Associated with Polyunsaturated Aldehyde Release in the Northern Adriatic Sea Franc. *PLoS One* 9, e85947. <https://doi.org/10.1371/journal.pone.0085947>

- Ribalet, F., Wichard, T., Pohnert, G., Ianora, A., Miralto, A., Casotti, R., 2007. Age and nutrient limitation enhance polyunsaturated aldehyde production in marine diatoms. *Franc. Phytochemistry* 68, 2059–2067. <https://doi.org/10.1016/j.phytochem.2007.05.012>
- Rose, J.M., Caron, D.A., 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol. Oceanogr.* 52, 886–895. <https://doi.org/10.4319/lo.2007.52.2.0886>
- Schmoker, C., Hernández-León, S., Calbet, A., 2013. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. *J. Plankton Res.* 35, 691–706. <https://doi.org/10.1093/plankt/fbt023>
- Sherr, E.B., Sherr, B.F., 2007. Heterotrophic dinoflagellates : a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar. Ecol. Prog. Ser.* 352, 187–197. <https://doi.org/10.3354/meps07161>
- Sherr, E.B., Sherr, B.F., Ross, C., 2013. Microzooplankton grazing impact in the Bering Sea during spring sea ice conditions. *Deep. Res. Part II* 94, 57–67. <https://doi.org/10.1016/j.dsr2.2013.03.019>
- Sherr, E.B., Sherr, B.F., Wheeler, P.A., Thompson, K., 2003. Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. *Deep. Res. Part I* 50, 557–571. [https://doi.org/10.1016/S0967-0637\(03\)00031-1](https://doi.org/10.1016/S0967-0637(03)00031-1)
- Sitta, K.A., Reed, M., Mortensen, R., Doll, C., Callahan, T., Green, D.I., 2018. The influences of nitrogen form and zooplankton grazing on phytoplankton assemblages in two coastal southeastern systems. *Limnol. Oceanogr.* 63, 2523–2544. <https://doi.org/10.1002/lno.10957>
- Staniewski, M.A., Short, S.M., 2018. Methodological review and meta-analysis of dilution assays for estimates of virus- and grazer-mediated phytoplankton mortality. *Limnol. Oceanogr. Methods* 649–668. <https://doi.org/10.1002/lom3.10273>
- Steinberg, D.K., Landry, M.R., 2017. Zooplankton and the Ocean Carbon Cycle. *Ann. Rev. Mar. Sci.* 9, 413–444. <https://doi.org/10.1146/annurev-marine-010814-015924>
- Strom, S.L., Brainard, M.A., Holmes, J.L., Olson, M.B., 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. *Mar. Biol.* 138, 355–368. <https://doi.org/10.1007/s002270000461>
- Strom, S.L., Fredrickson, K.A., 2008. Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep. Res. Part II* 55, 1761–1774. <https://doi.org/10.1016/j.dsr2.2008.04.008>
- Strom, S.L., Macri, E.L., Olson, M.B., 2007. Microzooplankton grazing in the coastal Gulf of Alaska: Variations in top-down control of phytoplankton. *Limnol.*

- Oceanogr. 52, 1480–1494. <https://doi.org/10.4319/lo.2007.52.4.1480>
- Tsai, A.-Y., Gong, G.-C., Huang, Y.W., Chao, C.F., 2015. Estimates of bacterioplankton and *Synechococcus* spp. mortality from nanoflagellate grazing and viral lysis in the subtropical Danshui River estuary. *Estuar. Coast. Shelf Sci.* 153, 54–61. <https://doi.org/10.1016/j.ecss.2014.11.032>
- Verity, P.G., 2002a. A Decade of Change in the Skidaway River Estuary. I. Hydrography and Nutrients. *Estuaries* 25, 944–960. <https://doi.org/10.1007/BF02691343>
- Verity, P.G., 2002b. A Decade of Change in the Skidaway River Estuary. II. Particulate Organic Carbon, Nitrogen, and Chlorophyll a. *Estuaries* 25, 961–975. <https://doi.org/10.1007/BF02691344>
- Verity, P.G., Borkman, D.G., 2010. A Decade of Change in the Skidaway River Estuary. III. Plankton. *Estuaries and Coasts* 33, 513–540. <https://doi.org/10.1007/s12237-009-9208-2>
- Wetz, M.S., Hayes, K.C., Lewitus, A.J., Wolny, J.L., White, D.L., 2006. Variability in phytoplankton pigment biomass and taxonomic composition over tidal cycles in a salt marsh estuary. *Mar. Ecol. Prog. Ser.* 320, 109–120. <https://doi.org/10.3354/meps320109>
- Worden, A.Z., Binder, B.J., 2003. Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquat. Microb. Ecol.* 30, 159–174. <https://doi.org/10.3354/ame030159>
- Zhou, L., Tan, Y., Huang, L., Hu, Z., Ke, Z., 2015. Seasonal and size-dependent variations in the phytoplankton growth and microzooplankton grazing in the southern South China Sea under the influence of the East Asian monsoon. *Biogeosciences* 12, 6809–6822. <https://doi.org/10.5194/bg-12-6809-2015>

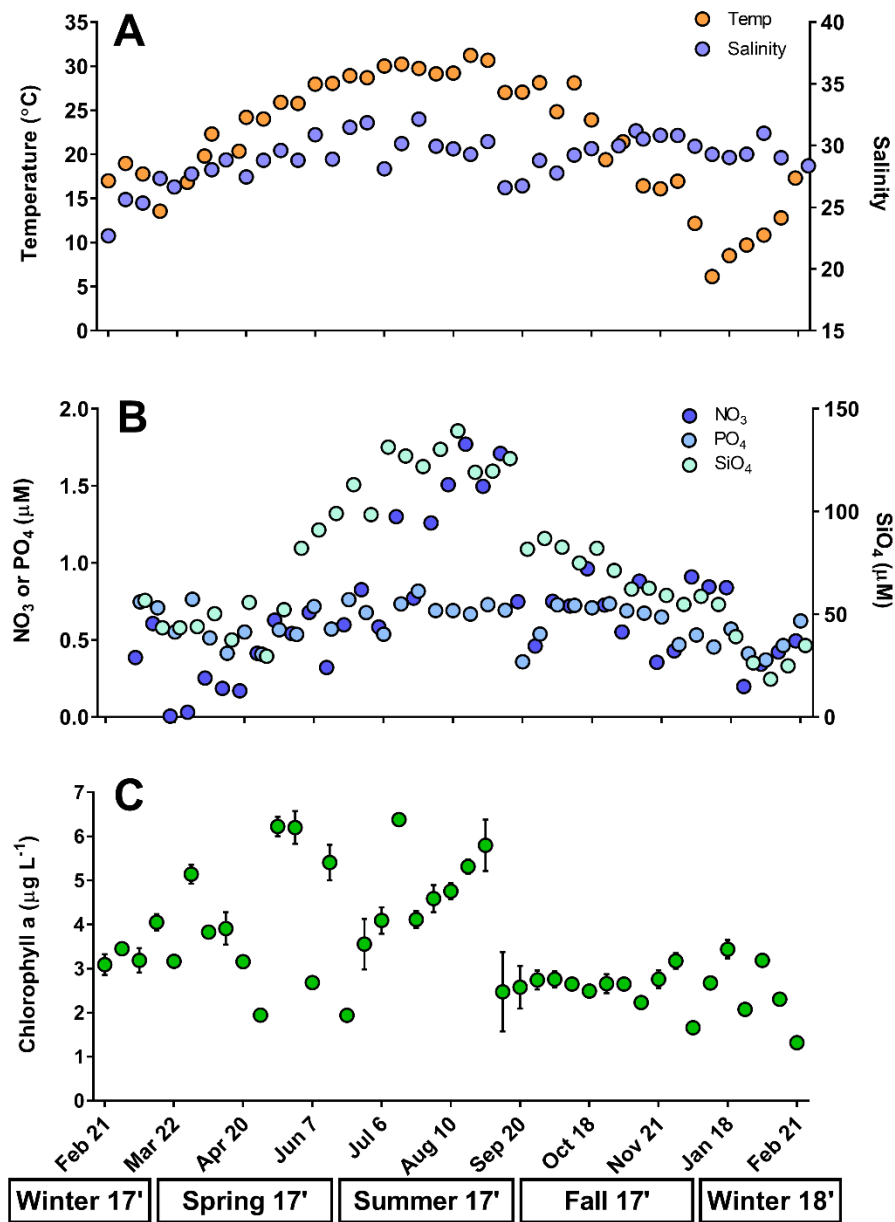
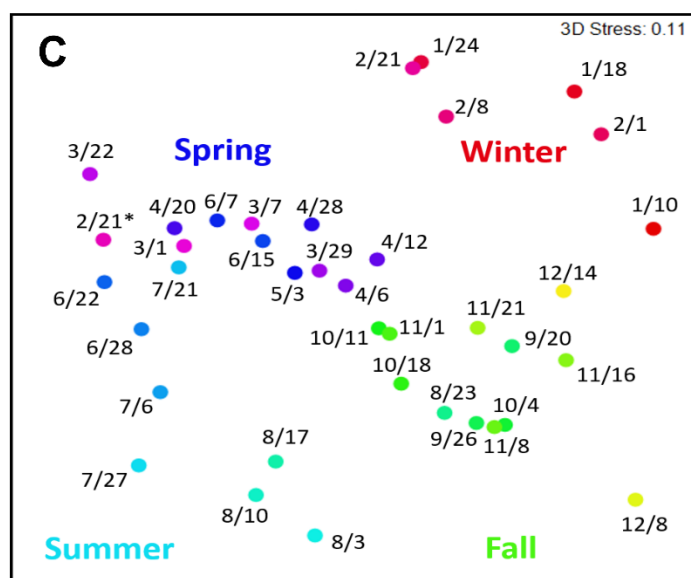
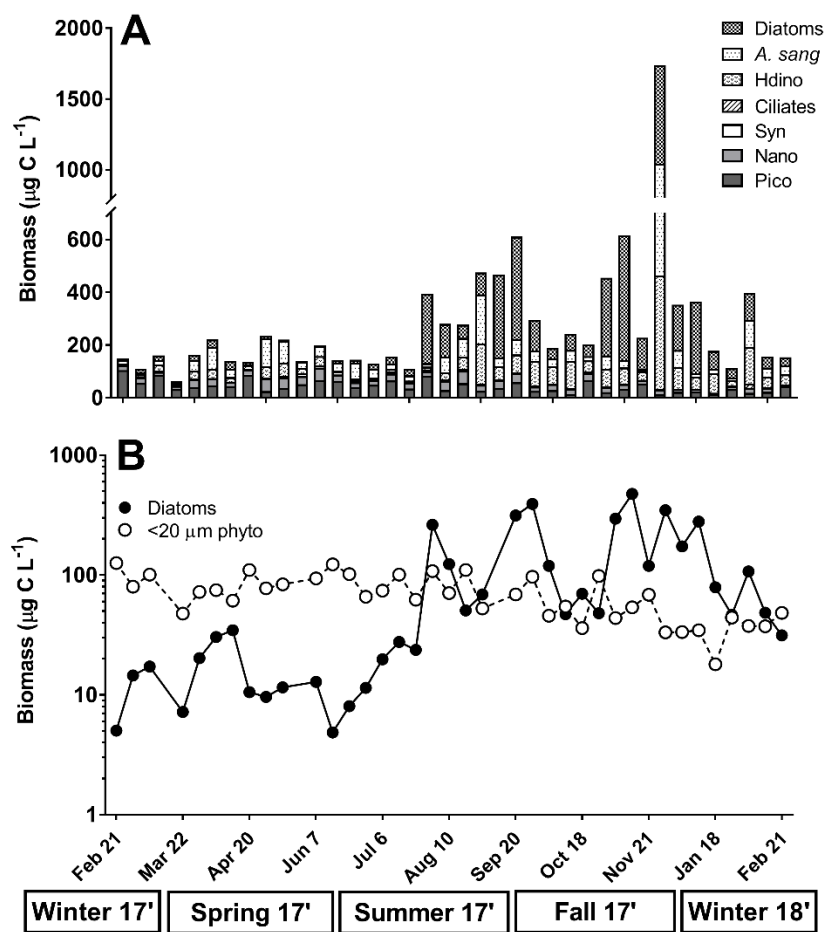


Figure 3.1: Surface water temperature and salinity (**A**), dissolved inorganic nutrients (μM ; nitrate, NO_3 ; phosphate, PO_4 ; silicate, SiO_4 ; **B**), and chlorophyll a ($\mu\text{g L}^{-1}$; **C**) at time zero (T0) of each weekly experiment. Error associated with chlorophyll represent ± 1 standard deviation of the triplicate sample mean. Temperature, salinity, and nutrients represent single replicates. Seasons are below x-axis.

Figure 3.2: (A) Carbon biomass ($\mu\text{g C L}^{-1}$) of plankton groups measured via FlowCam [diatoms; *A. sanguinea* (*A. sang*); heterotrophic dinoflagellates (Hdino); ciliates] and flow cytometry [*Synechococcus* spp. (Syn); Nanoeukaryotes (Nano); Picoeukaryotes (Pico)] at the start of each weekly experiment. (B) Log-transformed biomass of $< 20 \mu\text{m}$ combined phytoplankton (Syn, Nano, Pico; open symbols) versus diatoms (closed symbols). (C) Non-multidimensional scaling (NMDS) of total plankton composition on each sampling day (month/day) using log-transformed carbon biomass from (A). A color gradient based on day of year (red = winter; purple = spring; blue = summer; green = fall) was applied to observe weekly and seasonal differences in composition. Asterisk above 2/21 indicates first sampling day in 2017. Seasons are listed below the x-axis.



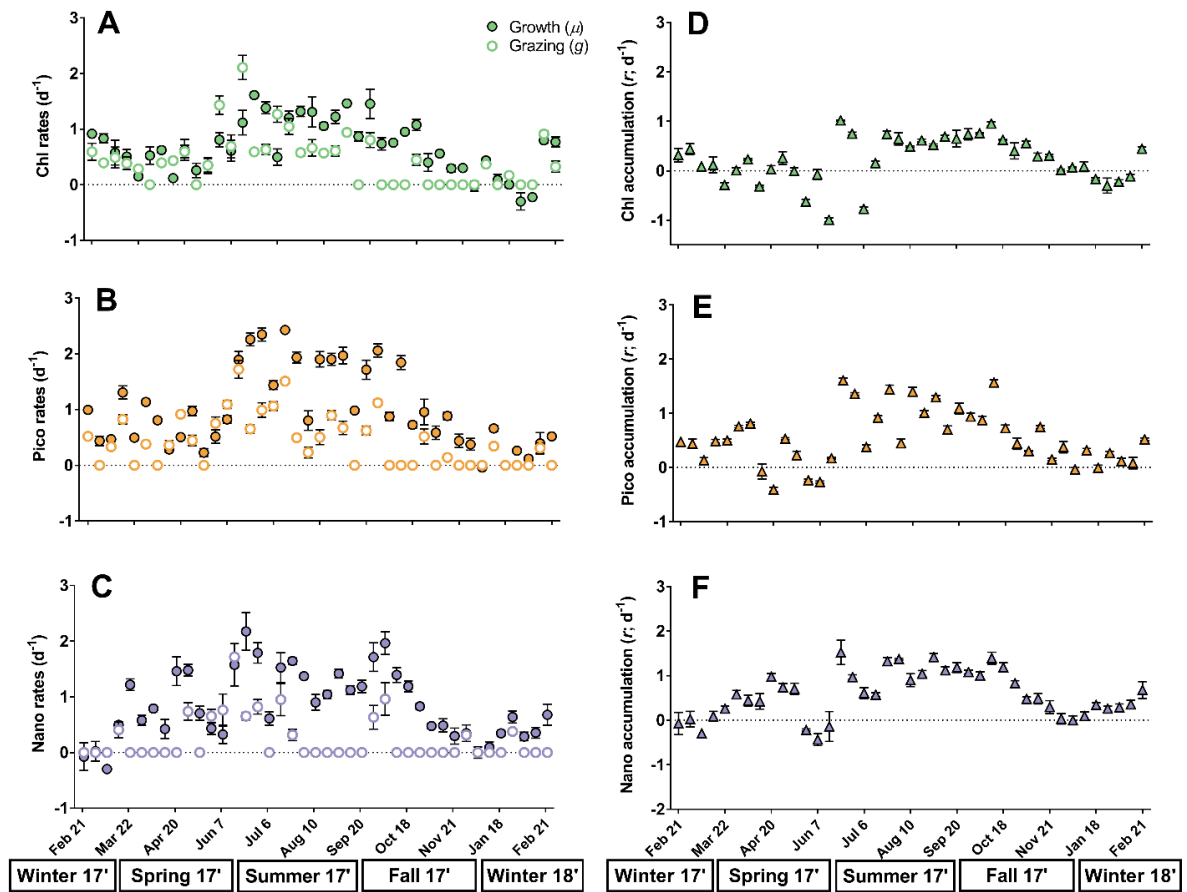


Figure 3.3: Rates (d^{-1}) of instantaneous phytoplankton growth (μ ; open circles), microzooplankton grazing (g ; closed circles), and net accumulation (r ; closed triangles) measured each week based on total chlorophyll (A, D; green), picoeukaryotes (B, E; orange), and nanoeukaryotes (C, F; purple). Seasons are shown below the x-axis. Error bars represent one standard deviation of the mean and small error is contained within symbols. Grazing rates equal to zero (dotted line) were not significant from zero ($p < 0.05$).

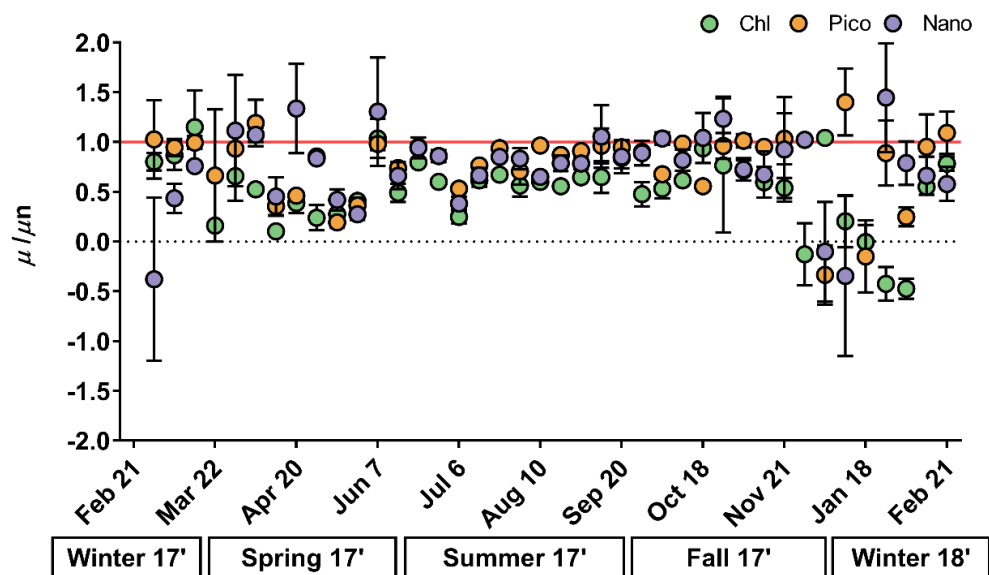


Figure 3.4: Ratio of instantaneous phytoplankton growth versus nutrient-amended growth rates (μ/μ_n) measured each week for chlorophyll (green), picoeukaryotes (orange), and nanoeukaryotes (purple). Seasons are below x-axis. Red dotted line indicates 1:1 growth ratio, with smaller values indicating enhanced phytoplankton nutrient limitation. Error bars represent one standard deviation of the mean. Ratios > 2 or -2 were measured once for picoeukaryotes (12/18) and twice for nanoeukaryotes (1/18, 3/22) and were excluded (see Supplementary Tables 4, 5).

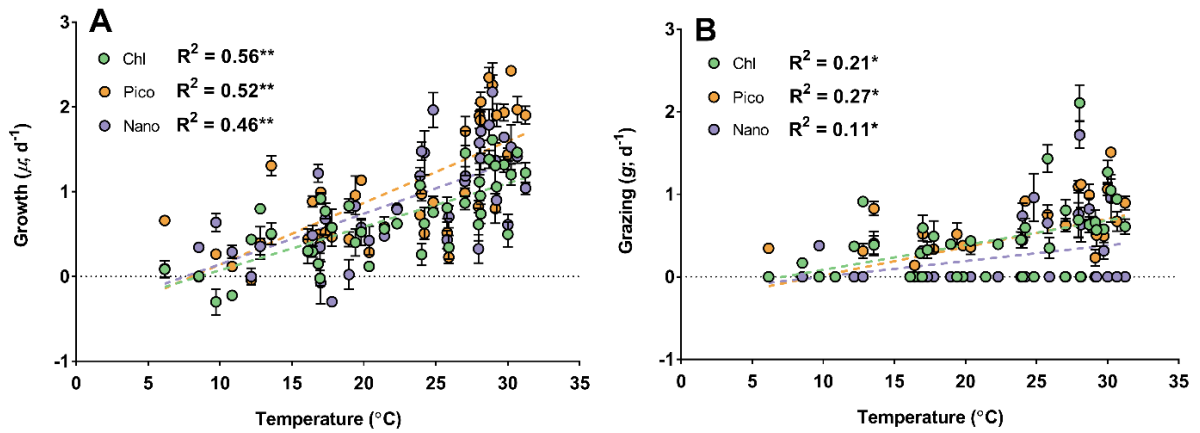


Figure 3.5: Model 1 linear regressions (dotted lines) of phytoplankton growth (**A**) and microzooplankton grazing (**B**) vs. surface water temperature ($^{\circ}\text{C}$) for chlorophyll (green), picoeukaryotes (orange), and nanoeukaryotes (purple). Values of coefficient of determination (R^2) indicate strength of linear relationship; regressions were significant for growth and grazing across all phytoplankton groups ($*p < 0.05$ and $**p < 0.001$).

CHAPTER 4

TEMPORAL VARIABILITY AND ECOLOGICAL INTERACTIONS OF MARINE
SYNDINIALES PARASITES IN COASTAL PROTIST COMMUNITIES¹

¹Anderson SR, and Harvey EL. Submitted to ISME J: 10/7/19.

ABSTRACT

Syndiniales are a ubiquitous group of protist parasites that infect a wide range of hosts, including harmful bloom-forming dinoflagellates. Despite the importance of parasitism as an agent of plankton mortality, parasite-host dynamics remain poorly understood, hindering the inclusion of parasitism in food web and ecosystem models. For a full year in the Skidaway River Estuary (GA), we employed weekly 18S rRNA sampling and co-occurrence network analysis to characterize temporal parasite-host infection dynamics of Syndiniales. Over the year, Syndiniales exhibited strong temporal variability, with higher relative abundance from June-October (7-28%) compared to other months in the year (0.01-6%). Non-metric dimensional scaling of Syndiniales composition revealed tight clustering in June-October that coincided with elevated temperatures (23-31°C), though in general, abiotic factors poorly explained composition and were less important in the network than interspecific relationships. Syndiniales ASVs were well-represented in the co-occurrence network (20% of edges) and exhibited significant positive associations (Spearman $r > 0.7$) with known dinoflagellate hosts (e.g., *Akashiwo* and *Gymnodinium*) and other protist groups (e.g., ciliates, radiolarians, and diatoms). Positive associations rarely involved a single Syndiniales and dinoflagellate species, implying flexible parasite-host infection dynamics. These findings reinforce the importance of parasitic interactions within protist communities and provide insight into the temporal infection dynamics of Syndiniales, which will benefit future testing of these interactions and the incorporation of parasitism in ecosystem models.

INTRODUCTION

Marine microbial eukaryotes (i.e., protists) occupy diverse and ecologically important roles within marine food webs, as they represent both primary producers and consumers of organic carbon [1, 2]. Protist abundance, diversity, and composition are driven by processes that either stimulate growth or those that promote mortality. In terms of mortality, the magnitudes of loss rates are often determined by interactions that occur between individual species (e.g., predator-prey or parasite-host) and with the surrounding environment. Identifying and quantifying these interactions are essential, as the type of interaction can dictate how carbon and nutrients are cycled through the food web [2, 3]. While grazing by heterotrophic protists has been identified as the main source of plankton mortality across global oceans [4], parasitism is also widespread within protist communities and may at times exceed grazing mortality [5, 6]. The ability to incorporate parasitism within food web and ecosystem models has remained challenging, hindered by a lack of understanding of *in situ* protist parasite dynamics, including temporal shifts in parasite populations and parasite-host interactions.

Parasites of phytoplankton and microzooplankton (including mixotrophs) represent important agents of mortality in marine systems, influencing plankton bloom dynamics, species succession, and host biodiversity and evolution [6, 7]. One major group of unicellular parasites, Syndiniales (marine alveolates), are ubiquitous, often representing the most abundant sequences in protist diversity surveys [8–11], and are highly represented among protist interaction networks inferred by correlation analyses [12–14]. The ubiquity of these protists may be related to their life-history, which typically involves a short-lived intracellular trophont stage (2-3 d), followed by the

release of hundreds of free-living dinospores [15]. Parasite dinospores can readily infect and kill a wide range of hosts, including other protists (e.g., dinoflagellates, ciliates, and radiolarians) and metazoans [8]. Moreover, the impact of parasitic infection on biogeochemical cycles differs from other forms of mortality, like grazing, as parasitism reroutes a portion (up to 50%) of host biomass away from the traditional food web (akin to viral lysis), supplying carbon and nutrients to the microbial loop [16–18]. Therefore, a deeper understanding of Syndiniales infection dynamics is warranted, especially given the impact their life-history (e.g., abundant progeny and short generation times) can have on plankton populations and ecosystem functioning.

Syndiniales are often associated with coastal plankton blooms, including frequent observations of the Syndiniales species complex, *Amoebophyra*, infecting harmful bloom-forming dinoflagellates [19–23]. In general, host density is thought to be the main driver of parasite abundance and infection rates, with elevated encounter and infection of hosts occurring under bloom conditions [17, 19]. Other factors may influence Syndiniales population dynamics such as temperature [14, 16], nutrients [24], and water column depth [19], though environmental drivers remain unclear. Syndiniales also exhibit complex interactions with their protist hosts, which raises interesting questions on parasite-host selection in the marine environment. For instance, *Amoebophyra* spp. have been shown to selectively parasitize a single dinoflagellate species in the field [25], though in culture, several *Amoebophyra* sp. strains are capable of infecting multiple hosts [15, 26, 27]. A generalist or opportunistic infection strategy may be common, as environmental sequences within Syndiniales are often correlated to multiple hosts (across different classes) via network analysis and are widely distributed in marine ecosystems

[8, 12, 13]. Unfortunately, it has remained challenging to broadly characterize Syndiniales infection dynamics, as despite their wide diversity (e.g., >50 clades across five main groups), most Syndiniales sequences have yet to be taxonomically classified (at species level) or brought into culture [8, 12, 28]. Establishing parasite-host dynamics in a natural setting will provide key insights into the top-down impacts of Syndiniales on their hosts (e.g., host diversity and abundance) and will benefit from consistent and high-throughput sampling of *in situ* protist communities.

Coastal estuaries are an ideal location to examine parasite-host dynamics, as they often exhibit strong temporal variation in abiotic factors, leading to ephemeral plankton blooms (including harmful blooms) that are subject to parasitic infection [20]. Here, we investigated temporal shifts in coastal Syndiniales communities, measuring Syndiniales relative abundance, diversity, and community composition through V4 18S rRNA gene tag sequencing. Our survey consisted of 33 weekly-biweekly surface water samples collected over a full year within the Skidaway River Estuary (GA, USA), a shallow and productive subtropical estuary with wide environmental gradients [29]. Protist parasites have not been well explored within the estuary, despite the presence of seasonal phytoplankton blooms and harmful plankton taxa [30]. To visualize significant interactions between Syndiniales and putative protist hosts, we applied co-occurrence analysis using the most abundant 152 amplicon sequences measured over the year. Our specific objectives through this work were to (1) identify abiotic and biotic drivers of Syndiniales over realistic time scales and (2) determine significant associations between Syndiniales and putative hosts. Given that infection by Syndiniales parasites can elicit strong top-down pressure on plankton populations, alter microbial diversity and co-

evolution, and influence carbon cycling [6], gathering baseline knowledge of protist parasite dynamics will be critical to inform parasitic mortality in coastal ecosystem and biogeochemical models.

MATERIAL AND METHODS

Sample collection

Surface water (1 m) was collected approximately every 1-2 weeks over a year (March 16, 2017 to February 21, 2018) from the Skidaway River Estuary (Lat: 31°99' N, Long: 81°02' W), which is accessible via the Skidaway Institute of Oceanography (GA, USA). Sampling always occurred at high tide. Seawater (10 l) was collected via Niskin bottles, filtered through a 200- μ m mesh (to limit mesozooplankton), and immediately transferred to the laboratory in clean 20-l carboys for filtration. Due to high particulate content in the estuary (see [31]) and to avoid filter clogging, smaller volumes (250 to 1000 ml) were filtered onto triplicate 47 mm 0.2 μ m polycarbonate filters (Millipore) and stored at -80°C until DNA extraction. Several environmental factors (e.g., temperature and chlorophyll) were measured simultaneously and used to establish relationships with protist composition (see [32] for details). Nutrients and organic carbon were not measured on September 6, 2017, and so this day was removed prior to correlation analyses.

DNA extraction and sequencing preparation

Triplicate filters were thawed on ice and total DNA was extracted with the Qiagen DNeasy PowerSoil Kit (Qiagen) as per the manufacturer's instructions, which included repeated steps of bead-beating for mechanical lysis of cells and a final elution step in 10 mM Tris-HCl (pH = 8.5). The concentration of eluted DNA was tested for each sample

using a Qubit dsDNA HS kit (Thermo Scientific) and submitted for sequencing at concentrations ranging from 2-5 ng μl^{-1} . The V4 region of the 18S rRNA gene was targeted using primers from Stoeck et al. [33]: forward (5'-CCAGCASCYGCGGTAATTCC-3') and reverse (5'-ACTTTCGTTCTTGATYRA-3'). We chose the V4 region because its length (ca. 400 bp) has been shown to increase phylogenetic resolution and diversity estimates compared to shorter regions of the 18S rRNA gene [34]. Amplicon libraries were prepared and sequenced using an Illumina Miseq (2 x 250 bp) at the Georgia Genomics and Bioinformatics Core at the University of Georgia. Sequences were deposited in NCBI SRA under BioProject ID PRJNA575563.

Analysis of 18S sequences

Amplicon sequence variants (ASVs) were inferred from raw sequences using the DADA2 program (<https://benjjneb.github.io/dada2/index.html>, V1.12) in R (<https://cran.r-project.org>, V3.6.1), which allows for high-resolution (1-2 bp difference) identification of sequences [35]. After inspection of read quality profiles, the following parameters were used to filter sequences (e.g., remove primers and phiX contamination) while maintaining sufficient overlap between paired reads: trimLeft = c(20,18), maxN = 0, maxEE = c(2,2), truncQ = 2, rm.phix = TRUE. Parametric error models were applied to the first 100 million bases of the forward and reverse reads, and following dereplication, error rates were used to infer ASVs. Finally, paired reads were merged and reads with unexpected lengths (<1% of reads) and chimeras (ca. 2% of reads) were removed using default DADA2 parameters.

Merged 18S rRNA sequences were classified against the Protistan Ribosomal Reference (PR2; V4.11.1; [36]) database using the “*assignTaxonomy*” function in DADA2, which implements a naïve Bayesian classifier method [37]. Additionally, we searched the most abundant Syndiniales sequences (distinguished from the network) in the NCBI database with BLASTn to assess further classification (e.g., *Amoebophyra*). Often, species level annotation was missing (e.g., *Chaetoceros* sp.) from the ASV table, and so we added this information if the genus level was annotated properly. Over the 33 sampling days included in the dataset, we had an average of 62,529 sequence reads per replicated sample (range = 29,160-104,327), which corresponded to a total of 9768 ASVs over the year (548 on average per replicate).

Tables produced by the DADA2 pipeline were imported into the phyloseq package in R (V1.28.0; [38]) to evaluate diversity metrics and community composition. Prior to diversity analysis, 18S ASVs that could not be identified at the supergroup level were removed. As we focused on diversity and associations among protists, ASVs were filtered to exclude material derived from metazoans (Opisthokonta) and expected parasites of metazoans that resided within Syndiniales group IV [8]. After these filtering steps, a total of 7984 ASVs remained across all samples. Using the filtered ASV table, rarefaction curves were generated for each replicate using the ggrare function and samples were grouped by month (Supplementary Fig. 1). Diversity plots were made for all ASVs (i.e., total 18S community) and for ASVs within Syndiniales. Tukey box plots displaying Shannon alpha diversity were produced using the plot_richness function. For remaining analysis, global singletons were removed (554 ASVs) and samples were normalized by calculating relative abundance for each ASV on a given day. Non-metric

dimensional scaling (NMDS) ordination was performed using Bray-Curtis distances. Temporal shifts in the community at the class and order level (e.g., within Syndiniales) were visualized using the `plot_bar` function in `phyloseq` and included groups with relative abundance >5% on any given day. The filtered ASV table containing reference sequences, taxonomic identification, and relative abundances for each replicate sample will be provided in online supplementary material.

Correlation between environmental factors and composition (total 18S and within-Syndiniales) was explored using canonical correspondence analysis (CCA) using the `vegan` package in R (V2.5; [39]). Contribution of environmental factors were added as arrows to the ordination plot. To further assess the importance of environmental factors in predicting class level relative abundances, a partial least squares (PLS) regression was applied using the R package `pls` (V2.7; [40]). PLS was included to mitigate collinearity of environmental factors, as a strong correlation between temperature and silicate was observed in the estuary (Spearman $r > 0.8$; [32]). Separate models were run using the predictor variables (environmental factors) and class level relative abundances averaged for each major protist group. All variables were log-transformed and standardized by centering and scaling to unit variance [41]. After initial validation, a 3-component model was used for each protist group, capturing 58-65% of the variance in the predictor variables. Finally, variable influence on the projection (VIP) scores were calculated for each predictor variable, with VIP values >1 considered more important to the model [41].

Protist network analysis

Co-occurrence networks were constructed using CoNet (V1.1.1 beta), which enabled visualization of significant interactions between Syndiniales and protist ASVs [42]. CoNet is an ensemble-based approach that combines multiple pairwise measures to restrict prediction of false positive relationships. Each measure assigns a positive (co-presence) or negative (exclusion) sign to a predicted relationship, which reflects whether relative abundance distributions of any two ASVs are significantly more similar or dissimilar than expected at random [42]. To focus on the most prevalent taxa and avoid ambiguous relationships, only ASVs present in >50% of all samples (152 ASVs) were included in the network. Filtered ASV tables were normalized to relative abundance and relationships between taxa and environmental variables were explored by loading metadata as a separate matrix. Sampling days had uneven intervals between them, and so, we did not consider time-lagged correlations.

We followed general CoNet settings as described in Faust and Raes [43]. Briefly, pairwise relationships were explored between ASVs using five measures (Bray Curtis and Kullback-Leibler dissimilarities, Pearson and Spearman correlations, and Mutual Information similarity), with each measure contributing 1000 positive and negative edges. To alleviate compositional bias of sequencing data, a renormalization ReBoot routine was applied [42], which generated 100 permutation and bootstrap scores for each edge and measure. Measure-specific p-values were then computed for each edge as the probability of observing the null value (i.e., mean of edge score distribution) under a Gauss curve generated from the bootstrap distribution [43]. After merging of p-values (resulting in q-value) using Brown's method [44] and correcting for multiple testing with

Benjamini-Hochberg's procedure, edges with q-values <0.05 were retained. Edges were only retained if they were supported by two or more measures or were unanimously given the same sign by all measures. Circular networks were visualized in Cytoscape (V3.7.1; [45]) and nodes (represented by individual ASVs) were colored based on class level annotation. The final network was filtered to include only positive and negative edges measured between Syndiniales ASVs and other major protist groups.

RESULTS

Temporal variability in Syndiniales

Over the year, the average relative abundance at the class level was dominated by Bacillariophyta (24%) and Dinophyceae (21%), while other groups such as Mamiellophyceae (14%), Syndiniales (10%), Cryptophyceae (9%), Spirotrichea (8%) and Filosa-Thecofilosea (2%) were less abundant (Fig. 1a). However, of all the major protist groups (i.e., relative abundance $>5\%$ on any day), only Syndiniales exhibited strong temporal variation, with highest relative abundance from June-October (7-28%) compared to other months in the year (<0.01 -6%; Fig. 1a). Syndiniales (total 658 ASVs) were composed of three main groups at the order level (hereafter termed Dino-Group-I, II, and III). The observed June-October peak in Syndiniales abundance was mainly attributed to ASVs in Dino-Groups-I and II ($>60\%$ of Syndiniales abundance), though ASVs from Group III exhibited temporal variability and emerged at this time (0.02-19%; Fig. 1b).

Canonical correspondence analysis (CCA) and partial least squares (PLS) regression were performed to assess relationships between environmental variables and protist composition, the latter accounting for observed collinearity in variables. CCA

revealed temporal variability in protist communities over the year, with environmental factors explaining 19% of the variance (sum of CCA1 and CCA2 axes) for total 18S and Syndiniales communities, respectively (Supplementary Fig. 2). For both CCA plots, communities in June-October corresponded most closely to temperature and silicate (Supplementary Fig. 2). Of the variables included, PLS models identified temperature (VIP = 1.45) and silicate (VIP = 1.57) to be the most important in explaining relative abundance of Syndiniales at the class level (Supplementary Table 1), confirming CCA analyses. Temperature was found to be an important factor (VIP > 1) explaining shifts in relative abundance for all other protist groups, except for Bacillariophyta abundance, which was explained by dissolved nutrient concentrations (Supplementary Table 1).

Given the wide temperature gradient in the estuary (6-31°C) and its importance as an environmental driver of composition [32], we used temperature in favor of arbitrarily binning samples (e.g., by season) to more accurately visualize temporal shifts in diversity (Fig. 2). Shannon diversity for both total 18S and Syndiniales-only sequences were stable over the year, except for a few samples in April and January, where alpha diversity decreased (Fig. 2 a, b). The diversity among Syndiniales was driven largely by Dino-Group-II, which was represented by 32 clades, while Dino-Group-I included only four clades. Non-metric dimensional scaling (NMDS) of the total 18S community revealed considerable clustering of samples collected in temperatures ranging from 23-31°C (June-October), whereas samples from colder temperatures were more scattered (Fig. 2c). Communities within Syndiniales exhibited even tighter temperature-based clustering from June-October, which coincided with the increased relative abundance of Syndiniales in the estuary at this time (Fig. 2d).

Protist co-occurrence networks

We constructed a co-occurrence network which described significant relationships between the most abundant 152 protist ASVs (represented as distinct nodes) observed in the estuary over the year. Environmental data were also included to assess relationships between specific ASVs and the abiotic factors we measured (e.g., 7 additional nodes). The initial network consisted of 1793 relationships (or edges) connected between 150 nodes, with the majority (64%) of edges being negative (exclusion) associations (Supplementary Fig. 3). Filtering of the network to include only the edges related to Syndiniales (12 ASVs) accounted for 20% of all edges found in the overall protist network (Fig. 3a; Supplementary Table 2). Significant edges (q -values < 0.05) were observed between Syndiniales nodes and nodes from a range of other abundant class level groups including Bacillariophyta (21 nodes), Dinophyceae (11 nodes), Cryptophyceae (9 nodes), and Spirotrichea (8 nodes; Fig. 3a). Syndiniales ASVs were most often associated with Bacillariophyta (85 total edges) and Cryptophyceae (60 total edges), with edges typically representing negative associations (Fig. 3b). Positive (copresence) relationships were most common between Syndiniales and Dinophyceae ASVs, accounting for ~70% of total edges between those groups. Significant associations of Syndiniales to Syndiniales (15 total edges), as well as Syndiniales to abiotic factors (6 total edges connected to ammonium, temperature, or silicate) were less frequent than associations to other protist groups (Fig. 3b).

Syndiniales-Dinophyceae ASV relationships

Given the frequency of positive Syndiniales-Dinophyceae associations, we constructed a presence/absence matrix of all significant edges found in the network

between these groups to better examine potential parasite-host dynamics (Fig. 4). Positive associations were revealed between Syndiniales and multiple hosts including those from the genera *Gyrodinium*, *Gymnodinium*, *Heterocapsa*, *Akashiwo*, and *Amphidoma*. We observed a single example of a one-to-one positive relationship between Dinophyceae ASV 20 (*Akashiwo sanguinea*) and Syndiniales ASV 43 (Dino-Group-II Clade 3), which interestingly, was further identified via BLASTn as an *Amoebophyra* strain infecting *A. sanguinea* (100% identity; Supplementary Table 3). Temporal dynamics of ASV 20 and 43 were strongly correlated over the year (Spearman $r > 0.8$; Pearson $r > 0.6$), with peaks in relative abundance in June-October (Fig. 5a). Other Syndiniales ASVs included in the network were further identified to previously annotated *Amoebophyra* sp. sequences (via BLASTn) infecting *Karlodinium micrum* (90-91% identity; ASV 50 and 386) and *Cochlodinium polykrikoides* (98-99% identity; ASV 77 and 646), while remaining Syndiniales ASVs were unassigned at the genus level (Supplementary Table 3).

Often, single Dinophyceae were associated with multiple Syndiniales ASVs and the converse was also observed (Fig. 4). For instance, significant positive relationships (q-value < 0.001) were observed between Dinophyceae ASV 28 (*Heterocapsa* sp.) and both Syndiniales ASV 27 (Dino-Group-III) and ASV 77 (Dino-Group-II Clade 12), which resulted in a strong correlation (Spearman $r > 0.7$) between ASVs over the year (Fig. 5b). Strong correlations (Spearman $r > 0.6$) between Syndiniales and ASVs in other major protists groups, like Bacillariophyta, were also well-represented. For example, Syndiniales ASV 27 (Dino-Group-III) exhibited a strong negative relationship with Bacillariophyta ASV 210 (*Thalassiosira minuscula*; Supplementary Fig. 4a), while a

positive interaction was measured between Syndiniales ASV 3 (Dino-Group-I Clade 1) and Bacillariophyta ASV 19 (*Cyclotella* sp.; Supplementary Fig. 4b).

DISCUSSION

Parasitism is an important source of mortality within marine protist communities [6], though it is seldom accounted for in ecosystem and biogeochemical models [2]. Syndiniales are a diverse group of protist parasites that often dominate 18S rRNA relative abundance [8–10], infect a range of dinoflagellates and ciliates [17, 28], and can terminate or prevent coastal plankton blooms [25, 46]. Studies of parasite-host interactions have typically involved few taxa - *Amoebophyra* spp. and dinoflagellates - revealing complex parasitic relationships with varying degrees of infectivity and host specificity [15, 26]. However, it has been difficult to characterize parasite-host infection dynamics in the marine environment, as most sequences within Syndiniales have yet to be taxonomically classified or brought into culture [8]. Additionally, poor resolution of Syndiniales communities over time has complicated our understanding of abiotic or biotic drivers of these parasite populations. We employed weekly 18S rRNA amplicon sequencing over a full year in the Skidaway River Estuary (GA), which allowed us to identify temporal shifts in Syndiniales abundance and composition relative to other major protist groups in the estuary. Furthermore, using relative abundance of dominant amplicon sequence variants (ASVs), we constructed a co-occurrence network to infer potential parasite-host interactions between Syndiniales (including unassigned sequences) and other protists. Our findings provide baseline information into temporal parasite dynamics, which will be important for accurate assessment of their ecological role within marine systems and in ecosystem models.

Temporal shifts in Syndiniales

We report strong temporal dynamics of Syndiniales in the estuary, accounting for up to 28% of total protist relative abundance from June-October and becoming less relatively abundant over the remainder of the year. Relative abundances of other major class level protists (e.g., Bacillariophyta and Dinophyceae) were more stable over the year, though temporal trends in total protist composition and diversity mirrored that of Syndiniales, with communities forming a distinct cluster with elevated surface temperatures (23-31°C). Despite temporal shifts in composition and environmental variables, Shannon diversity remained relatively stable over the year, which has been noted elsewhere [47, 48] and may indicate thresholds for species richness within protist communities. We acknowledge that comparing relative abundance among eukaryotic taxa remains tenuous, particularly as copy numbers per cell of the 18S rRNA gene can vary 4-fold among protist groups [49]. While copy numbers roughly scale to biovolume [50, 51], this relationship may not hold for certain protist groups such as alveolates (including Syndiniales), leading to overestimations of relative abundance [49, 52]. While higher gene copy numbers may have influenced the relative abundance of Syndiniales in our study, we observed clear temporal variability in Syndiniales, and in general, we present data on temporal shifts within a class to mitigate biases from gene copy number [14, 53].

Based on CCA and PLS analyses, temperature and nutrients (especially silicate) were identified as important abiotic correlates with Syndiniales relative abundance and composition. Temperature has been shown to determine physiological rates of marine microbial eukaryotes [54, 55] and warmer temperatures may accelerate the infectivity of Syndiniales and dinospore production [17, 56]. Conversely, high relative abundance of

Syndiniales sequences have been observed in polar regions (temperatures $<1^{\circ}\text{C}$) [10, 11]. This may reflect the presence of strain- or species-specific differences in thermal tolerance or distribution patterns among Syndiniales, however, the role of temperature in mediating Syndiniales-host interactions remains unclear. Environmental factors we included poorly explained the variance in parasite composition we observed (19%), which suggests abiotic factors may play a reduced role in structuring parasite communities or that other factors not considered here (e.g., turbulence or mixing) may be important [56]. We also observed strong collinearity of temperature and nutrients in the estuary, making it difficult to ascribe shifts in protist communities to any single environmental factor. It is more likely that temporal dynamics of Syndiniales (and total protists) in the estuary were driven by biological interactions between taxa instead of abiotic variables, which has been observed in other microbial networks [57, 58], and in our network, where significant abiotic interactions were scarce. Determining Syndiniales infection dynamics in the natural environment may rely more on complex interactions that occur between parasites and hosts, rather than relationships with any single environmental factor.

Syndiniales are highly influenced by host biomass and density [17, 19, 59], with parasitic encounter and infection expected to decrease under conditions that are unfavorable for autotrophic, heterotrophic, or mixotrophic hosts (e.g., temperature, nutrients, or prey availability). For instance, *Amoebophyra* spp. (Syndiniales group II) have been shown to produce 3-4 times fewer dinospores per infected host cell and have lower infectivity in nutrient-deplete vs. replete conditions [16]. The reduced Syndiniales relative abundance we observed in winter and spring may have been driven by poor

environmental conditions for host cells (e.g., low temperature and nutrients), inhibiting accumulation of host biomass available for parasitism. Prior work in the Skidaway River Estuary [32] measured significantly lower phytoplankton accumulation rates in winter-spring compared to summer-fall (-0.16 - 0.28 d^{-1} vs. 0.48 - 1.09 d^{-1}), with such shifts in biomass potentially affecting host: parasite ratios and encounter rates. Though we did not track the absolute abundance or biomass of host populations in this study, temperature and nutrients were revealed via PLS as important variables explaining shifts in putative hosts (Dinophyceae and Spirotrichea), suggesting host populations were influenced by changing environmental factors. Despite low relative abundance of parasites in winter-spring (0.01-6%), Syndiniales may have survived during this time by residing within host cysts or switching hosts [15, 26], allowing them to exploit intermittent or seasonal blooms. Though we cannot verify such survival strategies in our analyses, the Skidaway River Estuary is a shallow site (4-6 m) and has strong benthic-pelagic coupling [29, 31], which would facilitate the vertical transport of dinoflagellate cysts (and enclosed parasites) to the sediment. Additional temporal examinations of Syndiniales are needed to establish drivers of natural parasite communities and should take into consideration relative and absolute shifts in parasite-host systems, as well as parasite survival strategies during unfavorable conditions.

Syndiniales interactions via network analysis

Network inference via co-occurrence analysis has become a widely applied technique in microbial ecology [42, 60] and has been used to predict significant relationships or interactions among protist sequences [61–63]. Importantly, positive interactions inferred via co-occurrence networks can represent ecologically meaningful

associations between ASVs (e.g., parasitism, predation, or mutualism) or simply an overlapping niche between taxa [42]. We focused on positive correlations between Syndiniales and protists that may indicate parasitism [12]; however, we acknowledge limited causality of such interactions and the need for further testing to confirm them [52]. As with other temporal protist networks [12–14, 63], Syndiniales were well represented in our yearly network, accounting for 20% of all significant interactions between protist taxa. The most common interactions were found between Syndiniales and Bacillariophyta, though such associations were largely negative (75%), indicative of possible competition between taxa or the presence of different niches [42]. Positive interactions that were detected between these groups remain ambiguous, as there has yet to be empirical evidence of Syndiniales infecting diatoms. However, Syndiniales have been shown to positively associate with diatoms in other co-occurrence networks [12, 13] and diatoms are known hosts of other parasitic protists and fungi [64, 65], which warrants further investigation into their infection dynamics.

A handful of positive associations were observed between Syndiniales and known host taxa other than dinoflagellates, including Spirotrichea (e.g., *Tintinnidium* sp. and unidentified Strombididae) and Acantharea (unidentified radiolarians), both of which have been associated with Syndiniales in phylogenetic and empirical work [28, 66]. Tintinnid ciliates are commonly recognized hosts of the Syndiniales genera, *Euduboscquella*, with infection being prevalent in coastal ciliate populations [20, 28, 67]. Though *Euduboscquella* spp. were not identified among abundant Syndiniales sequences in our network, the presence of positive relationships between Syndiniales and ciliates may suggest parasitic relationships between these groups in the estuary. We recognize

that positive associations with ciliates could also infer predator-prey relationships, as there is evidence of ciliates consuming Syndiniales dinospores [68, 69]. Nevertheless, further work is needed to confirm parasite interactions between Syndiniales and these protist groups, as well as to differentiate between parasite-host and predator-prey relationships.

In our yearly network, positive associations with Syndiniales (mostly unassigned group I and II sequences) were most common with Dinophyceae ASVs (e.g., *Gyrodinium*, *Gymnodinium*, *Heterocapsa*, and *Akashiwo*), which confirms previous networks [12] and direct observations of infection in these dinoflagellate species in lab or field samples [17, 21]. Syndiniales were also found to positively interact with a recently identified toxin-producing (azaspiracid) species, *Amphidoma languida* (ASV 249) [70], which may infer a potential parasitic relationship, especially as Syndiniales are often associated with toxic dinoflagellates [71]. Further insight into the suspected interaction and co-evolution of Syndiniales parasites and toxin-producing dinoflagellates is essential given the potential top-down control of harmful blooms via parasitism and related ecosystem effects [25, 71]. While no direct evidence of infection was witnessed in this study, our findings of abundant Syndiniales-Dinophyceae interactions in the network, including those with harmful plankton, reinforces the importance of these parasitic interactions in coastal protist communities [17]. Overall, our network results provide further evidence for a wide host range among Syndiniales, though additional network analysis is needed to confirm these interactions and reveal novel parasitic interactions over spatiotemporal scales. The use of more targeted approaches (e.g., single-cell sorting, fluorescent in situ hybridization, or FlowCam imaging) may compliment future 18S

amplicon surveys and be useful in verifying parasite-host interactions proposed via co-occurrence networks.

Syndiniales host specificity

Given the high frequency of positive Syndiniales-Dinophyceae associations and the often-observed infection of coastal dinoflagellates [17, 20], we chose to further examine host specificity of these associations. We found only one example of a highly specific positive interaction involving Dinophyceae ASV 20 (*Akashiwo sanguinea*) and Syndiniales ASV 43 (identified as *Amoebophyra* sp. infecting *A. sanguinea*), confirming previous accounts of this highly specific parasitic relationship [15, 21]. *A. sanguinea* is common in the Skidaway River [30] and populations of this harmful species can exceed $1000 \mu\text{g C l}^{-1}$ in summer [72], during which time *A. sanguinea* is likely susceptible to parasitic infection. It was more common for Syndiniales ASVs in our network to be associated with multiple dinoflagellate hosts or for multiple Syndiniales to interact with the same host, suggesting flexible parasite-host dynamics. Other studies have noted a generalist infection strategy among Syndiniales and putative hosts [8, 13, 73], which may explain their ubiquity in marine systems and quick response to elevated host density. Though we focused on only the most abundant Syndiniales and protist sequences for our yearly network, we recognize there may be ephemeral parasite-host interactions that were overlooked (e.g., in winter-spring) and may reflect more specific interactions [25]. There are likely a myriad of infection strategies displayed among Syndiniales taxa that enables parasites to exploit a range of plankton host conditions.

Despite the diversity and ubiquity of Syndiniales in the global oceans [8, 9], large gaps in knowledge of their infection dynamics has made it difficult to place parasitism

within food web or ecosystem models [74]. In the data presented here, Syndiniales were temporally variable and well-represented in our protist network, exhibiting positive interactions with a range of putative hosts (e.g., dinoflagellates, ciliates, and diatoms) that largely involved multiple putative hosts per parasite. Given their importance within the protist community and broad host range, parasitism by Syndiniales is expected to have significant consequences for carbon cycling and ecosystem functioning in marine systems [6, 8]. Determining the contribution of parasitism to plankton mortality will be important, especially in relation to other forms of mortality (e.g., predation or viral lysis), which have contrasting effects on carbon and nutrient cycling in marine food webs. Gathering baseline information on the infection dynamics of Syndiniales is therefore critical to accurately predict the impact of parasitism on plankton populations and biogeochemical cycling.

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REFERENCES

1. Caron DA, Countway PD, Jones AC, Kim DY, Schnetzer A. Marine Protistan Diversity. *Ann Rev Mar Sci* 2012; **4**: 467–493.
2. Worden AZ, Follows MJ, Giovannoni SJ, Wilken S, Zimmerman AE, Keeling PJ. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science* 2015; **347**: 127594.
3. Fuhrman JA, Cram JA, Needham DM. Marine microbial community dynamics and their ecological interpretation. *Nat Rev Microbiol* 2015; **13**: 133–146.
4. Schmoker C, Hernández-León S, Calbet A. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. *J Plankton Res* 2013; **35**: 691–706.
5. Montagnes DJS, Chambouvet A, Guillou L, Fenton A. Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms. *Aquat Microb Ecol* 2008; **53**: 211–225.
6. Jephcott TG, Alves-de-Souza C, Gleason FH, van Ogtrop FF, Sime-Ngando T, Karpov SA, et al. Ecological impacts of parasitic chytrids, syndiniales and perkinsids on populations of marine photosynthetic dinoflagellates. *Fungal Ecol* 2016; **19**: 47–58.
7. Gleason FH, Küpper FC, Amon JP, Picard K, Gachon CMM, Marano A V., et al. Zoosporic true fungi in marine ecosystems: A review. *Mar Freshw Res* 2011; **62**: 383–393.
8. Guillou L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, Massana R, et al. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environ Microbiol* 2008; **10**: 3349–3365.
9. De Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. Eukaryotic plankton diversity in the sunlit ocean. *Science* 2015; **348**: 1261605.
10. Cleary AC, Durbin EG. Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *J Plankton Res* 2016; **38**: 401–417.
11. Clarke LJ, Bestley S, Bissett A, Deagle BE. A globally distributed Syndiniales parasite dominates the Southern Ocean micro-eukaryote community near the sea-ice edge. *ISME J* 2019; **13**: 734–737.
12. Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, et al. Determinants of community structure in the global plankton interactome. *Science* 2015; **348**: 1262073.
13. Torres-Beltrán M, Sehein T, Pachiadaki MG, Hallam SJ, Edgcomb V. Protistan parasites along oxygen gradients in a seasonally anoxic fjord: A network approach to assessing potential host-parasite interactions. *Deep Res Part II Top Stud Oceanogr* 2018; **156**: 97–110.

14. Berdjeb L, Parada A, Needham DM, Fuhrman JA. Short-term dynamics and interactions of marine protist communities during the spring-summer transition. *ISME J* 2018; **12**: 1907–1917.
15. Coats DW, Park MG. Parasitism of photosynthetic dinoflagellates by three strains of *Amoebophrya* (Dinophyta): parasite survival, infectivity, generation time, and host specificity. *J Phycol* 2002; **38**: 520–528.
16. Yih W, Wayne Coats D. Infection of *Gymnodinium sanguineum* by the dinoflagellate *Amoebophrya* sp.: Effect of nutrient environment on parasite generation time, reproduction, and infectivity. *J Eukaryot Microbiol* 2000; **47**: 504–510.
17. Park MG, Yih W, Coats DW. Parasites and phytoplankton, with special emphasis on dinoflagellate infections. *J Eukaryot Microbiol* 2004; **51**: 145–155.
18. Salomon PS, Granéli E, Neves MHCB, Rodriguez EG. Infection by *Amoebophrya* spp. parasitoids of dinoflagellates in a tropical marine coastal area. *Aquat Microb Ecol* 2009; **55**: 143–153.
19. Coats DW, Adam EJ, Gallegos CL, Hedrick S. Parasitism of photosynthetic dinoflagellates in a shallow subestuary of Chesapeake Bay, USA. *Aquat Microb Ecol* 1996; **11**: 1–9.
20. Coats DW. Parasitic life styles of marine dinoflagellates. *J Eukaryot Microbiol* 1999; **46**: 402–409.
21. Park MG, Kim S, Shin EY, Yih W, Coats DW. Parasitism of harmful dinoflagellates in Korean coastal waters. *Harmful Algae* 2013; **30**: S62–S74.
22. Alves-De-souza C, Varela D, Iriarte JL, González HE, Guillou L. Infection dynamics of Amoebophryidae Parasitoids on harmful dinoflagellates in a southern Chilean Fjord dominated by diatoms. *Aquat Microb Ecol* 2012; **66**: 183–197.
23. Gunderson JH, John SA, Boman WC, Coats DW. Multiple strains of the parasitic dinoflagellate *Amoebophrya* exist in Chesapeake Bay. *J Eukaryot Microbiol* 2002; **49**: 469–474.
24. Siano R, Alves-De-Souza C, Foulon E, M. Bendif E, Simon N, Guillou L, et al. Distribution and host diversity of Amoebophryidae parasites across oligotrophic waters of the Mediterranean Sea. *Biogeosciences* 2011; **8**: 267–278.
25. Chambouvet A, Morin P, Marie D, Guillou L. Control of Toxic Marine Dinoflagellate Blooms by Serial Parasitic Killers. *Science* 2008; **322**: 1254–1258.
26. Chambouvet A, Laabir M, Sengco M, Vaquer A, Guillou L. Genetic diversity of Amoebophryidae (Syndiniales) during *Alexandrium catenella/tamarense* (Dinophyceae) blooms in the Thau lagoon (Mediterranean Sea, France). *Res Microbiol* 2011; **162**: 959–968.
27. Kim S, Park MG. *Amoebophrya* spp. from the bloom-forming dinoflagellate *cochlodinium polykrikoides*: Parasites not nested in the '*amoebophrya ceratii*

- complex'. *J Eukaryot Microbiol* 2014; **61**: 173–181.
28. Bachvaroff TR, Kim S, Guillou L, Delwiche CF, Coats DW. Molecular diversity of the syndinean genus *euduboscquella* based on single-cell PCR analysis. *Appl Environ Microbiol* 2012; **78**: 334–345.
 29. Verity PG. A Decade of Change in the Skidaway River Estuary. I. Hydrography and Nutrients. *Estuaries* 2002; **25**: 944–960.
 30. Verity PG, Borkman DG. A Decade of Change in the Skidaway River Estuary. III. Plankton. *Estuaries and Coasts* 2010; **33**: 513–540.
 31. Bittar TB, Berger SA, Birsa LM, Walters TL, Thompson ME, Spencer RGM, et al. Seasonal dynamics of dissolved, particulate and microbial components of a tidal saltmarsh-dominated estuary under contrasting levels of freshwater discharge. *Estuar Coast Shelf Sci* 2016; **182**: 72–85.
 32. Anderson SR, Harvey EL. Seasonal variability and drivers of microzooplankton grazing and phytoplankton growth in a subtropical estuary. *Front Mar Sci* 2019; **6**: 1–13.
 33. STOECK T, BASS D, NEBEL M, CHRISTEN R, JONES MDM, BREINER H-W, et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol Ecol* 2010; **19**: 21–31.
 34. Hu SK, Liu Z, Lie AAY, Countway PD, Kim DY, Jones AC, et al. Estimating Protistan Diversity Using High-Throughput Sequencing. *J Eukaryot Microbiol* 2015; **62**: 688–693.
 35. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J* 2016; **11**: 2639–2643.
 36. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Res* 2013; **41**.
 37. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007; **73**: 5261–5267.
 38. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* 2013; **8**: e61217.
 39. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. R package version 2.5-2. *Cran R* 2018; **1**: 2.
 40. Mevik B, Wehrens R. The pls Package: Principal Component and Partial Least Squares Regression in R. *J Stat Softw* 2007; **18**: 1–24.
 41. Eriksson L, Byrne T, Johansson E, Trygg J, Vikström C. Process Analytical

Technology (PAT) and Quality by Design (QbD). *Multi- Megavariate Data Anal Value From Data* 2006; 323–355.

42. Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, et al. Microbial co-occurrence relationships in the Human Microbiome. *PLoS Comput Biol* 2012; **8**: e1002606.
43. Faust K, Raes J. CoNet app: Inference of biological association networks using Cytoscape [version 1; referees: 2 approved with reservations]. *F1000Research* 2016; **5**: 1–14.
44. Brown MB. A Method for Combining Non-Independent , One-Sided Tests of Significance. *Int Biometric Soc* 1975; **31**: 987–992.
45. Shannon P, Markiel A, Ozier O, Baliga N, Wang J, Ramage D, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res* 2003; **13**: 2498–2504.
46. Velo-Suárez L, Brosnahan ML, Anderson DM, McGillicuddy DJ. A quantitative assessment of the role of the parasite *Amoebophrya* in the termination of *Alexandrium fundyense* blooms within a small coastal embayment. *PLoS One* 2013; **8**: e81150.
47. Brannock PM, Ortmann AC, Moss AG, Halanych KM. Metabarcoding reveals environmental factors influencing spatio-temporal variation in pelagic micro-eukaryotes. *Mol Ecol* 2016; **25**: 3593–3604.
48. Piredda R, Tomasino MP, Erchia AMD, Manzari C, Pesole G, Montresor M, et al. Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. *FEMS Microbiol Ecol* 2016; **93**: 1–14.
49. Not F, del Campo J, Balagué V, de Vargas C, Massana R. New insights into the diversity of marine picoeukaryotes. *PLoS One* 2009; **4**: 1–5.
50. Zhu F, Massana R, Not F, Marie D, Vaultot D. Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiol Ecol* 2005; **52**: 79–92.
51. Godhe A, Asplund ME, Härnström K, Saravanan V, Tyagi A, Karunasagar I. Quantification of diatom and dinoflagellate biomasses in coastal marine seawater samples by real-time PCR. *Appl Environ Microbiol* 2008; **74**: 7174–7182.
52. Krabberød AK, Bjorbækmo MFM, Shalchian-Tabrizi K, Logares R. Exploring the oceanic microeukaryotic interactome with metaomics approaches. *Aquat Microb Ecol* 2017; **79**: 1–12.
53. Cram JA, Chow CET, Sachdeva R, Needham DM, Parada AE, Steele JA, et al. Seasonal and interannual variability of the marine bacterioplankton community throughout the water column over ten years. *ISME J* 2015; **9**: 563–580.
54. Eppley RW. Temperature and Phytoplankton Growth in the Sea. *Fish Bull* 1972;

70: 1063–1085.

55. Rose JM, Caron DA. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol Oceanogr* 2007; **52**: 886–895.
56. Wayne Coats D, Katrin Bockstahler R. Occurrence of the Parasitic Dinoflagellate *Amoebophrya ceratii* in Chesapeake Bay Populations of *Gymnodinium sanguineurn*. *J Eukaryot Microbiol* 1994; **41**: 586–593.
57. Steele JA, Countway PD, Xia L, Vigil PD, Beman JM, Kim DY, et al. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J* 2011; **5**: 1414–1425.
58. Needham DM, Fuhrman JA. Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. *Nat Microbiol* 2016; **1**: 16005.
59. Kim S, Myung GP, Yih W, Wayne Coats D. Infection of the bloom-forming thecate dinoflagellates *Alexandrium affine* and *Gonyaulax spinifera* by two strains of *Amoebophrya* (Dinophyta). *J Phycol* 2004; **40**: 815–822.
60. Weiss S, Van Treuren W, Lozupone C, Faust K, Friedman J, Deng Y, et al. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *ISME J* 2016; **10**: 1669–1681.
61. Needham DM, Fichot EB, Wang E, Berdjeb L, Cram JA, Fichot CG, et al. Dynamics and interactions of highly resolved marine plankton via automated high-frequency sampling. *ISME J* 2018; **12**: 2417–2432.
62. Needham DM, Sachdeva R, Fuhrman JA. Ecological dynamics and co-occurrence among marine phytoplankton, bacteria and myoviruses shows microdiversity matters. *ISME J* 2017; **11**: 1614–1629.
63. Hu SK, Connell PE, Mesrop LY, Caron DA. A Hard Day’s Night : Diel Shifts in Microbial Eukaryotic Activity in the North Pacific Subtropical Gyre. *Front Mar Sci* 2018; **5**: 1–17.
64. Tillmann U, Hesse KJ, Tillmann A. Large-scale parasitic infection of diatoms in the Northfrisian Wadden Sea. *J Sea Res* 1999; **42**: 255–261.
65. Kagami M, De Bruin A, Ibelings BW, Van Donk E. Parasitic chytrids: Their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia* 2007; **578**: 113–129.
66. Bråte J, Krabberød AK, Dolven JK, Ose RF, Kristensen T, Bjørklund KR, et al. Radiolaria Associated with Large Diversity of Marine Alveolates. *Protist* 2012; **163**: 767–777.
67. Coats DW, Bachvaroff TR, Delwiche CF. Revision of the family Duboscquellidae with description of *Euduboscquella crenulata* n. gen., n. sp. (Dinoflagellata, Syndinea), an intracellular parasite of the ciliate *Favella panamensis* Kofoid & Campbell. *J Eukaryot Microbiol* 2012; **59**: 1–11.

68. Maranda L. Infection of *Prorocentrum minimum* (Dinophyceae) by the parasite *Amoebophrya* sp. (Dinoflagellata). *J Phycol* 2001; **37**: 245–248.
69. Johansson M, Coats DW. Ciliate grazing on the parasite *Amoebophrya* sp. decreases infection of the red-tide dinoflagellate *Akashiwo sanguinea*. *Aquat Microb Ecol* 2002; **28**: 69–78.
70. Tillmann U, Jaén D, Fernández L, Gottschling M, Witt M, Blanco J, et al. *Amphidoma languida* (Amphidomatacea, Dinophyceae) with a novel azaspiracid toxin profile identified as the cause of molluscan contamination at the Atlantic coast of southern Spain. *Harmful Algae* 2017; **62**: 113–126.
71. Bai X, Adolf JE, Bachvaroff T, Place AR, Coats DW. The interplay between host toxins and parasitism by *Amoebophrya*. *Harmful Algae* 2007; **6**: 670–678.
72. Anderson SR, Diou-cass QP, Harvey EL. Short-term estimates of phytoplankton growth and mortality in a tidal estuary. *Limnol Oceanogr* 2018; **63**: 2411–2422.
73. Salomon PS, Janson S, Granéli E. Multiple species of the dinophagous dinoflagellate genus *Amoebophrya* infect the same host species. *Environ Microbiol* 2003; **5**: 1046–1052.
74. Warren CP, Pascual M, Lafferty KD, Kuris AM. The inverse niche model for food webs with parasites. *Theor Ecol* 2010; **3**: 285–294.

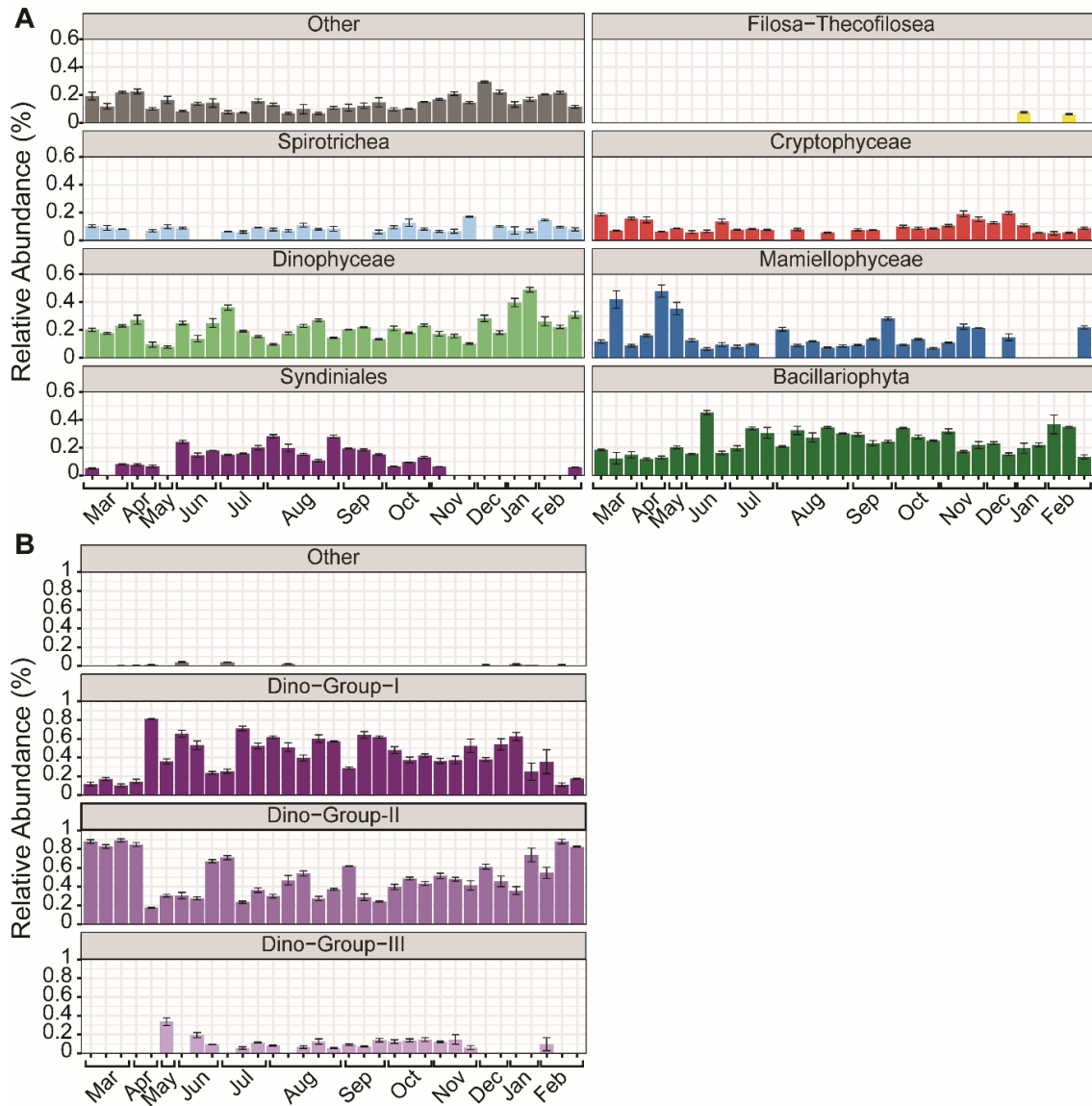


Figure 4.1: Relative abundance (%) bar plots of major taxa in the estuary over the year at the class level (a) and within the Syndiniales group (b). Error bars represent the standard deviation of replicate sample means. Taxa included in the “other” category represent <5% of the total protist or Syndiniales community on each respective day. Syndiniales were dominated by three groups (Dino-Groups I, II, and, III), with only a few sequences being unclassified at the order level. Samples from the same month are indicated by brackets on the x-axis for all temporal figures.

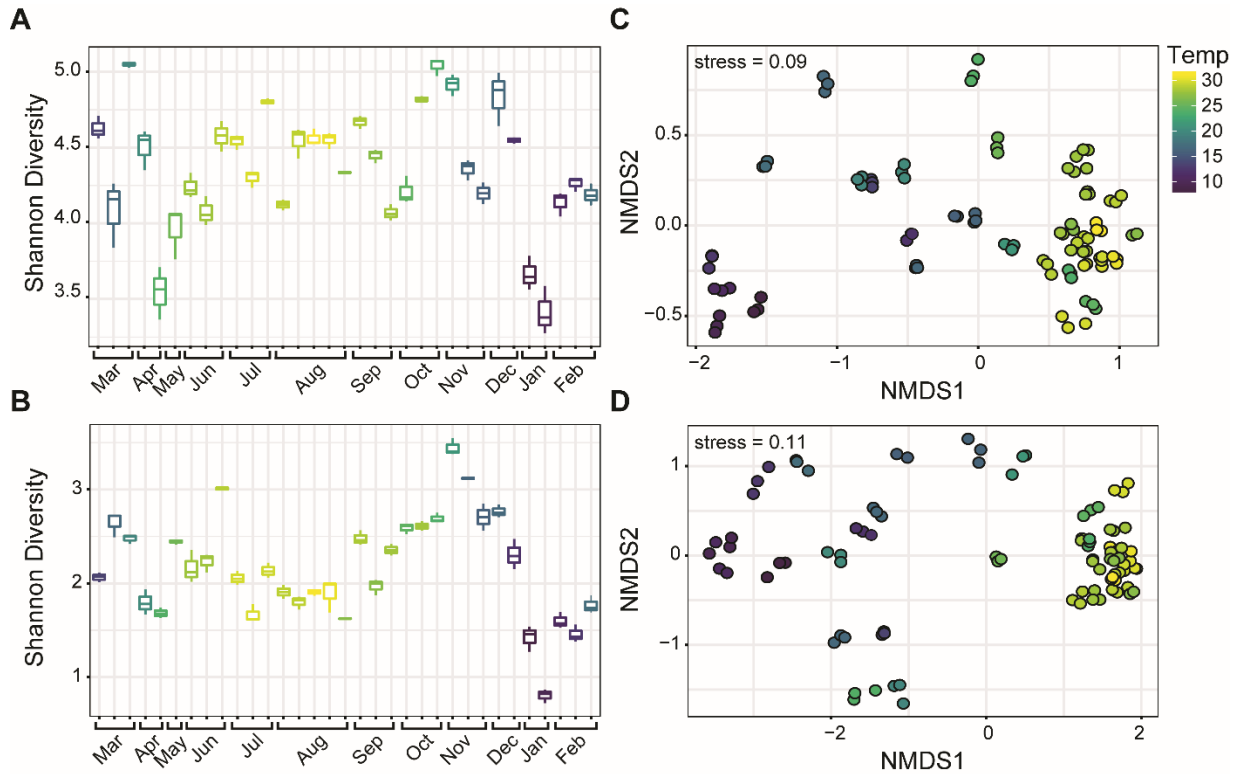


Figure 4.2: Temporal shifts in Shannon alpha diversity and ordination by non-metric dimensional scaling (NMDS) of the total protist community (**a, c**) and within the Syndiniales group (**b, d**). Alpha diversity values represent mean and standard deviation of replicate samples, while points in the NMDS represent Bray-Curtis distances of communities for each replicate per sample. Sampling days are colored similarly for both diversity metrics according to the observed temperature gradient in the estuary. Stress values for the NMDS are shown.

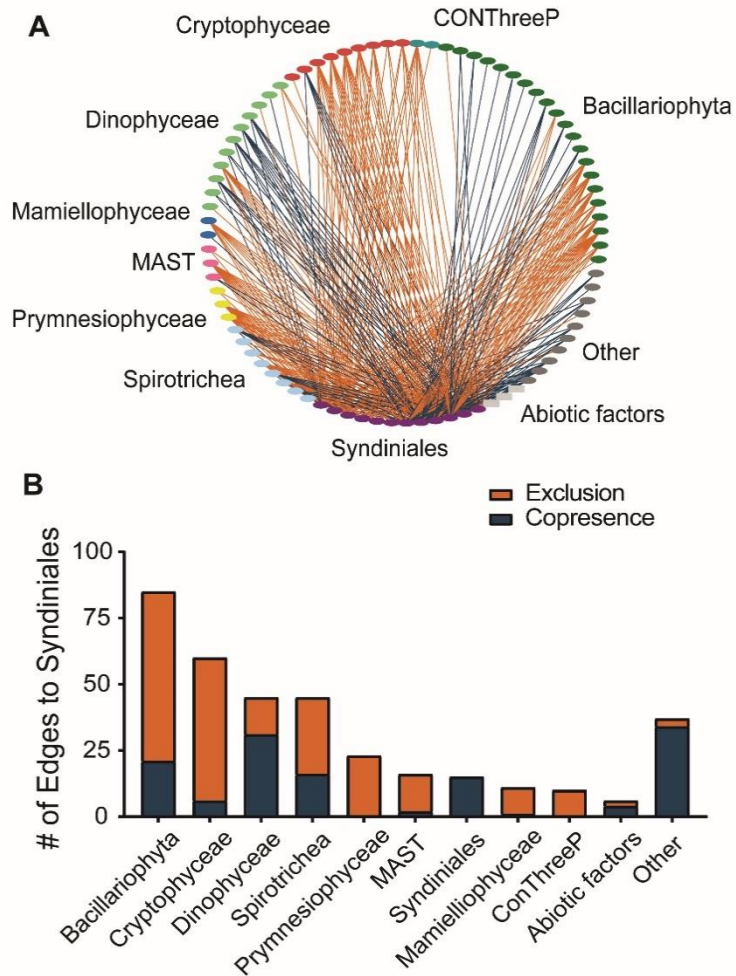


Figure 4.3: Filtered co-occurrence network of positive (copresence; blue) and negative (exclusion; orange) edges associated with dominant Syndiniales ASVs (**a**). Class level groups or abiotic factors associated with Syndiniales (labeled nodes) were represented by 2-21 ASVs per group. Taxa were included within the “other” category if represented by <2 ASVs (see Supplementary Table 2 for network details). Edges were computed between ASVs based on a suite of correlation and similarity metrics and included if statistically significant (merged q-value < 0.05). Total number of positive and negative edges connected between Syndiniales and other major groups are summarized in (**b**).

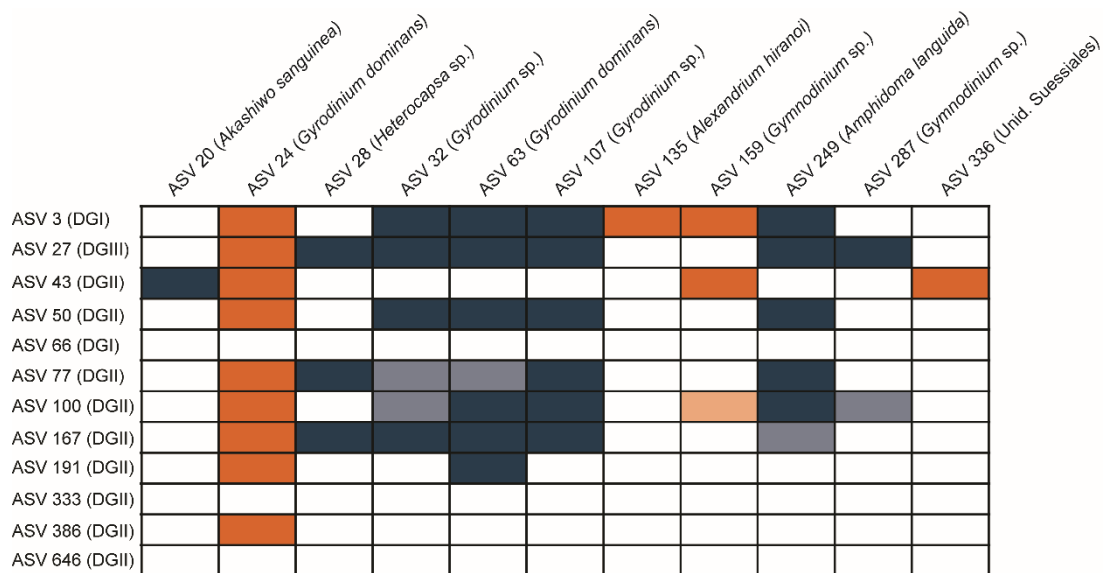


Figure 4.4: Diagram depicting the presence or absence of Syndiniales-Dinophyceae ASV pairings observed in the filtered network. Colored squares represent either a positive (copresence; blue) or negative (exclusion; orange) association for the respective pairing. Syndiniales parasites are referenced by group (DG I, II, or III), while species annotation is shown for dinoflagellates. Dinophyceae ASV 336 represents an unidentified Suessiales taxa. Dark shaded boxes represent edges with merged q-values < 0.001, while lighter boxes reflect q-values < 0.05.

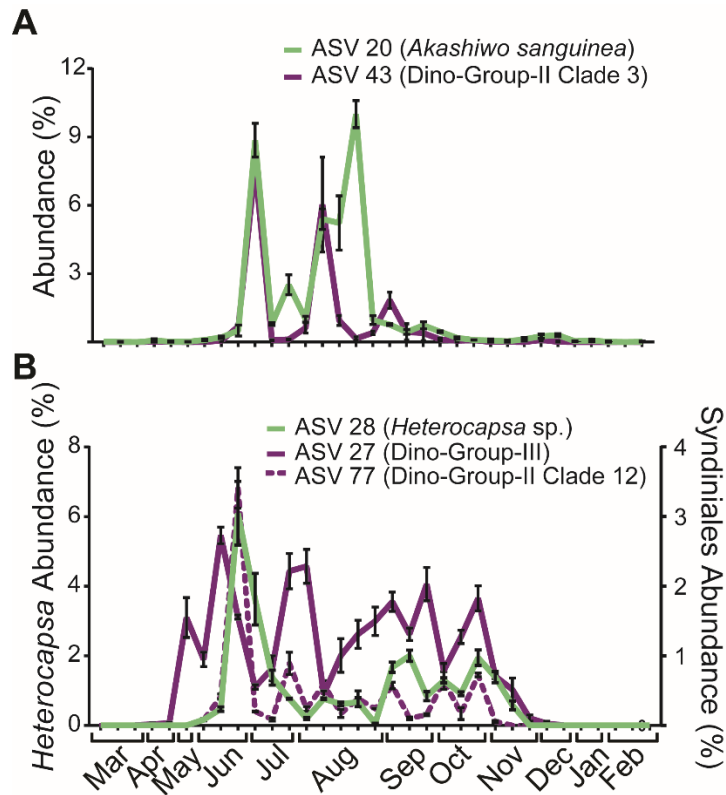


Figure 4.5: Relative abundance (%) plots of specific Syndiniales (purple) and Dinophyceae (green) ASVs over the year in the estuary, representing either single (**a**) or multiple parasite-host (**b**) positive relationships. All ASV associations were significant (q -values < 0.05) and derived from the filtered network. Error bars represent standard deviation of replicate sample means.

CHAPTER 5

CONCLUSIONS

Phytoplankton govern key ecosystem processes within marine food webs, and so, it is important to understand the causes and consequences of their mortality (Brum et al. 2014; Worden et al. 2015). Microzooplankton (<200 μm phagotrophic protists) have been identified as the dominant consumers of phytoplankton production (Calbet and Landry 2004; Schmoker et al. 2013), greatly influencing food web structure and trophic transfer (Steinberg and Landry 2017). Yet, microzooplankton grazing rates have rarely been measured with respect to time, which limits the ability to predict drivers of grazing mortality. Other forms of phytoplankton mortality, like viral lysis or parasitism, have also been poorly resolved over time, despite the importance of these loss processes in regulating host plankton abundance and diversity (Baudoux et al. 2008; Montagnes et al. 2008). This dissertation presented time-specific shifts in phytoplankton loss rates (grazing and viral lysis) and parasite-host relationships (via 18S genomic sequencing) over the course of a year-long coastal time series, which addressed some of the gaps in our understanding of temporal plankton mortality.

We performed dilution-based experiments with natural plankton assemblages from the Skidaway River Estuary, which revealed significant temporal variability in microzooplankton grazing rates over seasonal and tidal cycles. At all temporal scales, grazing rates were poorly explained by abiotic factors, an unexpected finding for seasonal rates that were measured over wide environmental gradients, especially surface water

temperature (6-31°C). Temperature has been thought to drive phytoplankton metabolism and growth rates (Eppley 1972; Bissinger et al. 2008) and may impact microzooplankton grazing (Rose and Caron 2007), yet we found a poor linear relationship between temperature and grazing and frequently observed low grazing rates at temperatures > 20°C. Instead, microzooplankton grazing rates were more likely influenced by observed shifts in grazer-prey biomass and community composition between same-day tides or seasons. For instance, grazing rates decreased in late summer-fall, coinciding with a transition in phytoplankton composition from a picoplankton to a diatom-dominated community (e.g., *Chaetoceros* and *Skeletonema*). Natural plankton assemblages are complex, with grazers exhibiting selectivity in grazing based on prey type or composition (Tillmann 2004), complicating the ability to extrapolate microzooplankton grazing dynamics from a controlled laboratory to field setting (Chen et al. 2012; Lawrence and Menden-Deuer 2012). Therefore, additional temporal grazing rate measurements, accompanied by estimates of plankton community composition (e.g., via FlowCam or genomic sequencing), will be critical to characterize patterns and drivers of *in situ* microzooplankton grazing.

Though grazing typically dominates phytoplankton mortality, it is important to consider other forms of mortality like viral lysis, as viruses can significantly contribute to daily production loss (10-20%) and influence biodiversity and function within planktonic communities (Baudoux et al. 2008; Breitbart 2012). Though accounted for in our modified dilutions, we rarely detected significant viral mortality rates. The absence of measurable viral mortality rates may be related to the length of incubation (24 hours), which may not cover the full lytic infection cycle of viruses (Jacquet et al. 2005). Further,

virus-host relationships are known to be highly specific and density dependent, and so it is possible that viral lysis was limited in our dilutions due to virus-host mismatches or dilute host concentrations (Kimmance et al. 2007). Lastly, the modified dilution approach may not be sensitive enough to capture viral mortality rates, as a recent meta-analysis revealed the modified version suffers from weak statistical power (Staniewski and Short 2018). These methodological issues may also apply to lysis via parasitic infection, as parasitism relies on density-dependent encounter with hosts and typically includes an infection cycle of 2-3 days (Coats and Park 2002). Reliable methods to estimate bulk viral or parasitic lysis rates have yet to be established for natural plankton communities, though such efforts will be important to advance our knowledge of plankton mortality and carbon transfer in marine food webs.

Though parasitic infection rates were not measured here, we used weekly amplicon surveys (18S rRNA) and network analysis over a full year in the estuary to assess baseline information into temporal parasite-host dynamics in coastal protist communities. Our study targeted Syndiniales (Alveolata), which are ubiquitous and diverse protist parasites (Guillou et al. 2008) that infect and kill a range of dinoflagellates, ciliates, and radiolarians (Coats 1999; Bachvaroff et al. 2012). Syndiniales were temporally variable in the estuary, accounting for up to 28% of protist relative abundance in June-October, which coincided with increased temperature in the estuary. However, as was the case with microzooplankton grazing rates, environmental factors (including temperature) poorly explained Syndiniales abundance or composition. Instead, biotic interactions were more important, as Syndiniales accounted for 20% of all interactions within the protist network and were positively correlated to known hosts such

as dinoflagellates and ciliates (Park et al. 2013), as well as putative hosts like diatoms (Torres-Beltran et al. 2018). Interestingly, parasitic relationships inferred from the network often involved a single Syndiniales and multiple dinoflagellate hosts, supporting the idea that Syndiniales exhibit a generalist or relaxed infection strategy (Guillou et al. 2008). Further experimental testing is needed to confirm parasite-host relationships inferred by our network. Nevertheless, Syndiniales exert strong top-down pressure on plankton populations and influence carbon cycling (Jephcott et al. 2016), making it imperative to gather baseline information on their infection dynamics.

Identifying and quantifying loss factors responsible for phytoplankton mortality is critical due to their contrasting effects on the structure and function of marine food webs and biogeochemical cycles. Unfortunately, it has remained challenging to separate the impacts of mortality on carbon cycling because loss processes occur concurrently in the natural environment and at different time scales (e.g., daily ingestion vs. 3-day infection cycle). Typically, grazing from microzooplankton channels carbon to higher trophic levels (Brum et al. 2014), whereas viral or parasitic lysis releases a portion of host dissolved organic matter (DOM), bypassing the traditional food web (Fuhrman 1999; Wilhem and Suttle 1999). Highly labile DOM released via host lysis enters the microbial loop, stimulating rapid bacterial production and the recycling of carbon and nutrients (Azam et al. 1983; Lønborg et al. 2013). Parasitism offers a more complex role in biogeochemical cycling, as unlike viruses, parasites incorporate up to half of their host biomass during infection, and following host lysis, release progeny (termed dinospores) that can be consumed by protist grazers (Johansson and Coats 2002). Quantifying how mortality is partitioned between microzooplankton grazing, viral lysis, and parasitism

will help to better inform model predictions of plankton population dynamics, as well as the transfer of organic matter through the food web.

Model predictions of *in situ* phytoplankton dynamics have been improved by the inclusion of microzooplankton grazing (and phytoplankton growth) rates measured via dilution assays (Landry et al. 2009; Li et al. 2011), however, such predictions often overlook temporal variability in rates. The research described in this dissertation has provided evidence of strong temporal variability in microzooplankton grazing rates over tidal and seasonal scales, as well as temporal shifts in parasite-host interactions.

Temporal shifts in phytoplankton mortality should be considered to accurately characterize plankton populations, especially within dynamic coastal regions (e.g., estuaries) that experience hourly-monthly periodicities in environmental factors and primary production (Cloern and Jassby 2012; Cloern et al. 2014). For instance, if only a single grazing rate measurement is used to parameterize daily phytoplankton mortality within a coastal food web model, this may not accurately capture variability in grazing between tides or seasons, misrepresenting model projections (Blauw et al. 2018). The ability to accurately monitor and predict primary production, carbon transfer, and ecosystem health of coastal systems will rely on continued measurements of phytoplankton mortality that account for different causes of mortality (grazing, viral lysis, and parasitism) over a range of temporal scales.

REFERENCES

- Azam, F., T. Fenchel, J. G. Field, J. C. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257-263. doi:10.3354/meps010257
- Bachvaroff, T. R., S. Kim, L. Guillou, C. F. Delwiche, and D. W. Coats. 2012. Molecular diversity of the syndinean genus *Euduboscquella* based on single-cell PCR analysis. *Appl. Environ. Microbiol.* 78: 334-345. doi:10.1128/AEM.06678-11
- Baudoux, A. C., M. J. W. Veldhuis, A. a. M. Noordeloos, G. Van Noort, and C. P. D. Brussaard. 2008. Estimates of virus- vs. grazing induced mortality of picophytoplankton in the North Sea during summer. *Aquat. Microb. Ecol.* 52: 69-82. doi:10.3354/ame01207
- Bissinger, J. E., D. J. S. Montagnes, J. Sharples, and D. Atkinson. 2008. Predicting marine phytoplankton maximum growth rates from temperature: Improving on the Eppley curve using quantile regression. *Limnol. Oceanogr.* 53: 487-493. doi:10.4319/lo.2008.53.2.0487
- Blauw, A. N., E. Benincà, R. W. Laane, N. Greenwood, and J. Huisman. 2018. Predictability and environmental drivers of chlorophyll fluctuations vary across different time scales and regions of the North Sea. *Prog. Oceanogr.* 161: 1-18. doi:10.1016/j.pocean.2018.01.005
- Breitbart, M. 2012. Marine viruses: Truth or dare. *Annu. Rev. Mar. Sci.* 4: 425-448. doi:10.1146/annurev-marine-120709-142805
- Brum, J. R., J. J. Morris, M. Décima, and M. R. Stukel. 2014. Mortality in the oceans: Causes and consequences, in *Eco-DAS IX Symposium Proceedings*, *Limnol. and Oceanogr.* 16-48. doi:10.4319/ecodas.2014.978-0-9845591-3-8.16
- Calbet, A., and M. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.* 49: 51-57. doi:10.4319/lo.2004.49.1.0051
- Chen, B., M. R. Landry, B. Huang, and H. Liu. 2012. Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? *Limnol. Oceanogr.* 57: 519-526. doi:10.4319/lo.2012.57.2.0519
- Cloern, J. E., and A. D. Jassby. 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. *Rev. Geophys.* 50: 1-33. doi:10.1029/2012RG000397

- Cloern, J. E., S. Q. Foster, and A. E. Kleckner. 2014. Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences* 11: 2477-2501. doi:10.5194/bg-11-2477-2014
- Coats, D. W. 1999. Parasitic life styles of marine dinoflagellates. *J. Eukaryot. Microbiol.* 46: 402-409. doi:10.1111/j.1550-7408.1999.tb04620.x
- Coats, D. W., and M. G. Park. 2002. Parasitism of photosynthetic dinoflagellates by three strains of *Amoebophrya* (Dinophyta): parasite survival, infectivity, generation time, and host specificity. *J. Phycol.* 38: 520-528. doi:10.1046/j.1529-8817.2002.01200.x
- Eppley, R. W. 1972. Temperature and Phytoplankton Growth in the Sea. *Fish. Bull.* 70: 1063-1085.
- Fuhrman, J. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* 399: 541-548. doi:10.1038/21119
- Guillou, L., and others. 2008. Widespread occurrence and genetic diversity of marine parasitoids belonging to syndiniales (alveolata). *Environ. Microbiol.* 10: 3349-3365. doi:10.1111/j.1462-2920.2008.01731.x
- Jacquet, S., and others. 2005. Estimates of protozoan- and viral-mediated mortality of bacterioplankton in Lake Bourget (France). *Freshw. Biol.* 50: 627-645. doi:10.1111/j.1365-2427.2005.01349.x
- Jephcott, T. G., and others. 2016. Ecological impacts of parasitic chytrids, syndiniales and perkinsids on populations of marine photosynthetic dinoflagellates. *Fungal Ecol.* 19: 47-58. doi:10.1016/j.funeco.2015.03.007
- Johansson, M. and D. W. Coats. 2002. Ciliate grazing on the parasite *Amoebophrya* sp. decreases infection of the red-tide dinoflagellate *Akashiwo sanguinea*. *Aquat. Microb. Ecol.* 28: 69-78. doi:10.3354/ame/028069
- Kimmance, S. A., W. H. Wilson, and S. D. Archer. 2007. Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: Limitations associated with method sensitivity in natural waters. *Aquat. Microb. Ecol.* 49: 207-222. doi:10.3354/ame01136
- Landry, M. R., M. D. Ohman, R. Goericke, M. R. Stukel, and K. Tsyrklevich. 2009. Lagrangian studies of phytoplankton growth and grazing relationships in a coastal upwelling ecosystem off Southern California. *Prog. Oceanogr.* 83: 208-216. doi:10.1016/j.pocean.2009.07.026
- Lawrence, C., and S. Menden-Deuer. 2012. Drivers of protistan grazing pressure: Seasonal signals of plankton community composition and environmental conditions. *Mar. Ecol. Prog. Ser.* 459: 39-52. doi:10.3354/meps09771

- Li, Q. P., P. J. S. Franks, and M. R. Landry. 2011. Microzooplankton grazing dynamics: parameterizing grazing models with dilution experiment data from the California Current Ecosystem. *Mar. Ecol. Prog. Ser.* 438: 59-69. doi:10.3354/meps09320
- Lønborg, C., M. Middelboe, and C. P. D. Brussaard. 2013. Viral lysis of *Micromonas pusilla*: Impacts on dissolved organic matter production and composition. *Biogeochemistry* 116: 231-240. doi:10.1007/s10533-013-9853-1
- Montagnes, D. J., A. Chambouvet, L. Guillou, and A. Fenton. 2008. Responsibility of microzooplankton and parasite pressure for the demise of toxic dinoflagellate blooms. *Aquat. Microb. Ecol.* 53: 211-225. doi:10.3354/ame01245
- Park, M. G., S. Kim, E. Y. Shin, W. Yih and D. W. Coats. 2013. Parasitism of harmful dinoflagellates in Korean coastal waters. *Harmful Algae*. 30: S62-S74. doi:10.1016/j.hal.2013.10.007
- Schmoker, C., S. Hernandez-Leon, and A. Calbet. 2013. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. *J. Plankton Res.* 35: 691-706. doi:10.1093/plankt/fbt023
- Staniewski, M. A., and S. M. Short. 2018. Methodological review and meta-analysis of dilution assays for estimates of virus- and grazer-mediated phytoplankton mortality. *Limnol. Oceanogr. Methods* 649-668. doi:10.1002/lom3.10273
- Steinberg, D. K., and M. R. Landry. 2017. Zooplankton and the Ocean Carbon Cycle. *Annu. Rev. Mar. Sci.* 9: 413-444. doi:10.1146/annurev-marine-010814-015924
- Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol. Oceanogr.* 53: 886-895. doi:10.4319/lo.2007.52.2.0886
- Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. *J. Eukaryot. Microbiol.* 51: 156-168. doi:10.1111/j.1550-7408.2004.tb00540.x
- Torres-Beltrán, M., T. Sehein, M. G. Pachiadaki, S. J. Hallam, and V. Edgcomb. 2018. Protistan parasites along oxygen gradients in a seasonally anoxic fjord: a network approach to assessing potential host-parasite interactions. *Deep-Sea Res. Pt II.* 156: 97-110. doi:10.1016/j.dsr2.2017.12.026
- Wilhelm, S. W., and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. *BioScience* 49: 781-788. doi:10.2307/1313569
- Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman, and P. J. Keeling. 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science* 347: 735. doi:10.1126/science.1257594

APPENDIX A

CHAPTER 2 SUPPLEMENTAL MATERIAL¹

¹ Anderson SR, Diou-Cass QP, and Harvey EL. 2018. Limnology and Oceanography 63: 2411-2422. Reprinted with permission of publisher.

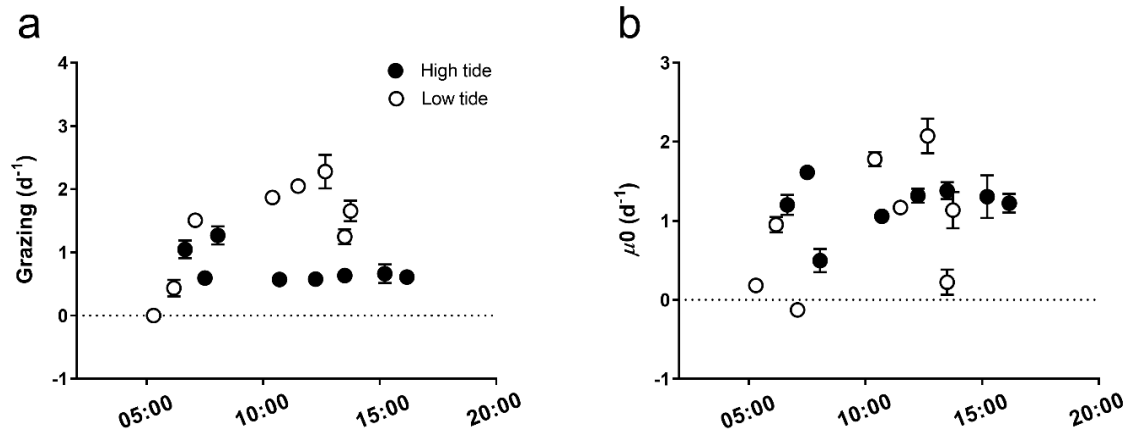


Figure 2.S1: Chlorophyll-based microzooplankton grazing rates (g ; **a**) and instantaneous growth rates (μ_0 ; **b**) versus sampling time of day (Eastern Standard Time) at high (black) and low (white) tides over sampling period. Error bars represent one standard deviation of the mean.

Table 2.S1: Daily rates of *Synechococcus* spp. instantaneous growth (μ_0), nutrient-amended growth (μ_n), grazing (g), viral lysis (v), and net accumulation (r) at low (L) and high tide (H) experiments. Standard deviation (± 1) of triplicate rates shown. Rates which were significantly different ($p < 0.05$) between same-day high and low tides are in bold.

Date	Tide	μ_0	μ_n	g	v	r
6/22/2017	L	0.98 (0.14)	1.4 (0.05)	0.61 (0.14)	0	0.38 (0.06)
	H	1.29 (0.1)	1.1 (0.14)	0	0	1.29 (0.1)
6/28/2017	L	1.18 (0.12)	2.18 (0.07)	1.11 (0.32)	0	0.06 (0.26)
	H	1.76 (0.18)	1.91 (0.04)	0	0.57 (0.11)	1.19 (0.07)
7/6/2017	L	1.83 (0.43)	2.44 (0.18)	2.67 (0.3)	0	-0.84 (0.15)
	H	0.45 (0.01)	1.7 (0.07)	0	0.43 (0.17)	0.01 (0.16)
7/21/2017	L	2.86 (0.46)	3.21 (0.11)	3.77 (0.26)	0	-0.92 (0.23)
	H	-0.5 (0.06)	1.78 (0.17)	0	0	-0.50 (0.06)
7/27/2017	L	2.01 (0.09)	2.04 (0.08)	0	0.8 (0.07)	1.21 (0.04)
	H	1.2 (0.17)	1.09 (0.02)	0	0.29 (0.04)	0.91 (0.14)
8/3/2017	L	1.30 (0.21)	1.49 (0.09)	2.15 (0.14)	0	-0.85 (0.12)
	H	0.87 (0.12)	1.05 (0.11)	0	0	0.87 (0.12)
8/10/2017	L	1.13 (0.57)	1.35 (0.16)	1.11 (0.35)	0	0.03 (0.3)
	H	0.65 (0.21)	0.76 (0.13)	0	0	0.65 (0.21)
8/17/2017	L	1.30 (0.46)	1.78 (0.14)	2.48 (0.32)	0	-1.18 (0.34)
	H	0.98 (0.43)	1.03 (0.1)	1.51 (0.3)	0	-0.53 (0.27)

Table S2.2: Daily rates of picoeukaryote instantaneous growth (μ_0), nutrient-amended growth (μ_n), grazing (g), viral lysis (v), and net accumulation (r) at low (L) and high tide (H) experiments. Standard deviation (± 1) of triplicate rates shown. Rates which were significantly different ($p < 0.05$) between same-day high and low tides are in bold.

Date	Tide	μ_0	μ_n	g	v	r
6/22/2017	L	2.23 (0.12)	3.08 (0.04)	1.55 (0.09)	0	0.68 (0.03)
	H	2.26 (0.12)	2.37 (0.06)	0.65 (0.08)	0	1.61 (0.05)
6/28/2017	L	1.51 (0.23)	2.04 (0.06)	1.86 (0.2)	0	-0.36 (0.06)
	H	2.35 (0.12)	2.73 (0.08)	0.99 (0.13)	0	1.36 (0.02)
7/6/2017	L	2.52 (0.12)	2.88 (0.02)	2.04 (0.06)	0	0.48 (0.08)
	H	1.44 (0.08)	2.7 (0.05)	1.06 (0.08)	0	0.38 (0.04)
7/21/2017	L	3.47 (0.17)	3.88 (0.09)	2.61 (0.13)	0	0.85 (0.09)
	H	2.43 (0.01)	3.17 (0.02)	1.51 (0.04)	0	0.92 (0.04)
7/27/2017	L	2.05 (0.05)	2.43 (0.02)	0.74(0.07)	0.37 (0.05)	1.31 (0.05)
	H	1.94 (0.1)	2.06 (0.07)	0.5 (0.05)	0	1.44 (0.08)
8/3/2017	L	0.36 (0.32)	0.77 (0.15)	0.88 (0.18)	0	-0.52 (0.14)
	H	0.8 (0.17)	1.26 (0.04)	0	0.36 (0.1)	0.45 (0.08)
8/10/2017	L	0.57 (0.11)	0.72 (0.05)	0	0	0.57 (0.11)
	H	1.9 (0.14)	1.97 (0.12)	0.5 (0.13)	0	1.4 (0.08)
8/17/2017	L	1.49 (0.17)	1.69 (0.18)	1.2 (0.07)	0	0.29 (0.12)
	H	1.9 (0.11)	2.18 (0.05)	0.9 (0.08)	0	1.01 (0.05)

Table S2.3: Daily rates of nanoeukaryote instantaneous growth (μ_0), nutrient-amended growth (μ_n), grazing (g), and net accumulation (r) at low (L) and high tide (H) experiments. Standard deviation (± 1) of triplicate rates shown. Rates which were significantly different ($p < 0.05$) between same-day high and low tides are in bold. Viral-induced mortality was not detected for nanoeukaryotes in any experiment.

Date	Tide	μ_0	μ_n	g	r
6/22/2017	L	1.7 (0.12)	1.48 (0.09)	0	1.7 (0.12)
	H	2.18 (0.34)	2.3 (0.13)	0.65 (0.07)	1.52 (0.27)
6/28/2017	L	0.19 (0.17)	1.05 (0.21)	0	0.19 (0.17)
	H	1.79 (0.18)	2.08 (0.12)	0.82 (0.13)	0.97 (0.05)
7/6/2017	L	1.54 (0.26)	1.92 (0.3)	0.81 (0.28)	0.74 (0.06)
	H	0.61 (0.12)	1.6 (0.15)	0	0.61 (0.12)
7/21/2017	L	2.54 (0.37)	3.11 (0.21)	1.68 (0.4)	0.85 (0.07)
	H	1.53 (0.27)	2.28 (0.16)	0.95 (0.29)	0.57 (0.05)
7/27/2017	L	1.27 (0.04)	1.43 (0.23)	0	1.27 (0.04)
	H	1.64 (0.05)	1.93 (0.06)	0.32 (0.1)	1.33 (0.08)
8/3/2017	L	1.31 (0.26)	1.93 (0.19)	0.63 (0.23)	0.68 (0.09)
	H	1.37 (0.02)	1.67 (0.21)	0	1.37 (0.02)
8/10/2017	L	1.24 (0.05)	1.33 (0.02)	0	1.24 (0.05)
	H	0.9 (0.14)	1.38 (0.16)	0	0.9 (0.14)
8/17/2017	L	2.1 (0.06)	2.44 (0.05)	0.83 (0.06)	1.27 (0.01)
	H	1.04 (0.07)	1.33 (0.06)	0	1.04 (0.07)

APPENDIX B

CHAPTER 3 SUPPLEMENTAL MATERIAL¹

¹Anderson SR, and Harvey EL. 2019. *Frontiers in Marine Science* 6: 174. Reprinted with permission of publisher.

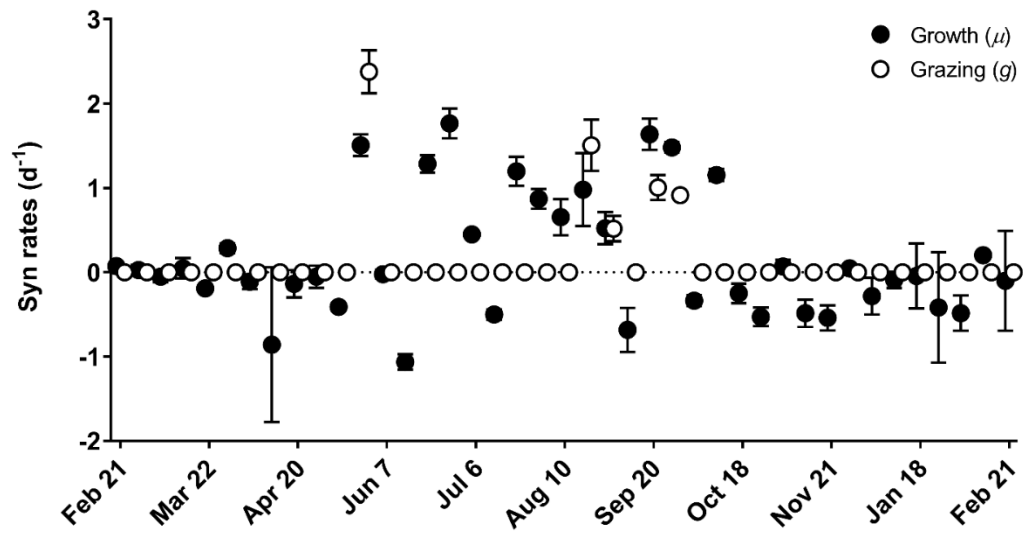


Figure S3.1: Rates (d^{-1}) of instantaneous phytoplankton growth (μ ; closed) and microzooplankton grazing (g ; open) for *Synechococcus* spp. over the year. Error bars represent one standard deviation of the mean and small error is contained within symbols. Grazing rates equal to zero (dotted line) were either negative or not significant from zero ($p < 0.05$).

Table S3.1: Weekly chlorophyll a concentration (Chl a; $\mu\text{g L}^{-1}$), temperature (T; $^{\circ}\text{C}$), salinity (S), and dissolved oxygen (DO; mg L^{-1}) in the estuary. Chlorophyll measured in triplicate samples (± 1 SD). Solar radiation (SR; MJ m^{-2}) measured on land at nearby weather station. Sampling time of day (TOD; eastern standard time) represents time of initial dilution set up.

Date	Chl a	T	S	DO	SR	TOD
2/21/2017	3.09 (0.24)	17	22.68	7.7	2.89	09:13
3/1/2017	3.45 (0.07)	18.96	25.62	7.74	9.72	09:52
3/7/2017	3.19 (0.28)	17.77	25.34	8.43	9.58	15:34
3/16/2017	4.05 (0.18)	13.56	27.34	9.84	13.49	11:45
3/22//2017	3.17 (0.13)	16.82	26.64	11.09	12.56	16:05
3/29/2017	5.14 (0.22)	19.82	27.7	8.98	14.09	09:50
4/6/2017	3.83 (0.11)	22.3	28.04	7.82	17.97	07:12
4/12/2017	3.91 (0.37)	20.36	28.84	9.49	17.41	09:33
4/20/2017	3.16 (0.08)	24.2	27.45	8.08	14.86	15:04
4/28/2017	1.94 (0.12)	24.01	28.81	4.29	19.21	09:14
5/3/2017	6.22 (0.22)	25.9	29.6	7.13	22.33	14:36
5/16/2017	6.2 (0.37)	25.78	28.8	7.7	22.34	13:22
6/7/2017	2.68 (0.08)	27.96	30.87	6.11	16.12	08:42
6/15/2017	5.41 (0.4)	28.03	28.89	7.69	19.86	13:35
6/22//2017	1.94 (0.06)	28.93	31.48	6.19	12.68	07:30
6/28/2017	3.56 (0.57)	28.7	31.85	5.49	17.86	13:30
7/6/2017	4.08 (0.3)	30.01	28.12	9.44	18.98	08:03
7/21/2017	6.38 (0.15)	30.23	30.15	6.22	16.39	06:39
7/27/2017	4.11 (0.19)	29.74	32.14	5.43	11.08	12:15
8/3/2017	4.59 (0.31)	29.13	29.94	7.75	15.47	15:13
8/10/2017	4.76 (0.18)	29.22	29.73	6.5	7.62	10:42
8/17/2017	5.32 (0.16)	31.23	29.28	6.82	18.51	16:10
8/23/2017	5.8 (0.58)	30.66	30.32	4.2	17.89	10:41
8/30/2017	2.47 (0.9)	27.02	26.58	21.67	10.79	16:31
9/20/2017	2.58 (0.48)	27.05	26.73	6.45	11.04	09:41
9/26/2017	2.74 (0.21)	28.13	28.79	7.7	11.03	14:56

10/4/2017	2.76 (0.19)	24.82	27.77	7.86	11.52	07:56
10/11/2017	2.65 (0.09)	28.1	29.23	6.85	11.13	14:15
10/18/2017	2.49 (0.1)	23.9	29.75	9.21	11.28	08:45
11/1/2017	2.66 (0.21)	19.4	29.97	12.37	8.63	07:24
11/8/2017	2.65 (0.08)	21.42	31.19	10.71	5.75	11:53
11/16/2017	2.23 (0.1)	16.43	30.53	13.37	6.48	07:53
11/21/2017	2.76 (0.2)	16.1	30.83	13.48	4.43	10:37
12/8/2017	3.17 (0.18)	16.95	30.82	12	0.86	10:48
12/14/2017	1.66 (0.09)	12.17	29.94	15.21	5.08	17:16
1/10/2018	2.67 (0.02)	6.15	29.28	19.45	4.81	15:29
1/18/2018	3.44 (0.21)	8.52	29.03	18.33	6.74	09:11
1/24/2018	2.07 (0.07)	9.71	29.3	17.2	6.58	12:50
2/1/2018	3.19 (0.08)	10.84	31	14.13	7.52	08:27
2/8/2018	2.31 (0.05)	12.79	29.02	14.86	3.45	13:10
2/21/2018	1.32 (0.07)	17.32	28.35	11.63	11.52	05:50

Table S3.2: Weekly nutrient concentrations (nitrate, NO₃; ammonium, NH₄; phosphate, PO₄; silicate, SiO₄; μM), total nitrogen (NO₃ + NH₄) to phosphate ratio (N:P), and particulate organic carbon (POC; μM C L⁻¹) and nitrogen (PON; μM N L⁻¹) measured in the estuary. Measurements not available (NA) over the first 2-3 weeks of sampling.

Date	NO ₃	NH ₄	PO ₄	N:P	SiO ₄	POC	PON
2/21/2017	NA	NA	NA	NA	NA	NA	NA
3/1/2017	NA	NA	NA	NA	NA	NA	NA
3/7/2017	0.39	1.74	0.75	2.85	56.71	NA	NA
3/16/2017	0.61	2.95	0.71	5.03	43.45	78.55	8.13
3/22//2017	0.01	1.39	0.55	2.53	43.45	68.64	12.83
3/29/2017	0.03	0.96	0.76	1.29	43.93	154.27	16.39
4/6/2017	0.25	2.13	0.51	4.64	50.28	74.56	13.77
4/12/2017	0.19	3.18	0.41	8.16	37.5	61.29	13.72
4/20/2017	0.17	1.13	0.55	2.37	55.72	50.22	13.4
4/28/2017	0.41	0.59	0.41	2.45	29.57	98.33	12.08
5/3/2017	0.63	0.48	0.57	1.96	52.25	56.71	12.09
5/16/2017	0.54	2.45	0.54	5.59	82.14	42.17	8.53
6/7/2017	0.68	1.3	0.72	2.76	91.04	52.57	11.92
6/15/2017	0.32	0.54	0.57	1.5	99.01	68.86	14.8
6/22//2017	0.6	2.14	0.76	3.59	113.06	81.42	18.26
6/28/2017	0.83	3.23	0.68	5.99	98.48	75.6	14.53
7/6/2017	0.58	0.59	0.54	2.19	131.36	80.09	14.1
7/21/2017	1.3	0.09	0.73	1.89	127.05	79.65	14.68
7/27/2017	0.77	0.19	0.82	1.18	121.86	70.61	11.19
8/3/2017	1.26	0	0.69	1.82	130.21	59.3	13.02
8/10/2017	1.51	0	0.69	2.18	139.24	69.3	12.69
8/17/2017	1.77	0	0.67	2.65	119.1	41.72	7.37
8/23/2017	1.5	0.35	0.73	2.54	119.65	38.91	10.53
8/30/2017	1.71	1.21	0.69	4.21	125.75	37.63	11.49
9/20/2017	0.75	2.85	0.36	10.05	81.62	56.33	7.33
9/26/2017	0.46	0.9	0.54	2.52	86.84	57.4	6.74

10/4/2017	0.75	1.63	0.73	3.27	82.66	79.48	7.01
10/11/2017	0.72	2.03	0.72	3.79	74.9	69.39	13.64
10/18/2017	0.96	2.18	0.71	4.44	82.14	62.67	7.8
11/1/2017	0.73	2.29	0.73	4.1	71.32	36.52	7.32
11/8/2017	0.55	2.04	0.69	3.76	62.21	41.71	6.37
11/16/2017	0.88	0.97	0.67	2.75	62.71	32.21	7.2
11/21/2017	0.35	3.25	0.65	5.55	59.2	53.17	6.22
12/8/2017	0.43	1.91	0.47	4.98	54.72	81.3	15.27
12/14/2017	0.91	4.82	0.53	10.77	58.7	34.36	8.16
1/10/2018	0.84	2.24	0.45	6.8	54.72	38.88	5.93
1/18/2018	0.84	4.99	0.57	10.23	39.1	58.26	7.16
1/24/2018	0.2	1.02	0.41	2.97	26.28	34.26	6.72
2/1/2018	0.34	2.36	0.37	7.29	18.38	80.48	6.76
2/8/2018	0.42	3.03	0.46	7.44	24.88	37.86	21.08
2/21/2018	0.49	3.86	0.62	6.97	34.79	40.86	11.3

Table S.3.3: Values of Global R statistic produced via similarity analysis (ANOSIM) to determine seasonal shifts in plankton composition in the estuary. Season comparisons ordered from highest to lowest R statistic; values closer to 1 indicate higher dissimilarity in composition between seasons. Differences in composition between any two seasons were significant (* $p < 0.05$; ** $p < 0.001$).

Groups	Global R Statistic
All Seasons	0.48**
Fall-Winter	0.64**
Spring-Fall	0.61**
Winter-Summer	0.52**
Summer-Fall	0.46**
Spring-Summer	0.37**
Winter-Spring	0.31*

Table S3.4: Percent contribution of the four most dominant plankton groups driving similarity within and dissimilarity between seasons (SIMPER), given average biomass estimates for each season (Biomass 1 vs. 2; $\mu\text{g C L}^{-1}$). Top four plankton groups contributed 48-71% of variability between seasons. Groups were identified down to genus or species level (diatoms and microzooplankton; FlowCam) or bulk phytoplankton group (flow cytometry).

Groups	Biomass 1	Biomass 2	Contribution%
Fall-Winter			
<i>Chaetoceros</i>	124.77	4.57	29.66
<i>Gyrodinium</i>	23.13	77.97	12.08
<i>A. sanguinea</i>	27.43	91.52	11.61
<i>Guinardia</i>	5.67	74.83	8.89
Spring-Fall			
<i>Chaetoceros</i>	0.62	124.77	33.47
<i>A. sanguinea</i>	45.49	91.52	14.93
<i>Gyrodinium</i>	15.3	77.97	13.52
<i>Guinardia</i>	0	74.83	8.77
Winter-Summer			
<i>A. sanguinea</i>	27.43	52.92	14.73
<i>Skeletonema</i>	21.48	26.71	12.15
Picoeukaryotes	44.93	48.45	12.07
<i>Gyrodinium</i>	23.13	13.62	9.22
Summer-Fall			
<i>Chaetoceros</i>	28.46	124.77	30.26
<i>Gyrodinium</i>	13.62	77.97	14.2
<i>A. sanguinea</i>	52.92	91.52	12.6
<i>Guinardia</i>	0	74.83	7.98
Spring-Summer			
<i>A. sanguinea</i>	45.49	52.92	21.75
Picoeukaryotes	48.03	48.45	10.73
<i>Skeletonema</i>	0.05	26.71	10.26
<i>Chaetoceros</i>	0.62	28.46	10.02
Winter-Spring			
<i>A. sanguinea</i>	27.43	45.49	20.09
Picoeukaryotes	44.93	48.03	16.68

Nanoeukaryotes	12.91	30.54	10.06
<i>Thalassiosira</i>	19.29	0.82	9.88

Table S3.5: Weekly chlorophyll-based rates (d^{-1}) of instantaneous growth (μ), nutrient-amended growth (μ_n), microzooplankton grazing (g), viral lysis (v), and net accumulation (r), as well as growth ratio (μ/μ_n). Standard deviation (± 1) of triplicate measurements shown. Measurements of μ_n were not available (NA) for the first experiment.

Date	μ	μ_n	μ/μ_n	g	v	r
2/21/2017	0.92 (0.07)	NA	NA	0.59 (0.15)	0	0.32 (0.14)
3/1/2017	0.83 (0.08)	1.04 (0.05)*	0.8 (0.09)	0.4 (0.04)	0	0.44 (0.11)
3/7/2017	0.58 (0.22)	0.65 (0.15)	0.87 (0.15)	0.49 (0.19)	0	0.09 (0.07)
3/16/2017	0.51 (0.13)	0.45 (0.05)	1.15 (0.37)	0.39 (0.11)	0	0.12 (0.16)
3/22/2017	0.15 (0.04)	0.93 (0.03)*	0.16 (0.04)	0.29 (0.05)	0	-0.14 (0.01)
3/29/2017	0.53 (0.16)	0.82 (0.07)*	0.66 (0.25)	0	0.52 (0.13)	0.01 (0.05)
4/6/2017	0.62 (0.05)	1.18 (0.02)*	0.53 (0.04)	0.39 (0.04)	0	0.23 (0.02)
4/12/2017	0.12 (0.03)	1.14 (0.03)*	0.1 (0.02)	0.43 (0.02)	0	-0.32 (0.02)
4/20/2017	0.62 (0.19)	1.61 (0.08)*	0.39 (0.1)	0.6 (0.11)	0	0.03 (0.08)
4/28/2017	0.26 (0.13)	1.1 (0.06)*	0.24 (0.13)	0	0	0.26 (0.13)
5/3/2017	0.34 (0.14)	1.22 (0.07)*	0.28 (0.11)	0.35 (0.12)	0	-0.01 (0.07)
5/16/2017	0.81 (0.13)	1.97 (0.14)*	0.41 (0.04)	1.43 (0.17)	0	-0.62 (0.05)
6/7/2017	0.61 (0.19)	0.58 (0.1)	1.04 (0.2)	0.69 (0.21)	0	-0.08 (0.11)
6/15/2017	1.12 (0.22)	2.28 (0.04)*	0.49 (0.09)	2.11 (0.22)	0	-0.99 (0.04)
6/22/2017	1.61 (0.03)	2.02 (0.02)*	0.8 (0.01)	0.59 (0.04)	0	1.02 (0.01)
6/28/2017	1.38 (0.11)	2.3 (0.06)*	0.6 (0.03)	0.63 (0.09)	0	0.75 (0.03)
7/6/2017	0.5 (0.15)	1.99 (0.04)*	0.25 (0.07)	1.27 (0.14)	0	-0.77 (0.04)
7/21/2017	1.2 (0.13)	1.95 (0.06)*	0.61 (0.06)	1.05 (0.14)	0	0.15 (0.04)
7/27/2017	1.32 (0.09)	1.96 (0.05)*	0.67 (0.04)	0.58 (0.06)	0	0.74 (0.03)
8/3/2017	1.31 (0.27)	2.32 (0.03)*	0.56 (0.11)	0.66 (0.15)	0	0.64 (0.12)
8/10/2017	1.06 (0.06)	1.76 (0.03)*	0.6 (0.02)	0.57 (0.04)	0	0.49 (0.02)
8/17/2017	1.22 (0.12)	2.2 (0.06)*	0.56 (0.04)	0.61 (0.09)	0	0.61 (0.03)
8/23/2017	1.46 (0.03)	2.26 (0.07)*	0.65 (0.02)	0.94 (0.05)	0	0.52 (0.02)
8/30/2017	0.87 (0.08)	1.37 (0.23)*	0.65 (0.16)	0	0.17 (0.05)	0.69 (0.03)
9/20/2017	1.45 (0.26)	1.73 (0.16)	0.84 (0.1)	0.81 (0.13)	0	0.65 (0.17)
9/26/2017	0.74 (0.11)	1.58 (0.18)*	0.48 (0.12)	0	0	0.74 (0.11)
10/4/2017	0.76 (0.01)	1.46 (0.29)*	0.53 (0.1)	0	0	0.76 (0.01)

10/11/2017	0.95 (0.04)	1.55 (0.16)*	0.62 (0.04)	0	0	0.95 (0.04)
10/18/2017	1.08 (0.1)	1.15 (0.07)	0.94 (0.03)	0.45 (0.1)	0	0.63 (0.01)
11/1/2017	0.4 (0.16)	0.72 (0.33)	0.77 (0.67)	0	0	0.41 (0.16)
11/8/2017	0.56 (0.04)	0.8 (0.15)	0.71 (0.1)	0	0	0.56 (0.04)
11/16/2017	0.29 (0.06)	0.51 (0.13)	0.6 (0.15)	0	0	0.29 (0.06)
11/21/2017	0.30 (0.03)	0.57 (0.09)*	0.54 (0.1)	0	0	0.3 (0.03)
12/8/2017	-0.02 (0.09)	0.35 (0.15)	-0.13 (0.31)	0	0	-0.02 (0.09)
12/14/2017	0.44 (0.05)	0.42 (0.05)	1.04 (0.03)	0.37 (0.04)	0	0.07 (0.02)
1/10/2018	0.08 (0.1)	0.33 (0.13)	0.2 (0.26)	0	0	0.08 (0.1)
1/18/2018	0.003 (0.05)	0.31 (0.04)*	-0.005 (0.17)	0.17 (0.04)	0	-0.17 (0.02)
1/24/2018	-0.3 (0.15)	0.35 (0.35)*	-0.42 (0.17)	0	0	-0.3 (0.15)
2/1/2018	-0.22 (0.04)	0.48 (0.1)*	-0.47 (0.1)	0	0	-0.22 (0.04)
2/8/2018	0.8 (0.05)	1.45 (0.01)*	0.55 (0.04)	0.91 (0.06)	0	-0.11 (0.04)
2/21/2018	0.77 (0.09)	0.99 (0.13)	0.78 (0.07)	0.33 (0.1)	0	0.44 (0.04)

* represents significant difference between μ and μ_n ($p < 0.05$)

Table S3.6: Weekly picoeukaryote rates (d^{-1}) of instantaneous growth (μ), nutrient-amended growth (μn), microzooplankton grazing (g), viral lysis (v), and net accumulation (r), along with the growth ratio ($\mu/\mu\text{n}$). Standard deviation (± 1) of triplicate rates shown. Measurements of μn were not available (NA) for the first experiment.

Date	μ	μn	$\mu/\mu\text{n}$	g	v	r
2/21/2017	0.99 (0.01)	NA	NA	0.52 (0.03)	0	0.47 (0.02)
3/1/2017	0.44 (0.09)	0.32 (0.19)	1.03 (0.39)	0	0	0.44 (0.09)
3/7/2017	0.47 (0.02)	0.5 (0.03)	0.95 (0.09)	0.33 (0.03)	0	0.13 (0.05)
3/16/2017	1.31 (0.12)	1.31 (0.04)	0.99 (0.07)	0.83 (0.08)	0	0.48 (0.04)
3/22//2017	0.5 (0.03)	0.93 (0.56)	0.66 (0.66)	0	0	0.5 (0.03)
3/29/2017	1.14 (0.04)	1.21 (0.04)	0.93 (0.03)	0.38 (0.05)	0	0.76 (0.02)
4/6/2017	0.81 (0.02)	0.7 (0.16)	1.19 (0.23)	0	0	0.81 (0.02)
4/12/2017	0.29 (0.05)	0.81 (0.07)*	0.36 (0.09)	0.36 (0.09)	0.52 (0.11)	-0.08 (0.14)
4/20/2017	0.51 (0.03)	1.1 (0.05)*	0.46 (0.01)	0.92 (0.01)	0	-0.41 (0.04)
4/28/2017	0.97 (0.08)	1.14 (0.12)	0.85 (0.03)	0.44 (0.09)	0	0.53 (0.02)
5/3/2017	0.23 (0.07)	1.16 (0.09)*	0.19 (0.05)	0	0	0.23 (0.08)
5/16/2017	0.52 (0.12)	1.42 (0.04)*	0.36 (0.08)	0.75 (0.11)	0	-0.24 (0.02)
6/7/2017	0.82 (0.07)	0.84 (0.02)	0.98 (0.06)	1.09 (0.07)	0	-0.27 (0.02)
6/15/2017	1.89 (0.15)	2.56 (0.14)*	0.74 (0.02)	1.72 (0.16)	0	0.17 (0.01)
6/22//2017	2.26 (0.12)	2.37 (0.06)	0.95 (0.03)	0.65 (0.08)	0	1.61 (0.05)
6/28/2017	2.35 (0.12)	2.73 (0.08)*	0.86 (0.02)	0.99 (0.13)	0	1.36 (0.02)
7/6/2017	1.44 (0.08)	2.7 (0.05)*	0.53 (0.02)	1.06 (0.08)	0	0.38 (0.04)
7/21/2017	2.43 (0.01)	3.17 (0.02)*	0.76 (0.01)	1.51 (0.04)	0	0.92 (0.04)
7/27/2017	1.94 (0.1)	2.06 (0.07)	0.94 (0.04)	0.5 (0.05)	0	1.44 (0.08)
8/3/2017	0.8 (0.17)	1.26 (0.04)*	0.71 (0.13)	0.23 (0.1)	0.36 (0.1)	0.45 (0.08)
8/10/2017	1.9 (0.14)	1.97 (0.12)	0.97 (0.03)	0.5 (0.13)	0	1.4 (0.08)
8/17/2017	1.9 (0.11)	2.18 (0.05)*	0.87 (0.03)	0.9 (0.08)	0	1.01 (0.05)
8/23/2017	1.97 (0.15)	2.16 (0.11)	0.91 (0.03)	0.67 (0.12)	0	1.3 (0.03)
8/30/2017	0.98 (0.01)	1.05 (0.2)	0.96 (0.18)	0	0.28 (0.06)	0.7 (0.06)
9/20/2017	1.71 (0.17)	1.81 (0.06)	0.95 (0.07)	0.62 (0.08)	0	1.09 (0.1)
9/26/2017	2.06 (0.12)	2.28 (0.07)	0.9 (0.02)	1.12 (0.05)	0	0.94 (0.07)
10/4/2017	0.87 (0.07)	1.29 (0.1)*	0.68 (0.01)	0	0	0.87 (0.07)

10/11/2017	1.84 (0.12)	1.87 (0.07)	0.99 (0.04)	0	0	1.57 (0.05)
10/18/2017	0.73 (0.06)	1.31 (0.15)*	0.56 (0.04)	0	0	0.73 (0.06)
11/1/2017	0.96 (0.23)	0.99 (0.1)	0.96 (0.13)	0.52 (0.14)	0	0.44 (0.1)
11/8/2017	0.58 (0.12)	0.57 (0.09)	1.01 (0.07)	0	0	0.3 (0.01)
11/16/2017	0.89 (0.07)	0.93 (0.07)	0.95 (0.04)	0.14 (0.04)	0	0.75 (0.03)
11/21/2017	0.44 (0.12)	0.43 (0.13)	1.03 (0.26)	0	0	0.15 (0.02)
12/8/2017	0.38 (0.11)	-0.09 (0.12)	-31.55 (41.05)	0	0	0.38 (0.11)
12/14/2017	-0.04 (0.04)	0.17 (0.09)	-0.33 (0.3)	0	0	-0.04 (0.04)
1/10/2018	0.66 (0.05)	0.49 (0.1)	1.4 (0.34)	0.35 (0.04)	0	0.32 (0.03)
1/18/2018	-0.01 (0.05)	-0.04 (0.17)	-0.15 (0.36)	0	0	-0.01 (0.05)
1/24/2018	0.26 (0.04)	0.32 (0.1)	0.89 (0.33)	0	0	0.26 (0.04)
2/1/2018	0.12 (0.05)	0.47 (0.06)*	0.25 (0.1)	0	0	0.12 (0.05)
2/8/2018	0.39 (0.19)	0.4 (0.08)	0.95 (0.33)	0.31 (0.09)	0	0.08 (0.1)
2/21/2018	0.52 (0.03)	0.75 (0.02)	1.09 (0.21)	0	0	0.52 (0.03)

* represents significant difference between μ and μ_n ($p < 0.05$)

Table S3.7: Weekly nanoeukaryote rates (d^{-1}) of instantaneous growth (μ), nutrient-amended growth (μn), microzooplankton grazing (g), viral lysis (v), and net accumulation (r), along with the growth ratio ($\mu/\mu\text{n}$). Standard deviation (± 1) of triplicate rates shown. Measurements of μn were not available (NA) for the first experiment.

Date	μ	μn	$\mu/\mu\text{n}$	g	v	r
2/21/2017	-0.07 (0.25)	NA	NA	0	0	-0.07 (0.25)
3/1/2017	0.02 (0.18)	0.11 (0.46)	-0.38 (0.82)	0	0	0.02 (0.18)
3/7/2017	-0.3 (0.04)	-0.52 (0.36)	0.43 (0.15)	0	0	-0.3 (0.04)
3/16/2017	0.49 (0.04)	0.65 (0.02)*	0.76 (0.04)	0.41 (0.15)	0	0.09 (0.11)
3/22/2017	1.22 (0.1)	-0.16 (0.06)*	-7.96 (2.27)	0	0.96 (0.04)	0.26 (0.06)
3/29/2017	0.58 (0.09)	0.62 (0.31)	1.12 (0.56)	0	0	0.58 (0.09)
4/6/2017	0.79 (0.06)	0.74 (0.06)	1.07 (0.07)	0	0.35 (0.07)	0.44 (0.13)
4/12/2017	0.42 (0.17)	0.95 (0.12)	0.45 (0.19)	0	0	0.42 (0.17)
4/20/2017	1.46 (0.26)	1.14 (0.23)	1.34 (0.45)	0	0.48 (0.18)	0.98 (0.07)
4/28/2017	1.48 (0.11)	1.77 (0.07)*	0.84 (0.03)	0.74 (0.16)	0.13 (0.04)	0.74 (0.09)
5/3/2017	0.7 (0.13)	1.69 (0.12)*	0.42 (0.11)	0	0	0.7 (0.13)
5/16/2017	0.43 (0.11)	1.56 (0.08)*	0.28 (0.06)	0.65 (0.12)	0	-0.22 (0.02)
6/7/2017	0.33 (0.17)	0.28 (0.2)	1.31 (0.54)	0.76 (0.29)	0	-0.44 (0.13)
6/15/2017	1.58 (0.38)	2.37 (0.14)*	0.66 (0.14)	1.72 (0.05)	0	-0.14 (0.34)
6/22/2017	2.18 (0.34)	2.3 (0.13)	0.94 (0.1)	0.65 (0.07)	0	1.52 (0.27)
6/28/2017	1.79 (0.18)	2.08 (0.12)	0.86 (0.06)	0.82 (0.13)	0	0.97 (0.05)
7/6/2017	0.61 (0.12)	1.6 (0.15)*	0.38 (0.07)	0	0	0.61 (0.12)
7/21/2017	1.53 (0.27)	2.28 (0.16)*	0.67 (0.07)	0.95 (0.29)	0	0.57 (0.05)
7/27/2017	1.64 (0.05)	1.93 (0.06)*	0.85 (0.01)	0.32 (0.1)	0	1.33 (0.08)
8/3/2017	1.37 (0.02)	1.66 (0.21)*	0.83 (0.11)	0	0	1.37 (0.02)
8/10/2017	0.9 (0.14)	1.38 (0.16)*	0.65 (0.03)	0	0	0.9 (0.14)
8/17/2017	1.04 (0.07)	1.33 (0.06)*	0.79 (0.08)	0	0	1.04 (0.07)
8/23/2017	1.42 (0.08)	1.81 (0.04)*	0.79 (0.06)	0	0	1.42 (0.08)
8/30/2017	1.12 (0.08)	1.12 (0.32)	1.06 (0.32)	0	0	1.12 (0.08)
9/20/2017	1.19 (0.11)	1.41 (0.15)	0.85 (0.17)	0	0	1.19 (0.11)
9/26/2017	1.71 (0.26)	1.93 (0.05)	0.89 (0.12)	0.63 (0.21)	0	1.08 (0.05)
10/4/2017	1.96 (0.21)	1.91 (0.31)	1.04 (0.06)	0.96 (0.29)	0	1 (0.08)

10/11/2017	1.39 (0.13)	1.71 (0.17)	0.82 (0.11)	0	0	1.39 (0.13)
10/18/2017	1.19 (0.1)	1.17 (0.21)	1.04 (0.25)	0	0	1.19 (0.1)
11/1/2017	0.83 (0.06)	0.69 (0.13)	1.23 (0.22)	0	0	0.83 (0.06)
11/8/2017	0.48 (0.05)	0.66 (0.05)*	0.73 (0.11)	0	0	0.48 (0.05)
11/16/2017	0.49 (0.12)	0.75 (0.18)	0.67 (0.23)	0	0	0.49 (0.12)
11/21/2017	0.3 (0.14)	0.15 (0.19)	0.93 (0.52)	0	0	0.3 (0.14)
12/8/2017	0.35 (0.15)	0.34 (0.13)	1.02 (0.03)	0.32 (0.03)	0	0.03 (0.12)
12/14/2017	-0.002 (0.1)	0.25 (0.07)*	-0.1 (0.5)	0	0	-0.002 (0.1)
1/10/2018	0.09 (0.1)	-0.12 (0.14)	-0.35 (0.8)	0	0	0.09 (0.1)
1/18/2018	0.34 (0.05)	0.28 (0.4)	5.79 (6.08)	0	0	0.34 (0.05)
1/24/2018	0.64 (0.11)	0.5 (0.22)	1.45 (0.55)	0.38 (0.05)	0	0.26 (0.05)
2/1/2018	0.29 (0.08)	0.4 (0.19)	0.79 (0.22)	0	0	0.29 (0.08)
2/8/2018	0.36 (0.1)	0.54 (0.04)*	0.66 (0.19)	0	0	0.36 (0.1)
2/21/2018	0.68 (0.19)	1.17 (0.07)*	0.58 (0.17)	0	0	0.68 (0.19)

* represents significant difference between μ and μ_n ($p < 0.05$)

Table S3.8: Weekly *Synechococcus* spp. rates (d^{-1}) of instantaneous growth (μ), nutrient-amended growth (μn), microzooplankton grazing (g), viral lysis (v), and net accumulation (r) over the year in the estuary. Standard deviation (± 1) of triplicate rates shown. Measurements of μn were not available (NA) for the first experiment.

Date	μ	μn	g	v	r
2/21/2017	0.07 (0.02)	NA	0	0	0.07 (0.02)
3/1/2017	0.03 (0.06)	0.09 (0.2)	0	0	0.03 (0.06)
3/7/2017	-0.05 (0.07)	-0.01 (0.11)	0	0	-0.05 (0.07)
3/16/2017	0.05 (0.12)	0.29 (0.16)	0	0	0.05 (0.12)
3/22/2017	-0.19 (0.02)	0.07 (0.29)	0	0	-0.19 (0.02)
3/29/2017	0.28 (0.06)	-0.28 (0.09)*	0	0	0.28 (0.06)
4/6/2017	-0.11 (0.09)	-0.77 (0.36)*	0	0	-0.11 (0.09)
4/12/2017	-0.86 (0.92)	-0.5 (0.26)	0	0	-0.86 (0.92)
4/20/2017	-0.14 (0.16)	-0.31 (0.2)	0	0	-0.14 (0.16)
4/28/2017	-0.05 (0.13)	-0.83 (0.36)*	0	0	-0.05 (0.13)
5/3/2017	-0.41 (0.04)	-1.22 (0.07)*	0	0	-0.41 (0.04)
5/16/2017	1.51 (0.13)	2.31 (0.09)*	2.38 (0.26)	0	-0.87 (0.16)
6/7/2017	-0.02 (0.05)	-0.05 (0.07)	0	0	-0.02 (0.05)
6/15/2017	-1.06 (0.09)	0.57 (0.46)*	0	0	-1.06 (0.09)
6/22/2017	1.29 (0.1)	1.1 (0.14)	0	0	1.29 (0.1)
6/28/2017	1.76 (0.18)	1.91 (0.04)*	0	0.57 (0.11)	1.2 (0.07)
7/6/2017	0.45 (0.01)	1.7 (0.07)*	0	0.43 (0.17)	0.01 (0.16)
7/21/2017	-0.5 (0.06)	1.78 (0.17)*	0	0	-0.5 (0.06)
7/27/2017	1.2 (0.17)	1.09 (0.02)*	0	0.29 (0.04)	0.91 (0.14)
8/3/2017	0.87 (0.12)	1.05 (0.11)	0	0	0.87 (0.12)
8/10/2017	0.65 (0.21)	0.76 (0.13)	0	0	0.65 (0.21)
8/17/2017	0.98 (0.43)	1.03 (0.1)	1.51 (0.3)	0	-0.53 (0.27)
8/23/2017	0.52 (0.19)	0.63 (0.09)	0.52 (0.15)	0	0.01 (0.1)
8/30/2017	-0.68 (0.26)	0.16 (0.34)*	0	0	-0.68 (0.26)
9/20/2017	1.64 (0.19)	1.71 (0.08)	1 (0.15)	0	0.63 (0.06)
9/26/2017	1.48 (0.06)	1.72 (0.07)*	0.91 (0.04)	0	0.57 (0.03)
10/4/2017	-0.34 (0.06)	1.12 (0.53)*	0	0	-0.34 (0.06)

10/11/2017	1.15 (0.07)	0.95 (0.19)	0	0	1.15 (0.07)
10/18/2017	-0.25 (0.12)	-0.04 (0.23)	0	0	-0.25 (0.12)
11/1/2017	-0.53 (0.11)	-0.1 (0.13)*	0	0	-0.53 (0.11)
11/8/2017	0.07 (0.08)	0.16 (0.08)	0	0	0.07 (0.08)
11/16/2017	-0.49 (0.16)	-0.28 (0.34)	0	0	-0.49 (0.16)
11/21/2017	-0.54 (0.14)	0.05 (0.36)	0	0	-0.54 (0.14)
12/8/2017	0.05 (0.04)	-0.17 (0.42)	0	0	0.05 (0.04)
12/14/2017	-0.28 (0.22)	-0.17 (0.17)	0	0.3 (0.07)	-0.58 (0.29)
1/10/2018	-0.09 (0.1)	-1.08 (0.53)*	0	0	-0.09 (0.1)
1/18/2018	-0.04 (0.38)	-0.86 (0.5)	0	0	-0.04 (0.38)
1/24/2018	-0.42 (0.65)	-0.2 (0.89)	0	0	-0.42 (0.65)
2/1/2018	-0.49 (0.21)	-0.43 (0.41)	0	0	-0.49 (0.21)
2/8/2018	0.2 (0.04)	-4.09 (13.12)	0	0	0.2 (0.04)
2/21/2018	-0.1 (0.59)	-0.46 (1.08)	0	0	-0.1 (0.59)

* represents significant difference between μ and μ_n ($p < 0.05$)

Table S3.9: Variable Influence on the Projection (VIP) and regression coefficients (Coeff) of environmental and biological parameters included in PLS models for chlorophyll-based growth (μ ; d⁻¹) and grazing rates (g ; d⁻¹). VIPs > 1 (marked in bold) are most important to the model. POC/PON = Particulate organic carbon or nitrogen; Hdino = heterotrophic dinoflagellates; *A. sang* = *Akashiwo sanguinea*.

Parameter	VIP _{μ}	Coeff _{μ}	VIP _{g}	Coeff _{g}
Temperature	1.75	0.11	1.2	0.05
Salinity	0.55	-0.05	0.74	-0.03
Chlorophyll	0.9	-0.05	0.94	0.05
Nitrate	1.16	0.05	0.47	0.02
Ammonium	1.01	0.01	1.01	-0.01
Phosphate	1.24	0.06	1.24	-0.12
Silicate	1.79	0.13	1.43	0.14
POC	0.5	-0.02	1.12	-0.1
PON	0.57	0.02	0.71	0.03
Solar radiation	0.92	0.04	1.3	0.07
Diatoms	0.62	0.04	0.75	-0.02
Hdino	0.64	-0.02	0.96	-0.06
<i>A. sang</i>	0.12	-0.01	0.65	0.02

Table S3.10: Coefficient of determination (R^2) values from Model 1 linear regression of the most important parameters versus phytoplankton growth rates for chlorophyll (Chl), picoeukaryotes (Pico), and nanoeukaryotes (Nano). Sign of the slope (+,-) of each relationship shown. Parameters are ordered based on highest importance as determined by VIP value.

Parameter	VIP	Chl R^2	Pico R^2	Nano R^2
Silicate	1.79	0.56**(+)	0.51**(+)	0.26*(+)
Temperature	1.75	0.56**(+)	0.51**(+)	0.46**(+)
Phosphate	1.24	0.27*(+)	0.19*(+)	0.08(+)
Nitrate	1.16	0.26*(+)	0.17*(+)	0.04(+)
Ammonium	1.01	0.08(-)	0.21*(-)	0.17*(-)

* represents significance of regression ($p < 0.05$; ** $p < 0.001$)

Table S3.11: Coefficient of determination (R^2) values from Model 1 linear regression of the most important parameters versus microzooplankton grazing rates for chlorophyll (Chl), picoeukaryotes (Pico), and nanoeukaryotes (Nano). Sign of the slope (+,-) of each relationship shown. Parameters are ordered based on highest importance as determined by VIP value.

Parameter	VIP	Chl R^2	Pico R^2	Nano R^2
Silicate	1.43	0.21*(+)	0.24*(+)	0.05(-)
Solar	1.3	0.27*(+)	0.24*(+)	0.14*(+)
Phosphate	1.24	0.01(+)	0.02(+)	0.01(+)
Temperature	1.2	0.21*(+)	0.27*(+)	0.11*(+)
POC	1.12	0.01(-)	0.02(+)	0.06(+)
Ammonium	1.01	0.05(-)	0.13*(-)	0.04(-)

* represents significance of regression ($p < 0.05$; ** $p < 0.001$)

APPENDIX C

CHAPTER 4 SUPPLEMENTAL MATERIAL¹

¹Anderson SR, and Harvey EL. Submitted to ISME J: 10/7/19.

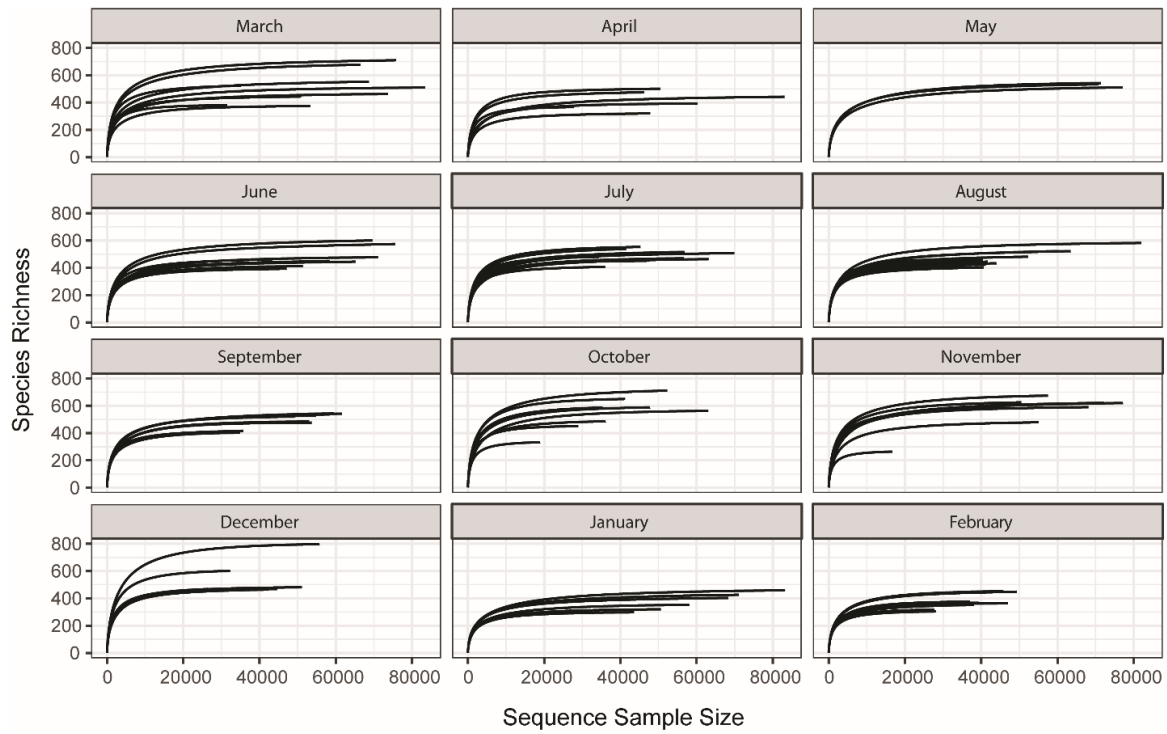
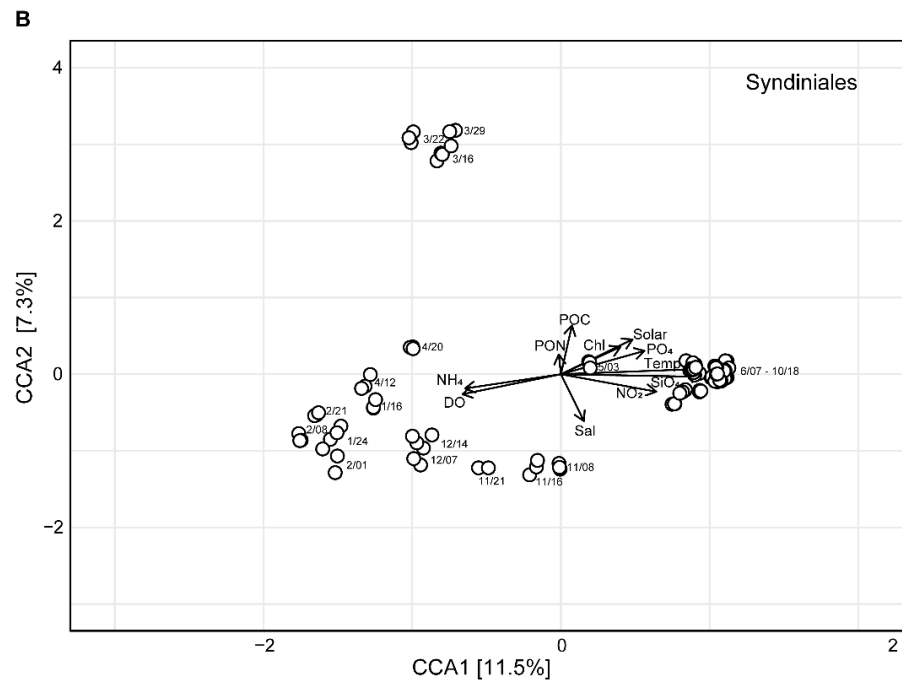
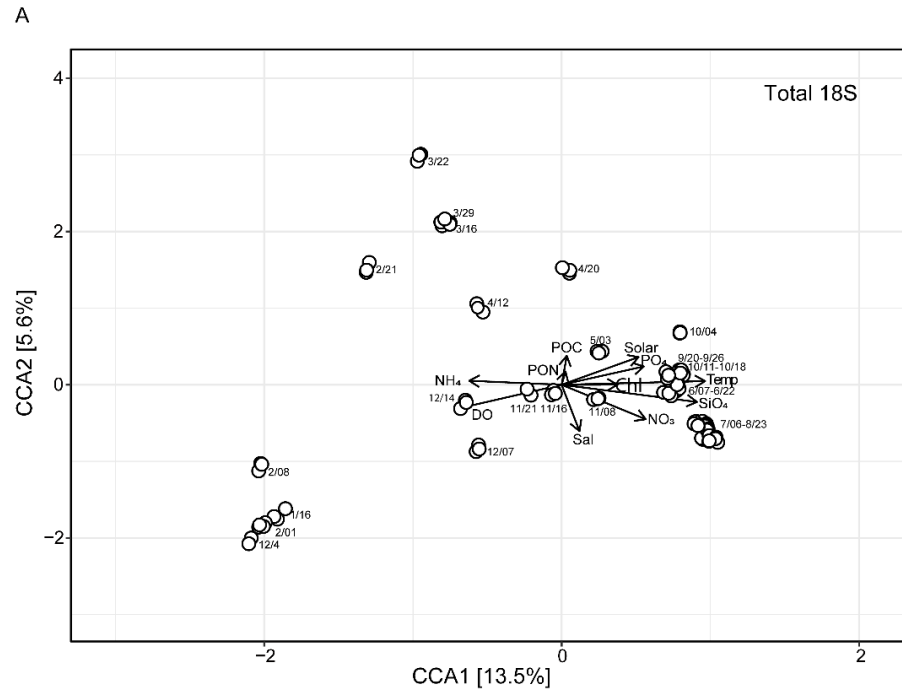


Figure S4.1: Rarefaction curves generated for the total 18S community. Curves are faceted by month and include all replicated samples within a given month.

Figure S4.2: Canonical correspondence analysis (CCA) based on total 18S composition **(a)** and Syndiniales composition **(b)**. Environmental factors are represented by arrows. Temp: Temperature ($^{\circ}\text{C}$), Sal: Salinity, SiO_4 : Silicate (μm), NO_3 : Nitrate (μm), NH_4 : Ammonium (μm), PO_4 : Phosphate (μm), Chl: Chlorophyll ($\mu\text{g L}^{-1}$), DO: Dissolved oxygen (mg L^{-1}), POC/PON: Particulate organic carbon or nitrogen ($\mu\text{g C or N L}^{-1}$), Solar: Solar radiation (MJ m^{-2}). Replicates are shown for each sampling day, with labeling reduced (i.e., one label per replicated sample or clustered grouping).



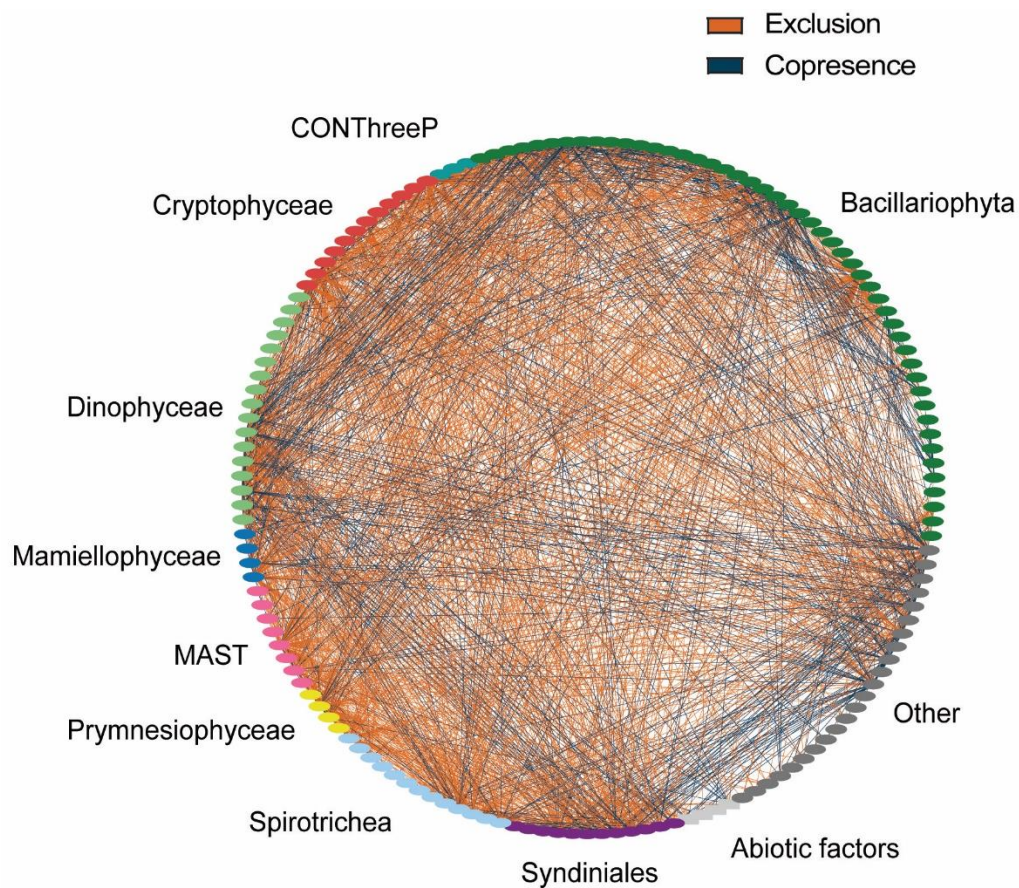


Figure S4.3: Overall co-occurrence network that includes significant (q -values < 0.05) positive (copresence; blue) and negative (exclusion; orange) edges between the most abundant 152 protist ASVs observed in the estuary over the year. Nodes were grouped by class (or abiotic factors) and poorly represented taxa were combined into “other” category. Full table with edge and node information will be accessible via online supplemental material.

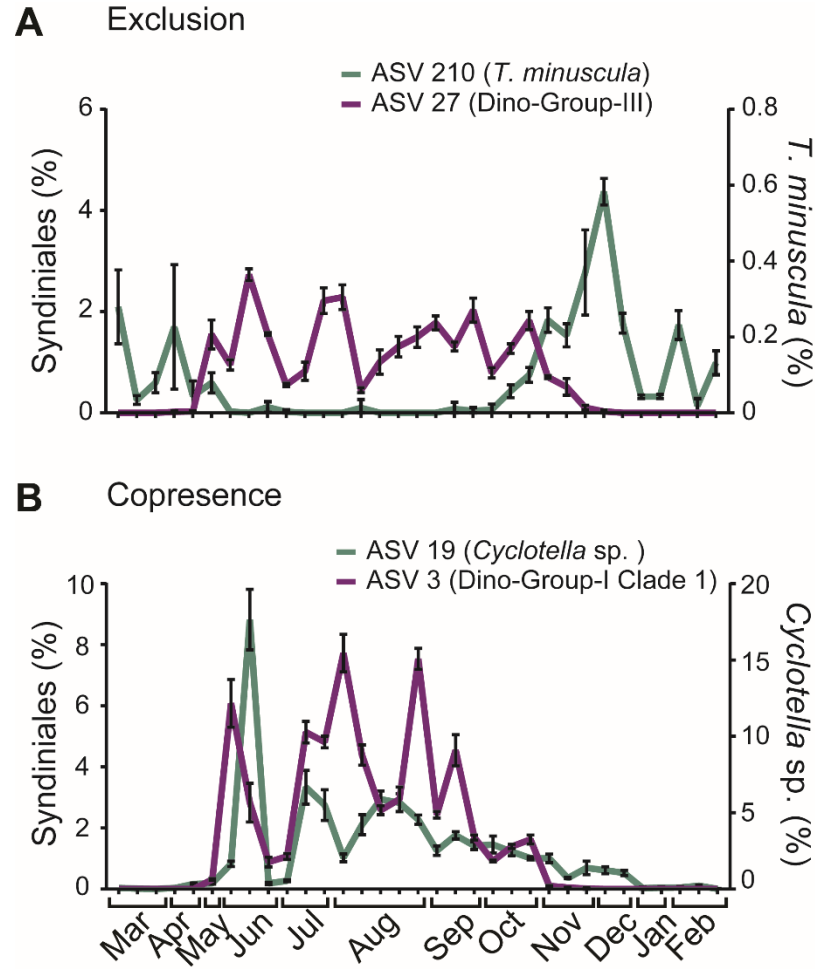


Figure S4.4: Relative abundance (%) plots of specific Syndiniales (purple) and Bacillariophyta (green) ASVs over the year in the estuary, representing single exclusion (**a**) or copresence relationships (**b**). All ASV associations were significant (q -values < 0.05) and derived from the filtered network. Error bars represent standard deviation of replicate sample means.

Table S4.1: Variable influence of the projection (VIP) values from predictor variables (environmental factors) and a single response variable (protist group) based on partial least squares (PLS) regression. POC/PON = Particulate organic carbon or nitrogen. Syn = Syndiniales; Dino = Dinophyceae; Crypto = Cryptophyceae; Bacill = Bacillariophyta; Spiro = Spirotrichea; Filo = Filosa-Thecofilosea. PLS models were run using average relative abundances. Most important VIP values (VIP > 1) are bolded.

Parameter	Syn	Dino	Crypto	Bacill	Spiro	Filo
Temperature	1.45	1.47	1.09	0.94	1.05	1.46
Salinity	0.27	0.37	0.8	0.91	0.7	0.21
Chlorophyll	0.96	1.15	0.82	1.16	0.89	1.1
Solar radiation	0.82	0.92	0.85	1.21	0.86	0.85
Dissolved oxygen	1.02	0.93	0.9	0.86	0.87	1.15
Silicate	1.57	1.16	0.97	1.03	1.22	1.45
Phosphate	0.88	1.08	1.44	0.92	1.37	0.86
Nitrate	1.1	1.2	0.68	1.32	0.7	0.86
Ammonium	1.02	0.88	1.47	1.15	0.82	1.07
POC	0.62	0.88	1.09	0.59	0.59	0.55
PON	0.57	0.42	0.42	0.62	1.49	0.74

Table S4.2: Significant edges ($q < 0.05$) determined from the filtered protist network, representing associations between protist groups (Node 1) and Syndiniales (Node 2). Nodes are described by ASV ID number and class level annotation. Edge type (copresence or mutual exclusion), number of methods supporting the edge (2-5), and merged q-value shown. Table is abbreviated here; full table will be available in supplemental material for article and will include species level annotation, method names (e.g., Pearson, Spearman, etc.), and method scores associated to each edge.

ASV		Class		Edge type	# Methods	q-value
Node 1	Node 2	Node 1	Node 2			
ASV 250	ASV 100	Acantharea	Syndiniales	copresence	2	0.016732
ASV 250	ASV 3	Acantharea	Syndiniales	copresence	5	0
ASV 250	ASV 167	Acantharea	Syndiniales	copresence	3	0
ASV 250	ASV 191	Acantharea	Syndiniales	copresence	4	0
ASV 250	ASV 27	Acantharea	Syndiniales	copresence	3	0
ASV 250	ASV 43	Acantharea	Syndiniales	copresence	4	0
ASV 250	ASV 50	Acantharea	Syndiniales	copresence	3	0
NA	ASV 43	Ammonium	Syndiniales	mutualExclusion	3	3.73E-04
NA	ASV 3	Ammonium	Syndiniales	mutualExclusion	4	7.15E-07
ASV 328	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 131	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	4	0
ASV 258	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	4	0
ASV 258	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 68	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 109	ASV 27	Bacillariophyta	Syndiniales	copresence	2	0
ASV 80	ASV 27	Bacillariophyta	Syndiniales	copresence	3	0
ASV 46	ASV 191	Bacillariophyta	Syndiniales	copresence	5	0
ASV 210	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 210	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 210	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	5	0
ASV 19	ASV 3	Bacillariophyta	Syndiniales	copresence	4	0
ASV 19	ASV 27	Bacillariophyta	Syndiniales	copresence	4	0
ASV 12	ASV 27	Bacillariophyta	Syndiniales	copresence	3	0

ASV 580	ASV 27	Bacillariophyta	Syndiniales	copresence	5	0
ASV 114	ASV 27	Bacillariophyta	Syndiniales	copresence	2	0
ASV 9	ASV 27	Bacillariophyta	Syndiniales	copresence	5	0
ASV 9	ASV 50	Bacillariophyta	Syndiniales	copresence	3	0
ASV 9	ASV 3	Bacillariophyta	Syndiniales	copresence	4	0
ASV 130	ASV 3	Bacillariophyta	Syndiniales	copresence	5	0
ASV 130	ASV 27	Bacillariophyta	Syndiniales	copresence	3	0
ASV 74	ASV 27	Bacillariophyta	Syndiniales	copresence	3	0
ASV 210	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	5	4.78E-16
ASV 328	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	4	1.41E-15
ASV 258	ASV 333	Bacillariophyta	Syndiniales	mutualExclusion	3	2.79E-15
ASV 47	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	3	9.48E-15
ASV 47	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	4	3.25E-14
ASV 258	ASV 386	Bacillariophyta	Syndiniales	mutualExclusion	3	4.06E-14
ASV 210	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	4	1.72E-12
ASV 47	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	3	6.42E-12
ASV 328	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	4	1.23E-10
ASV 68	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	4	2.47E-10
ASV 210	ASV 386	Bacillariophyta	Syndiniales	mutualExclusion	5	3.65E-09
ASV 47	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	3	5.68E-09
ASV 131	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	3	5.42E-08
ASV 131	ASV 333	Bacillariophyta	Syndiniales	mutualExclusion	3	1.42E-07
ASV 47	ASV 386	Bacillariophyta	Syndiniales	mutualExclusion	3	3.58E-07
ASV 328	ASV 191	Bacillariophyta	Syndiniales	mutualExclusion	2	1.10E-06
ASV 328	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	3	1.31E-06
ASV 68	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	2	1.35E-06
ASV 47	ASV 191	Bacillariophyta	Syndiniales	mutualExclusion	2	2.64E-06
ASV 328	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	4	2.79E-06
ASV 328	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	4	3.11E-06
ASV 502	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	2	1.28E-05

ASV 328	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	4	2.52E-05
ASV 502	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	2	3.69E-05
ASV 131	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	2	6.22E-05
ASV 239	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	3	2.94E-04
ASV 36	ASV 27	Bacillariophyta	Syndiniales	mutualExclusion	2	3.46E-04
ASV 502	ASV 386	Bacillariophyta	Syndiniales	mutualExclusion	3	7.37E-04
ASV 47	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	2	0.001312
ASV 502	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	2	0.001412
ASV 36	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	2	0.002752
ASV 68	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	2	0.003565
ASV 36	ASV 100	Bacillariophyta	Syndiniales	mutualExclusion	2	0.004333
ASV 502	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	2	0.014111
ASV 388	ASV 100	Bacillariophyta	Syndiniales	copresence	2	0.016751
ASV 114	ASV 167	Bacillariophyta	Syndiniales	copresence	2	0.048603
ASV 46	ASV 3	Bacillariophyta	Syndiniales	copresence	4	0.04952
ASV 328	ASV 66	Bacillariophyta	Syndiniales	mutualExclusion	2	0.04959
ASV 258	ASV 167	Bacillariophyta	Syndiniales	mutualExclusion	3	0
ASV 258	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	4	0
ASV 388	ASV 3	Bacillariophyta	Syndiniales	copresence	5	0
ASV 114	ASV 3	Bacillariophyta	Syndiniales	copresence	5	0
ASV 258	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	3	0
ASV 114	ASV 191	Bacillariophyta	Syndiniales	copresence	5	0
ASV 258	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	4	0
ASV 258	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	3	0
ASV 130	ASV 191	Bacillariophyta	Syndiniales	copresence	4	4.78E-16
ASV 131	ASV 3	Bacillariophyta	Syndiniales	mutualExclusion	3	5.05E-15
ASV 210	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	4	1.69E-13
ASV 131	ASV 50	Bacillariophyta	Syndiniales	mutualExclusion	3	3.57E-12
ASV 210	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	4	4.98E-12
ASV 210	ASV 191	Bacillariophyta	Syndiniales	mutualExclusion	4	4.23E-10

ASV 68	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	3	7.73E-10
ASV 258	ASV 191	Bacillariophyta	Syndiniales	mutualExclusion	2	8.71E-09
ASV 131	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	2	3.24E-08
ASV 502	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	2	3.65E-05
ASV 47	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	2	3.68E-05
ASV 131	ASV 77	Bacillariophyta	Syndiniales	mutualExclusion	2	6.23E-05
ASV 131	ASV 66	Bacillariophyta	Syndiniales	mutualExclusion	2	2.44E-04
ASV 258	ASV 66	Bacillariophyta	Syndiniales	mutualExclusion	2	3.50E-04
ASV 47	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	2	5.39E-04
ASV 73	ASV 43	Bacillariophyta	Syndiniales	mutualExclusion	2	5.50E-04
ASV 258	ASV 646	Bacillariophyta	Syndiniales	mutualExclusion	2	0.001568
ASV 241	ASV 333	Bicoecia	Syndiniales	mutualExclusion	2	0.018803
ASV 401	ASV 27	Chlorophyceae	Syndiniales	copresence	5	0
ASV 119	ASV 333	CONThreeP	Syndiniales	mutualExclusion	2	0.010496
ASV 119	ASV 167	CONThreeP	Syndiniales	mutualExclusion	2	0.004837
ASV 119	ASV 386	CONThreeP	Syndiniales	mutualExclusion	2	0.00482
ASV 119	ASV 50	CONThreeP	Syndiniales	mutualExclusion	2	0.00467
ASV 119	ASV 100	CONThreeP	Syndiniales	mutualExclusion	2	4.95E-05
ASV 119	ASV 43	CONThreeP	Syndiniales	mutualExclusion	2	3.48E-05
ASV 119	ASV 77	CONThreeP	Syndiniales	mutualExclusion	2	1.51E-05
ASV 119	ASV 3	CONThreeP	Syndiniales	mutualExclusion	2	4.72E-07
ASV 119	ASV 27	CONThreeP	Syndiniales	mutualExclusion	5	0
ASV 1018	ASV 333	Cryptophyceae	Syndiniales	mutualExclusion	2	0.020936
ASV 151	ASV 100	Cryptophyceae	Syndiniales	copresence	2	0.014971
ASV 17	ASV 100	Cryptophyceae	Syndiniales	mutualExclusion	2	0.001528
ASV 34	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	0.001444
ASV 1018	ASV 50	Cryptophyceae	Syndiniales	mutualExclusion	2	6.29E-04
ASV 1018	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	5.49E-04
ASV 34	ASV 333	Cryptophyceae	Syndiniales	mutualExclusion	2	2.44E-04
ASV 17	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	2	1.41E-04

ASV 54	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	2	1.86E-05
ASV 13	ASV 191	Cryptophyceae	Syndiniales	mutualExclusion	2	1.58E-05
ASV 1018	ASV 167	Cryptophyceae	Syndiniales	mutualExclusion	2	1.18E-05
ASV 26	ASV 50	Cryptophyceae	Syndiniales	mutualExclusion	2	7.17E-06
ASV 17	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	5.60E-06
ASV 26	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	1.22E-06
ASV 34	ASV 100	Cryptophyceae	Syndiniales	mutualExclusion	4	1.12E-06
ASV 26	ASV 191	Cryptophyceae	Syndiniales	mutualExclusion	2	5.94E-07
ASV 151	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	2	5.07E-07
ASV 23	ASV 167	Cryptophyceae	Syndiniales	mutualExclusion	2	4.07E-07
ASV 54	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	2.73E-07
ASV 17	ASV 167	Cryptophyceae	Syndiniales	mutualExclusion	3	2.63E-07
ASV 23	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	2	1.60E-07
ASV 1018	ASV 100	Cryptophyceae	Syndiniales	mutualExclusion	2	8.52E-08
ASV 17	ASV 191	Cryptophyceae	Syndiniales	mutualExclusion	2	3.55E-08
ASV 54	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	2	2.96E-08
ASV 23	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	3	2.61E-08
ASV 26	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	2	8.52E-09
ASV 1018	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	2	1.09E-09
ASV 26	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	2	9.82E-10
ASV 1018	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	3	4.91E-10
ASV 23	ASV 100	Cryptophyceae	Syndiniales	mutualExclusion	3	2.37E-10
ASV 34	ASV 167	Cryptophyceae	Syndiniales	mutualExclusion	4	7.12E-11
ASV 13	ASV 333	Cryptophyceae	Syndiniales	mutualExclusion	4	7.49E-12
ASV 34	ASV 50	Cryptophyceae	Syndiniales	mutualExclusion	3	6.52E-12
ASV 17	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	3	1.41E-12
ASV 17	ASV 50	Cryptophyceae	Syndiniales	mutualExclusion	3	2.60E-13
ASV 23	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	3	5.05E-15
ASV 17	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	3	0
ASV 13	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	5	0

ASV 151	ASV 3	Cryptophyceae	Syndiniales	copresence	3	0
ASV 13	ASV 386	Cryptophyceae	Syndiniales	mutualExclusion	5	0
ASV 13	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	4	0
ASV 151	ASV 27	Cryptophyceae	Syndiniales	copresence	4	0
ASV 13	ASV 50	Cryptophyceae	Syndiniales	mutualExclusion	5	0
ASV 151	ASV 167	Cryptophyceae	Syndiniales	copresence	5	0
ASV 151	ASV 191	Cryptophyceae	Syndiniales	copresence	5	0
ASV 13	ASV 100	Cryptophyceae	Syndiniales	mutualExclusion	5	0
ASV 13	ASV 167	Cryptophyceae	Syndiniales	mutualExclusion	5	0
ASV 34	ASV 3	Cryptophyceae	Syndiniales	mutualExclusion	3	0
ASV 34	ASV 27	Cryptophyceae	Syndiniales	mutualExclusion	3	0
ASV 401	ASV 167	Cryptophyceae	Syndiniales	copresence	4	0.011746
ASV 401	ASV 50	Cryptophyceae	Syndiniales	copresence	3	0
ASV 401	ASV 77	Cryptophyceae	Syndiniales	copresence	4	0
ASV 299	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	2	0.020951
ASV 26	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	2	0.013643
ASV 1018	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	2	0.012354
ASV 23	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	2	0.005409
ASV 34	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	3	0.002172
ASV 17	ASV 77	Cryptophyceae	Syndiniales	mutualExclusion	2	2.93E-04
ASV 1018	ASV 77	Cryptophyceae	Syndiniales	mutualExclusion	2	1.42E-04
ASV 34	ASV 77	Cryptophyceae	Syndiniales	mutualExclusion	2	1.30E-04
ASV 34	ASV 191	Cryptophyceae	Syndiniales	mutualExclusion	2	3.60E-05
ASV 13	ASV 43	Cryptophyceae	Syndiniales	mutualExclusion	4	1.72E-08
ASV 151	ASV 77	Cryptophyceae	Syndiniales	copresence	5	0
ASV 13	ASV 77	Cryptophyceae	Syndiniales	mutualExclusion	4	0
ASV 107	ASV 27	Dinophyceae	Syndiniales	copresence	5	0
ASV 107	ASV 50	Dinophyceae	Syndiniales	copresence	3	0
ASV 107	ASV 77	Dinophyceae	Syndiniales	copresence	5	0
ASV 107	ASV 100	Dinophyceae	Syndiniales	copresence	5	0

ASV 107	ASV 167	Dinophyceae	Syndiniales	copresence	5	0
ASV 107	ASV 3	Dinophyceae	Syndiniales	copresence	5	0
ASV 63	ASV 100	Dinophyceae	Syndiniales	copresence	3	0
ASV 63	ASV 167	Dinophyceae	Syndiniales	copresence	3	0
ASV 63	ASV 27	Dinophyceae	Syndiniales	copresence	3	0
ASV 32	ASV 167	Dinophyceae	Syndiniales	copresence	3	0
ASV 32	ASV 27	Dinophyceae	Syndiniales	copresence	4	0
ASV 24	ASV 27	Dinophyceae	Syndiniales	mutualExclusion	5	0
ASV 24	ASV 50	Dinophyceae	Syndiniales	mutualExclusion	4	0
ASV 24	ASV 100	Dinophyceae	Syndiniales	mutualExclusion	4	0
ASV 24	ASV 167	Dinophyceae	Syndiniales	mutualExclusion	4	0
ASV 249	ASV 27	Dinophyceae	Syndiniales	copresence	5	0
ASV 249	ASV 50	Dinophyceae	Syndiniales	copresence	3	0
ASV 249	ASV 3	Dinophyceae	Syndiniales	copresence	3	0
ASV 24	ASV 3	Dinophyceae	Syndiniales	mutualExclusion	5	0
ASV 28	ASV 167	Dinophyceae	Syndiniales	copresence	5	0
ASV 28	ASV 27	Dinophyceae	Syndiniales	copresence	3	0
ASV 287	ASV 27	Dinophyceae	Syndiniales	copresence	4	0
ASV 24	ASV 386	Dinophyceae	Syndiniales	mutualExclusion	4	8.99E-12
ASV 24	ASV 191	Dinophyceae	Syndiniales	mutualExclusion	2	2.79E-04
ASV 249	ASV 100	Dinophyceae	Syndiniales	copresence	4	3.19E-04
ASV 336	ASV 43	Dinophyceae	Syndiniales	mutualExclusion	2	0.001466
ASV 159	ASV 100	Dinophyceae	Syndiniales	mutualExclusion	2	0.006425
ASV 32	ASV 100	Dinophyceae	Syndiniales	copresence	2	0.014946
ASV 249	ASV 167	Dinophyceae	Syndiniales	copresence	3	0.034662
ASV 287	ASV 100	Dinophyceae	Syndiniales	copresence	2	0.035653
ASV 32	ASV 3	Dinophyceae	Syndiniales	copresence	5	0
ASV 63	ASV 3	Dinophyceae	Syndiniales	copresence	5	0
ASV 32	ASV 50	Dinophyceae	Syndiniales	copresence	4	0
ASV 63	ASV 50	Dinophyceae	Syndiniales	copresence	3	0

ASV 63	ASV 191	Dinophyceae	Syndiniales	copresence	5	0
ASV 20	ASV 43	Dinophyceae	Syndiniales	copresence	4	0
ASV 24	ASV 43	Dinophyceae	Syndiniales	mutualExclusion	4	0
ASV 24	ASV 77	Dinophyceae	Syndiniales	mutualExclusion	4	0
ASV 28	ASV 77	Dinophyceae	Syndiniales	copresence	5	0
ASV 249	ASV 77	Dinophyceae	Syndiniales	copresence	4	0
ASV 159	ASV 3	Dinophyceae	Syndiniales	mutualExclusion	4	8.04E-13
ASV 159	ASV 43	Dinophyceae	Syndiniales	mutualExclusion	3	5.17E-09
ASV 135	ASV 3	Dinophyceae	Syndiniales	mutualExclusion	2	1.75E-06
ASV 63	ASV 77	Dinophyceae	Syndiniales	copresence	2	0.005535
ASV 32	ASV 77	Dinophyceae	Syndiniales	copresence	2	0.007002
ASV 406	ASV 386	Filosa-Thecofilosea	Syndiniales	copresence	5	0
ASV 406	ASV 3	Filosa-Thecofilosea	Syndiniales	copresence	4	0
ASV 406	ASV 27	Filosa-Thecofilosea	Syndiniales	copresence	5	0
ASV 406	ASV 50	Filosa-Thecofilosea	Syndiniales	copresence	5	0
ASV 129	ASV 43	Katablepharidaceae	Syndiniales	mutualExclusion	2	0.038708
ASV 129	ASV 66	Katablepharidaceae	Syndiniales	mutualExclusion	3	1.41E-05
ASV 5	ASV 333	Mamiellophyceae	Syndiniales	mutualExclusion	2	3.13E-06
ASV 5	ASV 386	Mamiellophyceae	Syndiniales	mutualExclusion	3	2.83E-09
ASV 1	ASV 27	Mamiellophyceae	Syndiniales	copresence	3	2.08E-13
ASV 5	ASV 100	Mamiellophyceae	Syndiniales	mutualExclusion	3	9.47E-16
ASV 5	ASV 3	Mamiellophyceae	Syndiniales	mutualExclusion	3	0
ASV 5	ASV 27	Mamiellophyceae	Syndiniales	mutualExclusion	3	0
ASV 5	ASV 50	Mamiellophyceae	Syndiniales	mutualExclusion	3	0
ASV 5	ASV 167	Mamiellophyceae	Syndiniales	mutualExclusion	3	0
ASV 5	ASV 43	Mamiellophyceae	Syndiniales	mutualExclusion	3	1.31E-07
ASV 5	ASV 191	Mamiellophyceae	Syndiniales	mutualExclusion	2	3.47E-09
ASV 5	ASV 77	Mamiellophyceae	Syndiniales	mutualExclusion	2	0
ASV 132	ASV 333	MAST	Syndiniales	mutualExclusion	2	4.74E-04
ASV 132	ASV 167	MAST	Syndiniales	mutualExclusion	4	8.46E-09

ASV 132	ASV 386	MAST	Syndiniales	mutualExclusion	4	1.38E-09
ASV 132	ASV 100	MAST	Syndiniales	mutualExclusion	4	8.25E-12
ASV 132	ASV 27	MAST	Syndiniales	mutualExclusion	5	0
ASV 132	ASV 50	MAST	Syndiniales	mutualExclusion	4	0
ASV 132	ASV 3	MAST	Syndiniales	mutualExclusion	4	0
ASV 412	ASV 27	MAST	Syndiniales	copresence	5	0
ASV 412	ASV 167	MAST	Syndiniales	copresence	4	0.037277
ASV 132	ASV 66	MAST	Syndiniales	mutualExclusion	2	0.032296
ASV 132	ASV 646	MAST	Syndiniales	mutualExclusion	2	0.002391
ASV 123	ASV 43	MAST	Syndiniales	mutualExclusion	2	1.34E-05
ASV 123	ASV 3	MAST	Syndiniales	mutualExclusion	2	7.36E-07
ASV 132	ASV 77	MAST	Syndiniales	mutualExclusion	4	1.38E-09
ASV 132	ASV 191	MAST	Syndiniales	mutualExclusion	4	1.43E-14
ASV 132	ASV 43	MAST	Syndiniales	mutualExclusion	4	0
ASV 281	ASV 167	Nephroselmidophyceae	Syndiniales	copresence	4	0.035693
ASV 281	ASV 386	Nephroselmidophyceae	Syndiniales	copresence	5	0
ASV 281	ASV 3	Nephroselmidophyceae	Syndiniales	copresence	3	0
ASV 281	ASV 27	Nephroselmidophyceae	Syndiniales	copresence	5	0
ASV 221	ASV 386	Pedinophyceae	Syndiniales	copresence	4	0.002383
ASV 221	ASV 3	Pedinophyceae	Syndiniales	copresence	5	0
ASV 221	ASV 27	Pedinophyceae	Syndiniales	copresence	5	0
ASV 221	ASV 50	Pedinophyceae	Syndiniales	copresence	5	0
ASV 369	ASV 27	Porphyridiophyceae	Syndiniales	copresence	2	0
ASV 448	ASV 333	Prymnesiophyceae	Syndiniales	mutualExclusion	2	0.001262
ASV 448	ASV 646	Prymnesiophyceae	Syndiniales	mutualExclusion	2	8.50E-04
ASV 602	ASV 66	Prymnesiophyceae	Syndiniales	mutualExclusion	2	6.82E-04
ASV 448	ASV 66	Prymnesiophyceae	Syndiniales	mutualExclusion	2	5.02E-04
ASV 602	ASV 333	Prymnesiophyceae	Syndiniales	mutualExclusion	2	2.88E-04
ASV 602	ASV 100	Prymnesiophyceae	Syndiniales	mutualExclusion	3	2.58E-06
ASV 602	ASV 386	Prymnesiophyceae	Syndiniales	mutualExclusion	4	4.59E-10

ASV 448	ASV 386	Prymnesiophyceae	Syndiniales	mutualExclusion	4	5.42E-12
ASV 448	ASV 191	Prymnesiophyceae	Syndiniales	mutualExclusion	3	4.09E-12
ASV 602	ASV 77	Prymnesiophyceae	Syndiniales	mutualExclusion	3	1.30E-13
ASV 602	ASV 167	Prymnesiophyceae	Syndiniales	mutualExclusion	4	1.41E-15
ASV 209	ASV 3	Prymnesiophyceae	Syndiniales	mutualExclusion	4	4.78E-16
ASV 602	ASV 3	Prymnesiophyceae	Syndiniales	mutualExclusion	5	0
ASV 602	ASV 27	Prymnesiophyceae	Syndiniales	mutualExclusion	5	0
ASV 602	ASV 50	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 100	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 167	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 3	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 27	Prymnesiophyceae	Syndiniales	mutualExclusion	5	0
ASV 448	ASV 43	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 50	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 448	ASV 77	Prymnesiophyceae	Syndiniales	mutualExclusion	4	0
ASV 209	ASV 43	Prymnesiophyceae	Syndiniales	mutualExclusion	2	0.007132
ASV 348	ASV 191	Pyramimonadales	Syndiniales	copresence	3	5.84E-09
ASV 348	ASV 27	Pyramimonadales	Syndiniales	copresence	5	0
ASV 348	ASV 3	Pyramimonadales	Syndiniales	copresence	5	0
NA	ASV 27	Silicate	Syndiniales	copresence	5	1.40E-09
ASV 70	ASV 167	Spirotrichea	Syndiniales	mutualExclusion	2	0.045825
ASV 70	ASV 100	Spirotrichea	Syndiniales	mutualExclusion	2	0.001509
ASV 338	ASV 191	Spirotrichea	Syndiniales	copresence	4	0.001476
ASV 240	ASV 100	Spirotrichea	Syndiniales	mutualExclusion	2	2.66E-04
ASV 70	ASV 43	Spirotrichea	Syndiniales	mutualExclusion	2	2.25E-04
ASV 70	ASV 3	Spirotrichea	Syndiniales	mutualExclusion	3	6.48E-05
ASV 69	ASV 191	Spirotrichea	Syndiniales	mutualExclusion	3	3.73E-11
ASV 69	ASV 50	Spirotrichea	Syndiniales	mutualExclusion	4	9.91E-15
ASV 338	ASV 100	Spirotrichea	Syndiniales	copresence	3	0
ASV 338	ASV 167	Spirotrichea	Syndiniales	copresence	5	0

ASV 338	ASV 3	Spirotrichea	Syndiniales	copresence	5	0
ASV 338	ASV 27	Spirotrichea	Syndiniales	copresence	5	0
ASV 338	ASV 50	Spirotrichea	Syndiniales	copresence	4	0
ASV 338	ASV 77	Spirotrichea	Syndiniales	copresence	3	0
ASV 289	ASV 100	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 289	ASV 27	Spirotrichea	Syndiniales	mutualExclusion	5	0
ASV 69	ASV 100	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 69	ASV 167	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 69	ASV 3	Spirotrichea	Syndiniales	mutualExclusion	5	0
ASV 69	ASV 27	Spirotrichea	Syndiniales	mutualExclusion	5	0
ASV 69	ASV 386	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 75	ASV 27	Spirotrichea	Syndiniales	copresence	5	0
ASV 75	ASV 167	Spirotrichea	Syndiniales	copresence	3	0
ASV 69	ASV 66	Spirotrichea	Syndiniales	mutualExclusion	2	0.044314
ASV 289	ASV 66	Spirotrichea	Syndiniales	mutualExclusion	2	0.037913
ASV 240	ASV 3	Spirotrichea	Syndiniales	mutualExclusion	2	0.006712
ASV 332	ASV 43	Spirotrichea	Syndiniales	mutualExclusion	2	0.005152
ASV 289	ASV 191	Spirotrichea	Syndiniales	mutualExclusion	2	0.001095
ASV 289	ASV 333	Spirotrichea	Syndiniales	mutualExclusion	2	8.42E-04
ASV 332	ASV 3	Spirotrichea	Syndiniales	mutualExclusion	2	5.43E-06
ASV 289	ASV 386	Spirotrichea	Syndiniales	mutualExclusion	5	1.16E-08
ASV 289	ASV 77	Spirotrichea	Syndiniales	mutualExclusion	4	3.61E-12
ASV 289	ASV 50	Spirotrichea	Syndiniales	mutualExclusion	3	2.78E-12
ASV 289	ASV 43	Spirotrichea	Syndiniales	mutualExclusion	4	1.94E-13
ASV 289	ASV 167	Spirotrichea	Syndiniales	mutualExclusion	5	4.71E-14
ASV 289	ASV 3	Spirotrichea	Syndiniales	mutualExclusion	5	0
ASV 71	ASV 3	Spirotrichea	Syndiniales	copresence	5	0
ASV 75	ASV 3	Spirotrichea	Syndiniales	copresence	5	0
ASV 75	ASV 50	Spirotrichea	Syndiniales	copresence	3	0
ASV 71	ASV 27	Spirotrichea	Syndiniales	copresence	3	0

ASV 75	ASV 191	Spirotrichea	Syndiniales	copresence	4	0
ASV 69	ASV 43	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 69	ASV 77	Spirotrichea	Syndiniales	mutualExclusion	4	0
ASV 75	ASV 77	Spirotrichea	Syndiniales	copresence	3	0
ASV 71	AS 100	Spirotrichea	Syndiniales	copresence	4	0
ASV 27	ASV 167	Syndiniales	Syndiniales	copresence	5	0
ASV 100	ASV 167	Syndiniales	Syndiniales	copresence	5	0
ASV 27	ASV 386	Syndiniales	Syndiniales	copresence	5	0
ASV 100	ASV 3	Syndiniales	Syndiniales	copresence	4	0
ASV 27	ASV 3	Syndiniales	Syndiniales	copresence	5	0
ASV 167	ASV 3	Syndiniales	Syndiniales	copresence	3	0
ASV 333	ASV 50	Syndiniales	Syndiniales	copresence	5	0
ASV 167	ASV 50	Syndiniales	Syndiniales	copresence	3	0
ASV 3	ASV 50	Syndiniales	Syndiniales	copresence	5	0
ASV 27	ASV 50	Syndiniales	Syndiniales	copresence	4	0
ASV 100	ASV 27	Syndiniales	Syndiniales	copresence	5	0
ASV 3	ASV 191	Syndiniales	Syndiniales	copresence	5	0
ASV 27	ASV 77	Syndiniales	Syndiniales	copresence	3	0
ASV 100	ASV 77	Syndiniales	Syndiniales	copresence	4	0
ASV 167	ASV 77	Syndiniales	Syndiniales	copresence	5	0
NA	ASV 77	Temp	Syndiniales	copresence	2	0.002685
NA	ASV 191	Temp	Syndiniales	copresence	2	0.013178
NA	ASV 43	Temp	Syndiniales	copresence	2	0.003888
ASV 361	ASV 3	Trebouxiophyceae	Syndiniales	copresence	5	0
ASV 361	ASV 50	Trebouxiophyceae	Syndiniales	copresence	4	0
ASV 361	ASV 77	Trebouxiophyceae	Syndiniales	copresence	3	0
ASV 361	ASV 386	Trebouxiophyceae	Syndiniales	copresence	4	0.003655
ASV 361	ASV 27	Trebouxiophyceae	Syndiniales	copresence	5	0
ASV 361	ASV 100	Trebouxiophyceae	Syndiniales	copresence	3	0
ASV 361	ASV 167	Trebouxiophyceae	Syndiniales	copresence	5	0

Table S4.3: Annotation results via NCBI BLASTn of dominant Syndiniales ASVs included in the filtered network. For each successful ASV annotation, query cover (%), E-value, and percent identity (%) are shown. Literature references associated with each annotation are marked by lettered superscripts (a-c).

ASV	Species	NCBI	Query	E-value	% Identity
3	Dino-Group-I-Clade-1_sp.	Unassigned	na	na	na
27	Dino-Group-III_sp.	Unassigned	na	na	na
43	Dino-Group-II-Clade-3_sp.	<i>Amoebophyra</i> sp. ex. <i>Akashiwo sanguinea</i> ^a	100%	0	100%
50	Dino-Group-II-Clade-1_sp.	<i>Amoebophyra</i> sp. ex. <i>Karlodinium micrum</i> ^b	100%	2.00E-141	90%
66	Dino-Group-I-Clade-4_sp.	Unassigned	na	na	na
77	Dino-Group-II-Clade-12_sp.	<i>Amoebophyra</i> sp. ex. <i>Cochlodinium polykrikoides</i> ^c	100%	0	99.74%
100	Dino-Group-II-Clade-44_sp.	Unassigned	na	na	na
167	Dino-Group-II-Clade-14_sp.	Unassigned	na	na	na
191	Dino-Group-II-Clade-30_sp.	Unassigned	na	na	na
333	Dino-Group-II-Clade-17_sp.	Unassigned	na	na	na
386	Dino-Group-II-Clade-1_sp.	<i>Amoebophyra</i> sp. ex. <i>Karlodinium micrum</i> ^b	100%	7.00E-148	91.05%
646	Dino-Group-II-Clade-12_sp.	<i>Amoebophyra</i> sp. ex. <i>Cochlodinium polykrikoides</i> ^c	100%	0	98.16%

(a) Park et al. 2013; (b) Gunderson et al. 2002; (c) Kim and Park 2014