EFFECTS OF CHANGES IN SALINITY

Algal blooms in a river-dominated estuary and nearshore region of Florida, USA: the influence of regulated discharges from water control structures on hydrologic and nutrient conditions

E. J. Phlips · S. Badylak · A. L. Mathews · E. C. Milbrandt · L. R. Montefiore · E. S. Morrison · N. Nelson · B. Stelling

Abstract Flow-regulated discharges of water from control structures into estuaries result in hydrologic and water chemistry conditions that impact spatial and temporal variability in the structure and biomass of phytoplankton communities, including the potential for harmful algal blooms (HABs). The relationships between regulated Caloosahatchee River (i.e., C-43 Canal) discharges and phytoplankton communities in the Caloosahatchee Estuary and adjacent nearshore regions on the southwest coast of Florida were investigated during two study periods, 2009–2010 and 2018–2019. During periods of low to moderate discharge rates, when mesohaline conditions predominated in the estuary, and water residence times were comparatively long, major blooms of the HAB dinoflagellate species *Akashiwo sanguinea* were observed in the estuary. Periods of high discharge were characterized by comparatively low phytoplankton biomass in the estuary and greater influence of a wide range of freshwater taxa in the upper reaches. By contrast, intense blooms of the toxic dinoflagellate *Karenia brevis* in the nearshore region outside of the estuary were observed during high discharge periods in 2018–2019. The latter events were significantly associated with elevated levels of nitrogen in the estuary compared to lower average concentrations in the 2009–2010 study period. The relationships observed in this study provide insights into the importance of managing regulated discharge regimes to minimize adverse impacts of HABs on the health of the estuary and related coastal environments.

Keywords Caloosahatchee Estuary · Lake Okeechobee · Red tides · Karenia · Akashiwo · Eutrophication · Climate change

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Introduction

River-dominated estuaries are characterized by highly variable salinity regimes, hydrologic conditions, and plankton communities (Jassby et al., 1997; Lucas et al., 1999a, b; Cloern, 2001; Bledsoe et al., 2004; Quinlan & Phlips, 2007; Cloern & Jassby, 2008; Sin & Jeong, 2015). The dynamics of river flow strongly affect the structure and function of biological communities in the estuary and nearshore environment (Bunn & Arthington, 2002; Poff & Zimmermann, 2010; Van Niekerk et al., 2019; Chilton et al., 2021), including phytoplankton populations (Snow et al., 2000; Dorado et al., 2015; Cloern et al., 2017; Phlips et al., 2020). These dynamic elements are strongly influenced by climatic conditions and the character of watershed inputs (Lancelot & Muylaert, 2011; Hallett et al., 2018; Chilton et al., 2021). In many estuaries around the world, high rainfall periods are associated with enhanced nutrient-enriched river discharges, which can influence phytoplankton composition and biomass in both the estuary and associated coastal regions (Eyre, 2000; Paerl et al., 2006; Quinlan & Phlips, 2007; Costa et al., 2009; Lancelot & Muylaert, 2011; Glibert et al., 2014; Winder et al., 2017; Bharathi et al., 2018). In hydrologically restricted estuaries, periods of nutrient-rich inflows can result in algal blooms due to extended water residence times (Knoppers et al., 1991; Monbet, 1992; Phlips et al., 2004, 2015, 2021; Robson & Hamilton, 2004; Ralston et al., 2015). By contrast, in well-flushed estuaries, high rates of river discharge can result in declines in water residence times and water clarity, reducing the buildup of phytoplankton biomass, despite elevated nutrient levels (Mallin et al., 1998; Phlips et al., 2004; Dix et al., 2013; Hart et al., 2015; Wang et al., 2016; Phlips et al., 2020). In some estuaries, river discharges can contain high freshwater phytoplankton biomass resulting in the introduction of freshwater harmful algal blooms (HABs) (Phlips et al., 2012; Rosen et al., 2018; Metcalf et al., 2021). The latter scenario is illustrated by the re-occurring toxic cyanobacteria blooms observed in the St. Lucie estuary on the east coast of Florida and Caloosahatchee Estuary on the west coast, both of which emanate from canals connected to Lake Okeechobee, a lake subject to frequent intense cyanobacteria blooms (Phlips et al., 2012, 2020; Rosen et al., 2018).

The already complex and dynamic nature of river-dominated estuaries is further complicated in ecosystems associated with rivers containing engineered water control structures designed to manage water levels, flows, and navigation (Doering & Chamberlain, 1999; Sin et al., 2013; Sin & Jeong, 2015; Jo et al., 2019; Kim & Kim, 2020). The Caloosahatchee River and estuary is one such system. In the nineteenth century, the Caloosahatchee River was channelized to create the C-43 Canal and the canal was extended to connect with Lake Okeechobee in order to manage water levels in the lake and provide a navigable passage from the east to the west coasts of Florida (SFWMD, 2014). Connection of the lake to the Caloosahatchee Estuary via the C-43 Canal has significantly altered salinity gradients in the estuary. Due to the eutrophic character of Lake Okeechobee and high nutrient levels in the watersheds that feed directly into the C-43 Canal, the connection has increased nutrient loads to the estuary and coastal environment, as well as increased inter- and intra-annual variability in salinity and water residence times in the estuary (Doering & Chamberlain 1999; SFWMD 2009; Wan et al. 2013; Mathews et al., 2015; Sun et al. 2022). For example, periods of high discharge from the C-43 Canal via the S-79 control structure can turn much of the upper estuary fresh or oligohaline and reduce water residence times (Mathews et al., 2015). The frequent occurrence of intense cyanobacteria blooms in Lake Okeechobee (Phlips et al., 1993; Havens et al., 2016; Rosen et al., 2018) elevates the potential for introduction of high levels of toxic cyanobacteria into the estuary via the canal (Phlips et al., 2012, 2020; Metcalf et al., 2021). Downstream of the Lake's input, several tributaries from the watershed associated with the C-43 discharge water into the Canal, introducing additional nutrients to the canal (Doering & Chamberlain, 1999; Doering et al., 2006; SFWMD 2009). In addition, rapid human development in the watershed surrounding the Caloosahatchee Estuary proper has increased the area of impervious surface cover, adding to nutrient runoff into the estuary (Doering et al., 2006). Increases in nutrient loads to the estuary and nearshore regions of southwest Florida have raised concerns about the potential for intensification of harmful algal blooms, such as the toxic red tide events that frequent the eastern Gulf

of Mexico (Heil et al., 2014b; Medina et al., 2021, 2022).

The objective of this study was to examine changes in the character of phytoplankton communities in the Caloosahatchee Estuary and adjacent nearshore regions within the context of variability in salinity and nutrient levels associated with different flow-regulated discharge levels from the C-43 Canal into the estuary. Salinity, nutrient levels, and water residence times all play major roles in defining spatial and temporal patterns in the composition and biomass of phytoplankton in the region, including harmful bloom-forming species, such as red tides dominated by the toxic dinoflagellate *Karenia brevis* (Steidinger, 2009; Heil et al., 2014). It was hypothesized that the range of residence times in the estuary, which are closely tied to discharge rates from control structures upstream of the estuary, would play a major role in defining phytoplankton biomass, composition, and the potential for blooms. By contrast, the volume and nutrient concentrations in water leaving the estuary would define the potential impact on major nearshore blooms, such as red tides that frequent the west coast of Florida. The relationships observed in this study provide insights into the importance of managing regulated discharge regimes to minimize the occurrence, and intensity of HABs, not just in the Caloosahatchee estuary, but in similar systems around the world. Other studies have highlighted the need to better understand the unique challenges posed by the presence of water control structures and their impact on phytoplankton dynamics in estuaries (Sin et al., 2013). Expected future changes in rainfall levels, storm frequencies, and sea level driven by climate change will exacerbate the need to better understand these relationships in order to inform future management efforts.

Methods

Sampling site description

The Caloosahatchee Estuary is located on the southwest coast of Florida (Fig. 1) and has an area of 62 km². The upper boundary of the estuary is defined by the Franklin lock and dam water control structure (S-79), which is the point of release of water from the C-43 Canal. The width of the estuary ranges from

160 m near S-79 to 2,500 m at the entrance to San Carlos Bay (Scarlatos, 1988). Upstream of the lock, the C-43 Canal extends up to Lake Okeechobee, where the S-77 water control structure regulates water releases from the lake into the canal. Between the S-77 and S-79, there is an additional control structure (S-78), and several tributaries from the C-43 basin watershed that discharge into the C-43 Canal. The mouth of the lower estuary is bordered by the San Carlos Bay, which is open to the Gulf of Mexico.

The average depths through the Caloosahatchee Estuary are near 2.0 m, and a narrow navigation channel of 3–4 m depth extends from the S-79 through the estuary (Scarlatos, 1988). The estuary experiences a combination of diurnal and mixed semi-diurnal tides with a mean tidal range of 0.3 m in the middle of the estuary near downtown Fort Myers (Scarlatos, 1988; NOAA, 2010).

Freshwater from the C-43 Canal is released into the estuary through S-79 in order to maintain prescribed water levels in Lake Okeechobee and control flooding in the watersheds (C-43 basin) between Lake Okeechobee and the Caloosahatchee Estuary (Fig. 1) (Flaig & Capece, 1998; Doering & Chamberlain, 1999). The C-43 basin watershed covers 3,625 km² and is made up of agricultural areas in the east and urban areas surrounding the Caloosahatchee Estuary west of the S-79 (Knight & Steele, 2005). Additional sources of freshwater entering the estuary downstream of S-79 include surface water runoff and several regional tributaries, including Orange River, Popash Creek, and Whiskey Creek, which contribute to annual freshwater inputs to the estuary, although the inputs are relatively small compared to annual inputs from S-79 (Scarlatos, 1988; Flaig & Capece 1998; Knight & Steele, 2005; SFWMD, 2014).

Rainfall and S-79 discharge rates

Monthly rainfall levels at the Ft. Meyers Page Field meteorological station were obtained from the NOAA National Center for Environmental Data, Climatological Data—Florida (www.ncdc.noaa.gov). Daily rates of freshwater discharge from the S-79 Franklin Lock and Dam were obtained from DBHYDRO, a database maintained by the South Florida Water Management District (https://my.sfwmd.gov/dbhydroplsql/show_dbkey_info.main_menu). For statistical analyses of the relationships between discharge and
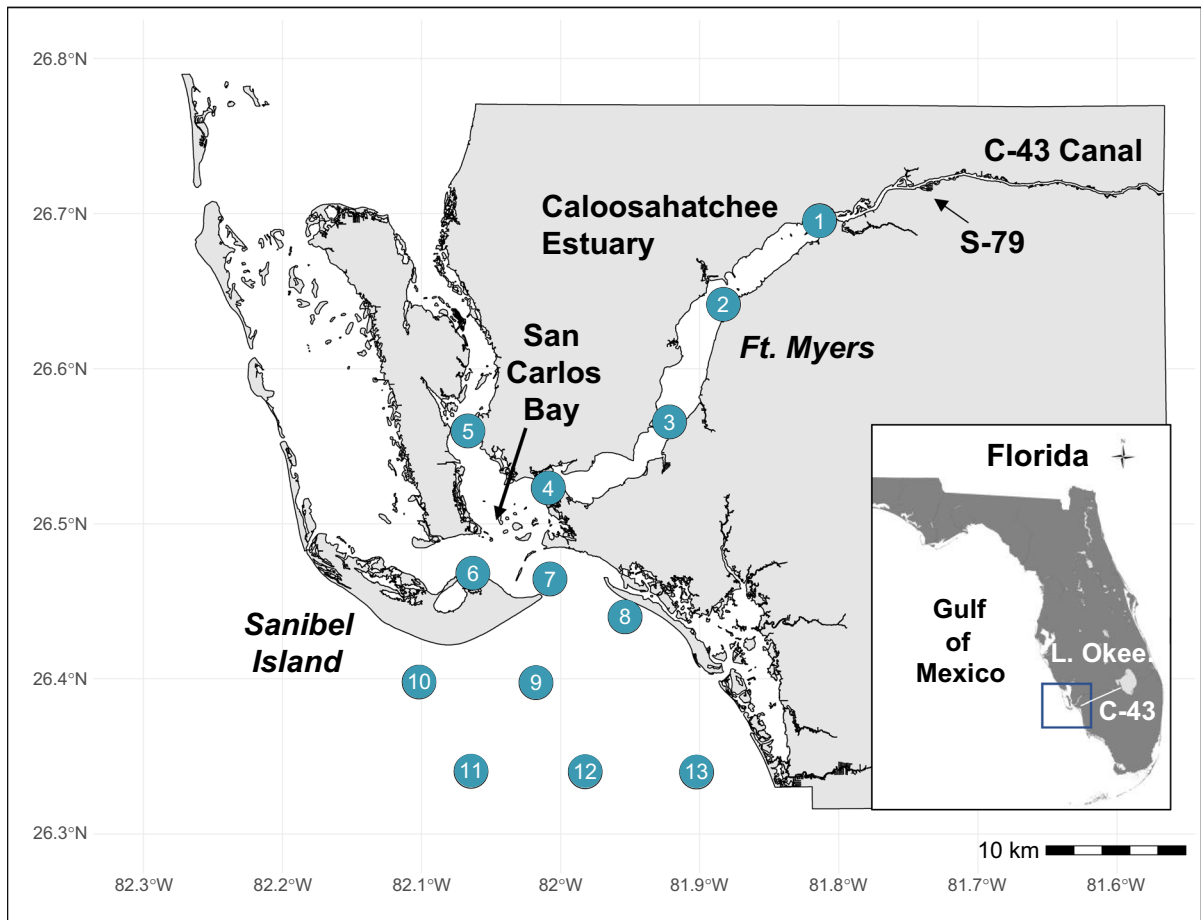
Fig. 1 Locations of sampling sites in the Caloosahatchee Estuary and nearshore regions

phytoplankton biomass, mean discharge rates for the 14 days prior to water sampling dates were used.

Water sampling

Data used in this paper were based on two separate studies of the estuary and nearshore region, the first from February of 2009 to February of 2010 (funded by the South Florida Water Management District, W. Palm Beach, Florida), and the second from September of 2018 to November of 2019 (funded by the National Science Foundation, Washington DC).

The 2009–2010 study period included four sampling sites (1–4) (Fig. 1). Site 1 was located in the Upper Caloosahatchee Estuary just downstream of Beautiful Island. Site 2 was located in the Middle Estuary near downtown Fort Myers. Site 3 was located in the Lower Estuary. Site 4 was located in San Carlos Bay, just outside the estuary. Sampling was carried out just outside the main navigation channel. Depths at the sampling sites were between 2 and 3 m. The sites were sampled once a month from February 2009 to February 2010 (excluding March 2009).

The 2018–2019 study period included 13 sites sampling sites (Fig. 1). The locations of Sites 1–4 matched those used in the 2009/2010 study period. The depths of the sampling sites in San Carlos Bay (Sites 5–7) were between 2 and 3 m. Depths of Sites 8–13 ranged from 3 to 6 m. Because the sampling frequency was based on capturing responses to different flow regimes and sources, the samples were collected in Sept. 2018, Oct. 2018, May 2019, June 2019, August 2019, Sept. 2019, Oct. 2019, and Nov.

2019. The selection of the sampling dates was based on the priorities of the NSF-Rapid research grant that funded the 2018–2019 study period.

Water samples were collected with a vertically integrating pole sampler (Wetzel & Likens, 1991) that evenly collected water from the water column down to approximately 0.5 m from the bottom of sites up to 3.5 m in depth. For deeper sites (Sites 9–13), the top 3 m of the water column was sampled using the pole. A minimum of three pole draws of water were combined in a mixing vessel. From each collection, aliquots of sample water were bottled, stored, and preserved for chemical and phytoplankton analysis according to the protocols set forth in the Phlips Laboratory (University of Florida) National Environmental Laboratory Accreditation Program manual (NELAP Certification # E72883).

Field measurements

Water temperature and salinity were measured during each sampling event at the surface and near the bottom of the water column using a HACH (Loveland, Colorado) HjQ40D or YSI EXO2 sonde. Salinity was calibrated using a two-point calibration following the manufacturers' guidelines. Temperature and salinity data for the 2018–2019 study period included gaps in coverage from November 2018 to April 2019. The gaps were partially filled in with information from continuous water quality monitoring platforms equipped with YSI EXO2 sondes established by the Sanibel Captiva Conservation Commission RECON program. The platforms are located near Sites 1, 2, and 4.

Chemical analyses

Chemical analyses during the 2009–2010 study period were conducted at the Dr. Edward J. Phlips Laboratory at the University of Florida, using methods certified by the National Environmental Laboratory Accreditation Program (NELAP Certification # E72883). Aliquots of whole unfiltered sample water were transported on ice and stored frozen until chemical analysis, within NELAP-specified holding times. Samples for TN and TP analyses were digested using the persulfate oxidation method (Strickland & Parsons, 1972; APHA, 2005) and measured colorimetrically on a Bran-Luebbe (Norderstedt, Germany) AA3

auto-analyzer and a Hitachi (Tokyo, Japan) U2810 dual-beam spectrophotometer, respectively.

Chemical analyses during the 2018–2019 study period were conducted at Benchmark EA, using NELAP-certified methods (NELAP Certification #E85086). Aliquots of whole unfiltered water were fixed with sulfuric acid (pH < 2) and placed on ice (4 °C) for transportation to the laboratory. Samples were analyzed for TN and TP following EPA methods (http://www.benchmarkea.com). For TP, samples were analyzed following persulfate digestion (EPA 365.3), and for TN, a sulfuric acid digestion (EPA 351.2) followed by analysis on a Systea Easychem Plus (Systea, Italy).

Phytoplankton analyses

Integrated, whole water samples were preserved on-site with Lugol's solution (APHA, 2005) and analyzed microscopically for phytoplankton abundance and species composition (S. Badylak & E. J. Phlips at the University of Florida). General phytoplankton abundance and composition was determined using the Utermöhl method (Utermöhl, 1958), as described in Badylak et al. (2014a). Samples preserved in Lugol's were settled in 19 mm diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400× and 100× with a Leica phase contrast inverted microscope. At 400×, a minimum of 100 cells of a single taxon and 30 grids were counted. If 100 cells were not counted by 30 grids, up to a maximum of 100 grids were counted until 100 cells of a single taxon were reached. At 100×, a total bottom count was completed for taxa > 30 μm in size.

Picocyanobacteria abundances were determined using a Zeiss Axio compound microscope, using green and blue light excitation (Fahnenstiel & Carrick, 1992; Phlips et al., 1999). Samples were preserved with buffered glutaraldehyde. Subsamples of water were filtered onto 0.2 μm Nucleopore filters and mounted between a microscope slide and cover slip with immersion oil and picoplankton counted at × 1000 magnification.

Count data were converted to phytoplankton biovolume, using the closest geometric shape method (Smayda 1978; Sun & Liu 2003). Phytoplankton carbon values (as μg carbon l^{-1}) were estimated by applying conversion factors for different taxonomic groups to biovolume estimates (expressed as

10^6 μm^3 ml^{-1}): i.e., 0.065 × biovolume of diatoms, 0.22 × biovolume for cyanobacteria, and 0.16 × biovolume for dinoflagellates or other taxa (Strathmann, 1967; Ahlgren, 1983; Sicko-Goad et al., 1984; Verity et al., 1992; Work et al., 2005).

Statistical methods

Non-parametric Spearman's correlation analysis (P-value ≤ 0.10) was used to explore the relationships between the biomass of phytoplankton and the environmental factors for the estuary and nearshore regions.

Nonmetric multidimensional scaling (NMDS) was performed to investigate potential environmental predictors of phytoplankton assemblage patterns. NMDS is an indirect gradient analysis which creates an ordination based on a dissimilarity or distance matrix (Kruskal, 1964). NMDS is a non-parametric ordination technique based on the ranking of dissimilarity values rather than actual values of dissimilarity. NMDS is considered one of the most effective ordination methods for ecological data (McCune et al., 2002) and has been widely applied to phytoplankton composition analysis (Winter & Hunter, 2008; Rothenberger et al., 2009; Sun et al., 2011; Kim et al., 2020). Prior to the analysis, phytoplankton data were transformed by log ($x + 1$), and water temperature, salinity, TN, TP, TN:TP ratio, and mean discharge were included in the analysis. TN:TP ratios are expressed on a weight basis, and were used to examine deviations from the Redfield ratio (i.e., N:P, 7.2:1 by weight) (Reynolds, 2006), as an indicator of broad spatial and temporal shifts in relative nutrient levels. The NMDS ordinations were carried out using the statistical software R (R Core Team, 2022) with the "vegan" package (Oksanen et al., 2007). A Bray–Curtis distance matrix was used (Beals, 1984). NMDS was run for the samples collected in the estuary, and a separate NMDS was run for samples collected in the nearshore. NMDS arranged samples in ordination space according to their similarity or dissimilarity in species composition. Environmental variables or phytoplankton species were represented via vectors on the ordination maps to indicate the strength and direction of maximum increase (Rothenberger et al., 2009).

Results

Local rainfall and S-79 discharge

During the 2009–2010 study period, rainfall levels in the local region of the Caloosahatchee Estuary increased substantially from the beginning of the wet season in late May through September (Fig. 2). S-79 discharge rates from the S-79 control structure were below 2000 cfs from February through April 2009, then increased to between 2000 and 5000 cfs through September (Fig. 2). In October and November, discharges were negligible, then picked up to moderate levels (i.e., 500–2000 cfs) through February of 2010, in association with a moderate peak in rainfall. Discharge rates followed the same general pattern as local rainfall; however, during certain time periods, local rainfall levels were proportionally higher than discharge, such as October and December of 2009.

The 2018–2019 study period was primarily confined to the wet season in the region (May–October) (Fig. 2). The September/October 2018 sampling
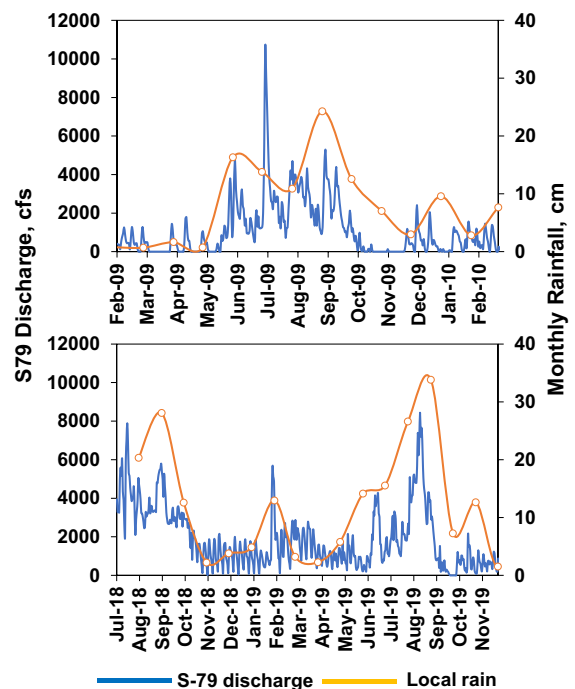

Fig. 2 Rates of water discharge from the Caloosahatchee Canal at the S-79 lock (blue line) and monthly rainfall totals (orange line) at the Ft. Myers Airport in the local region of the Caloosahatchee Estuary

period was associated with high rainfall and the June–November 2019 sampling period was also characterized by high rainfall. S-79 discharge rates were high from July through September 2018, with most values between 2000 and 6000 cfs (Fig. 2). From October 2018 through May 2019, discharge rates were primarily below 2000 cfs, then increased in June through August to between 2000 to 6000 cfs. From September through November 2019, discharge rates were below 1000 cfs. However, during certain time periods, local rainfall levels were proportionally higher than discharge, such as May and October of 2019.

Temperature

The Caloosahatchee Estuary is located in the sub-tropical region of the eastern Gulf of Mexico along the south-western coast of Florida. As such, it is typically characterized by relatively modest seasonal range in water temperatures compared to estuaries in temperate latitudes. This is illustrated by water temperatures observed during the 2009–2010 and 2018–2019 study periods, which ranged from 15 °C in the winter of 2010 to near 30 °C during the summer months (Fig. 3). Temperatures did not drop below 20 °C except in mid-winter (Dec.–Feb.).

Salinity

During the 2009–2010 study period, salinities encountered at Sites 1–4 increased from the upper estuary to the nearshore reaches of the sampling region in San Carlos Bay (Site 4) (Fig. 4). All four sites exhibited similar temporal pattern of variability, with higher salinities during periods of low S-79 discharge and local rainfall levels, which were generally associated with the dry season in central Florida (November–April) (Fig. 2). The upper-most sites in the estuary (Sites 1) were the first to respond to changes in rainfall and S-79 discharge, reaching near freshwater surface water salinities (<1 psu) from June through September of 2009. The largest range in salinity values was observed at Site 3 in the lower estuary, as illustrated by the decline in salinity from 28 psu in May to 3 psu in July. The smallest range in salinity was observed at Site 4 in San Carlos Bay near the mouth of the estuary, with a low of 25 psu in July and August, and a peak of
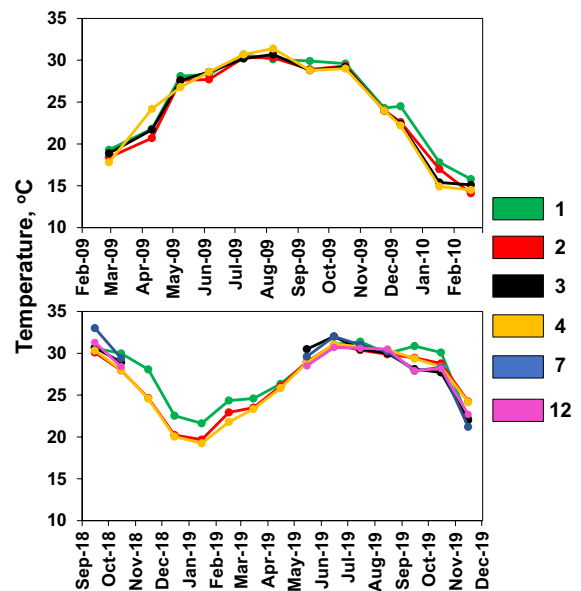

Fig. 3 Surface water temperatures at the four sampling sites (Sites 1–4) during the 2009–2010 study period, and six sites (Sites 1–4, 7 and 12) during the 2018–2019 sampling period. Gaps in the time series at Sites 3, 7, and 12 in the 2018–2019 study period are because of the lack of in situ sampling events from November 2018 to April 2019. Temperatures for Sites 1, 2, and 4 during the latter time period were filled in with data from the RECON continuous water quality monitoring stations located near the sites

34 psu in May. Sites 1–3 exhibited periods of differences between surface and bottom water salinity during the wet season.

During the 2018–2019 study period, the salinities encountered at Sites 1–4 increased from the upper to the nearshore reaches of the sampling region (Fig. 5). All four sites exhibited similar temporal patterns of variability, with lower salinities during periods of high local rainfall and S-79 discharge (Fig. 2). All three sites in the estuary had near freshwater salinities (<1 psu) in September 2018 and August 2019, reflecting the high S-79 discharge rates and local rainfall levels during the wet seasons of 2018 and 2019 (Fig. 2). The depressed salinity levels observed during these periods were even observed at the outer edge of San Carlos Bay (Site 7) (Fig. 5), demonstrating the extended influence of outflows from the Caloosahatchee Estuary on nearshore waters of the Gulf of Mexico. The nearshore sites outside of San Carlos Bay exhibited less salinity variation, as illustrated by Site 12 (Fig. 5).
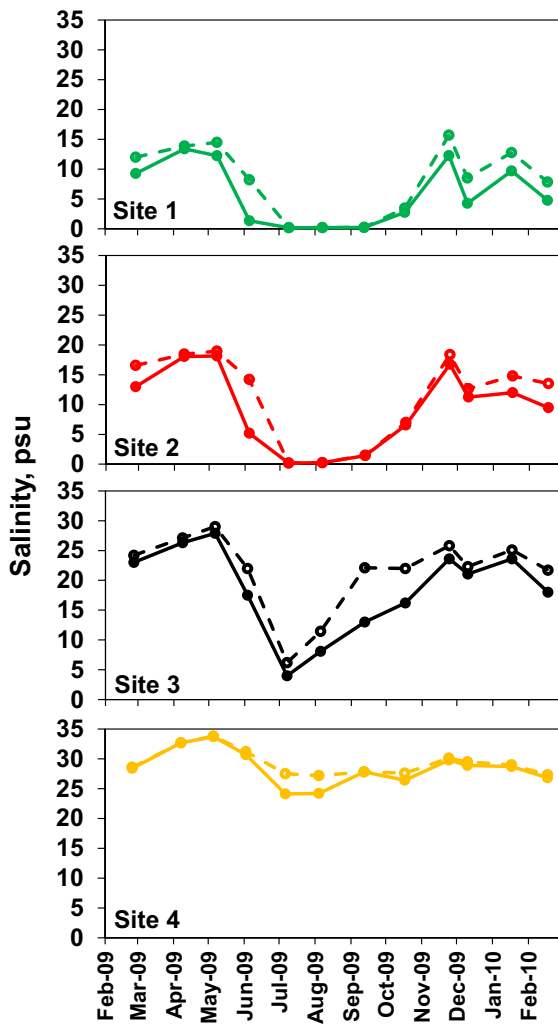
Fig. 4 Salinity (psu) at the four sampling sites during the 2009–2010 study period. Solid lines are the surface water salinities and dashed lines the bottom water salinities
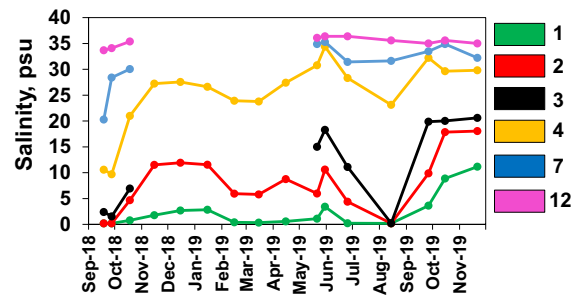

Fig. 5 Surface water salinities (psu) at six sampling sites during the 2018–2019 study period. Gaps in the time series at Sites 3, 7, and 12 are because of the lack of in situ sampling events during the dry season from November 2018 to April 2019. Salinities for Sites 1, 2, and 4 were filled in with data from the RECON continuous water quality monitoring stations located near the sites
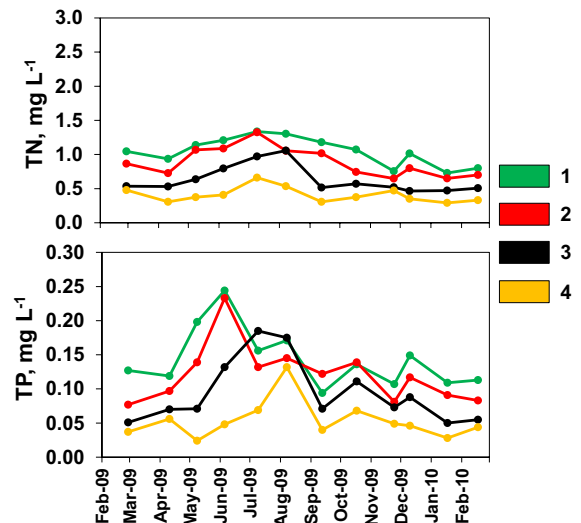

Fig. 6 Total nitrogen concentration (TN) and total phosphorus concentration (TP) at the four sampling sites during the 2009–2010 study period

Total nitrogen and total phosphorus

In the 2009–2010 study period, temporal trends in total nitrogen (TN) were similar at all four sites (Fig. 6). With the onset of the wet season in May, increases in local rainfall levels and discharge from the C-43 Canal coincided with increases in TN concentrations at all four sites until July–August, after which concentrations declined to near pre-wet season levels. Mean TN concentrations in the dry season ranged from 0.37 mg l^{-1} at Site 4 in San Carlos Bay to 0.90 mg l^{-1} at Site 1 in the upper Caloosahatchee Estuary near the S-79 structure (Table 1). In the wet

season, mean TN concentrations were higher, ranging from 0.44 mg l^{-1} at Site 4 to 1.21 mg l^{-1} at Site 1.

Total phosphorus (TP) concentrations in the 2009–2010 study period exhibited the same general trend as TN (Fig. 6). TP concentrations increased at Sites 1 and 2 in the upper Caloosahatchee Estuary at the beginning of the wet season in May, followed by increases at Sites 3 and 4 in June. By June–July, TP concentrations increased by 2-fold-to-3-fold at all four sites, then declined to near pre-wet season levels by August–September. Mean TP during

Table 1 Mean total nitrogen (TN) and total phosphorus (TP) concentrations (mg l^{-1}) during the dry (November–April) and wet periods (May–October) in the Caloosahatchee Estuary (Sites 1–3) and nearshore regions of San Carlos Bay (Sites 4 and 7)

Site	TN			TP			TN/TP		
	2009/10		2018/19	2009/10		2018/19	2009/10		2018/19
	Dry	Wet	Wet	Dry	Wet	Wet	Dry	Wet	Wet
1	0.90	1.21	2.08	0.12	0.17	0.14	7.3	7.9	14.8
	(0.13)	(0.20)	(0.48)	(0.02)	(0.05)	(0.03)	(0.6)	(2.7)	(3.6)
2	0.71	1.05	1.83	0.09	0.15	0.14	8.2	7.2	14.5
	(0.13)	(0.22)	(0.43)	(0.02)	(0.03)	(0.04)	(1.6)	(2.0)	(4.7)
3	0.51	0.76	1.71	0.07	0.12	0.12	8.2	6.5	15.3
	(0.07)	(0.22)	(0.41)	(0.02)	(0.05)	(0.04)	(1.9)	(1.4)	(3.8)
4	0.37	0.44	1.04	0.04	0.06	0.06	8.9	8.5	20.1
	(0.08)	(0.13)	(0.30)	(0.01)	(0.03)	(0.02)	(2.6)	(4.0)	(6.6)
7	NA	NA	0.88	NA	NA	0.04	NA	NA	24.2
			(0.22)			(0.02)			(8.7)

Standard deviations are shown in parentheses

the dry season ranged from 0.04 mg l^{-1} at Site 4 in San Carlos Bay to 0.12 mg l^{-1} at Site 1 in the upper estuary (Table 1). Mean TP concentrations in the wet season were higher, ranging from 0.06 mg l^{-1} at Site 4 to 0.17 mg l^{-1} at Site 1.

The 2018–2019 study period was primarily confined to the wet periods of the 2 years. Temporal trends in TN concentrations in the estuary at Sites 1–3, and Site 4 in San Carlos Bay were similar to those observed in 2009–2010 (Fig. 7), with marked increases during the onset of the wet season in 2019. In the nearshore region outside of San Carlos Bay, TN concentrations were generally lower than in the Bay, as illustrated by Site 12 (Fig. 7). Mean TN concentrations for the wet seasons of 2018 and 2019 ranged from 0.88 mg l^{-1} at Site 7 at the southern edge of San Carlos Bay to 2.03 mg l^{-1} at Site 1 in the upper Caloosahatchee Estuary (Table 1). It is noteworthy that the mean wet season TN concentrations in the 2018–2019 period were nearly twice as high as the mean concentrations during the wet season of 2009–2010 at the shared sites (Table 1).

TP concentrations in the 2018–2019 study period exhibited the same general trends as TN (Fig. 7). Mean TP concentrations for the wet seasons of 2018/19 ranged from 0.04 mg l^{-1} at Site 7 to 0.14 mg l^{-1} at Site 1 (Table 1). Unlike mean TN concentrations, mean wet season TP concentrations for 2018–2019 were similar to those observed in 2009–2010 at all shared sites, suggesting that nitrogen loads to the estuary were higher in the former
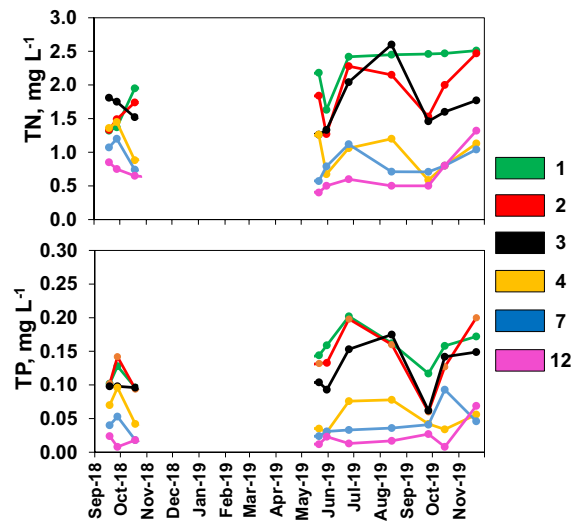

Fig. 7 Total nitrogen concentration (TN), and total phosphorus concentration (TP) at the five sampling sites in the 2018–2019 study period. Gaps in the time series are because of the lack of in situ sampling events during the dry season from November 2018 to April 2019

time period, but phosphorus loads remained similar (Table 1).

Mean TN/TP ratios in the 2009–2010 portion of the study period were similar at all four sites in both the dry and wet period, ranging from 6.5 to 8.9, near the Redfield ratio (i.e., 7.2 on a weight basis) (Table 1). In the 2018–2019 portion of the study period, TN/TP ratios for the wet period ranged from 14.5 to 24.2. Ratios at Sites 1–4 for the wet season

were twice as high in 2018–2019 than in 2009–2010, largely due to higher TN values. The higher ratios in San Carlos Bay (i.e., Sites 4 and 7) than in the estuary reflect the incrementally larger decrease of TP than TN concentrations in the bay. The observed spatial and temporal changes in ratios provide broad insights into shifts in total nutrient loads to the ecosystem. However, it is premature to specifically define changes in nutrient limiting status for the phytoplankton community due to insufficient detailed information on the percent of each total that is in bioavailable forms.

Phytoplankton biomass and bloom composition

During the 2009–2010 study period, the range of phytoplankton biomass was highest at Sites 1 and 2 in the upper Caloosahatchee Estuary, with peak values of 7.2 and 4.4 mg carbon l^{-1}, respectively (Fig. 8). Peak biomass at Site 3 in the lower estuary was 0.8 mg carbon l^{-1}. The dominant species during the major bloom peaks at the three sites was the HAB dinoflagellate *Akashiwo sanguinea* (K. Hirasaka) Gert Hansen & Moestrup. Bloom levels of *A. sanguinea* were first observed at Site 1 in February

Fig. 8 Biomass (mg carbon l^{-1}) of phytoplankton by group in samples from the first collection time on the day of the monthly sampling in the Caloosahatchee Estuary (Sites 1–3) and San Carlos Bay (Site 4), i.e., pink—dinoflagellates (excluding *K. brevis*); red—*Karenia brevis*; orange—diatoms; light blue—picocyanobacteria; blue—other cyanobacteria; green—all other phytoplankton taxa. Letters designate dominant species in sample with major bloom, i.e., A—*Akashiwo sanguinea*; E—*Euglena*
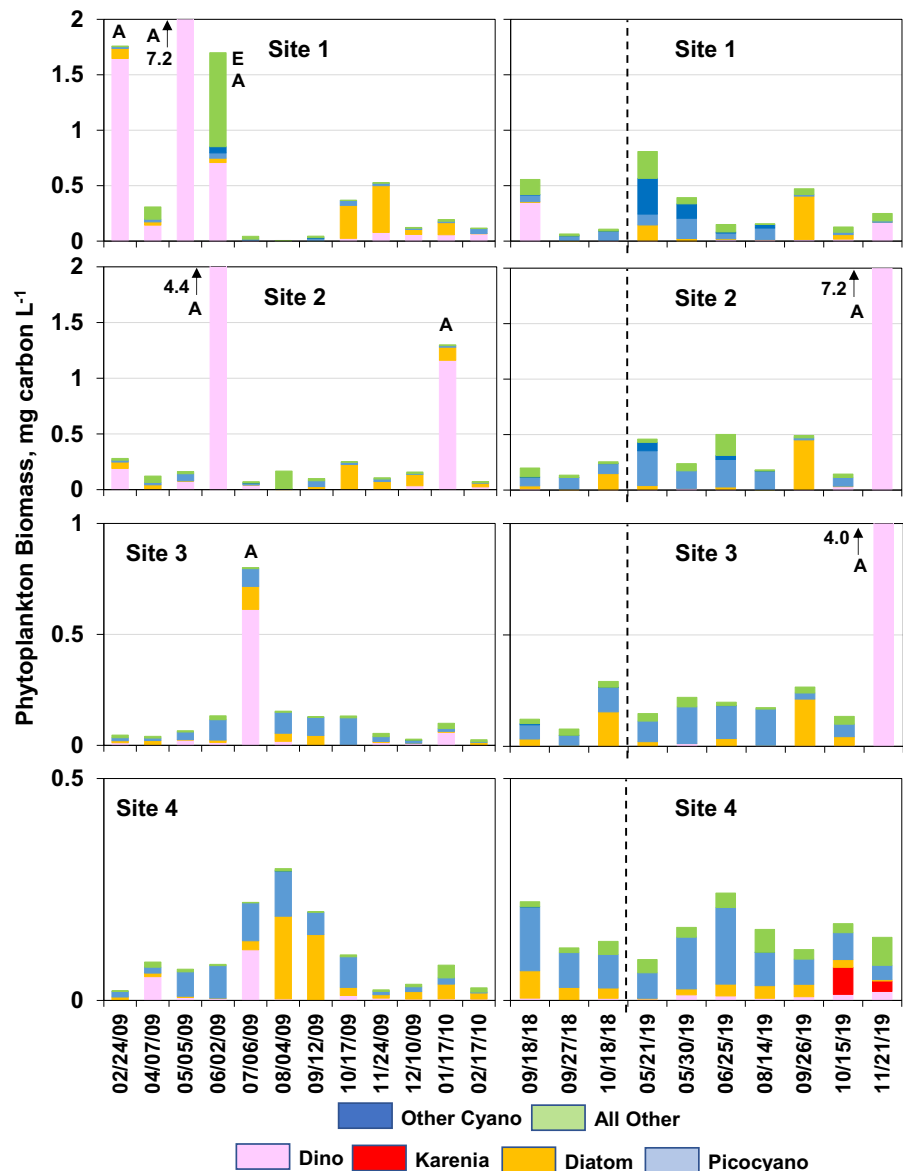
2009, then peaked in early May. Bloom levels of *A. sanguinea* were first observed at Site 2 in June, and subsequently in July at Site 3, suggesting movement of the bloom peaks downstream. Phytoplankton biomass levels at Site 4 in San Carlos Bay peaked in the mid-summer of 2009 at 0.25 mg carbon l^{-1} and were primarily dominated by diatoms (e.g., *Skeletonema* spp.) and picoplanktonic cyanobacteria (i.e., spherical picocyanobacteria and *Synechococcus* spp.). Diatoms and picoplanktonic cyanobacteria were also major contributors to total phytoplankton biomass at Sites 1–3 outside of periods of *A. sanguinea* blooms.

During the 2018–2019 study period, the highest biomass levels in the Caloosahatchee Estuary were observed at Sites 2 and 3, with peak values of 7.2 and 4.0 mg carbon l^{-1}, respectively (Fig. 8). The peaks were dominated by *A. sanguinea* and were only observed the last month of the sampling period in November 2019. In the other months of the sampling period, total phytoplankton biomass levels at Sites 1–3 were generally less than 0.5 mg carbon l^{-1} and dominated by cyanobacteria, nanoplanktonic eukaryotes, or diatoms. At Site 4 in San Carlos Bay, biomass levels were consistently below 0.25 mg carbon l^{-1} and most months were dominated by picoplanktonic cyanobacteria, nanoplanktonic eukaryotes, or diatoms. At the end of the sampling period, low concentrations of the toxic 'red tide' dinoflagellate *Karenia brevis* (C.C. Davis) Gert Hansen & Moestrup were observed.

At the nearshore sites outside of the boundary of the Caloosahatchee Estuary (7–13), a major red tide event dominated by the dinoflagellate *K. brevis* was observed in September of 2018 (as illustrated by representative sites, Fig. 9). Sites 7–13 were all involved in the event, with biomass levels up to 6.0 carbon l^{-1} (cell density of 5.6×10^6 cells l^{-1}). Another *K. brevis* bloom event was observed in the same region in November 2019, with peak biomass of 5.1 mg carbon l^{-1} (Fig. 9). During the rest of the sampling dates, total phytoplankton biomass values were below 0.5 mg carbon l^{-1} and generally dominated by picoplanktonic cyanobacteria and/or diatoms.

Mean and median total phytoplankton biomass levels were higher during the wet than dry season in the 2009–2010 study period (Table 2). Mean and median biomass levels were higher in the upper (Sites 1 and 2) than the lower estuary, in part
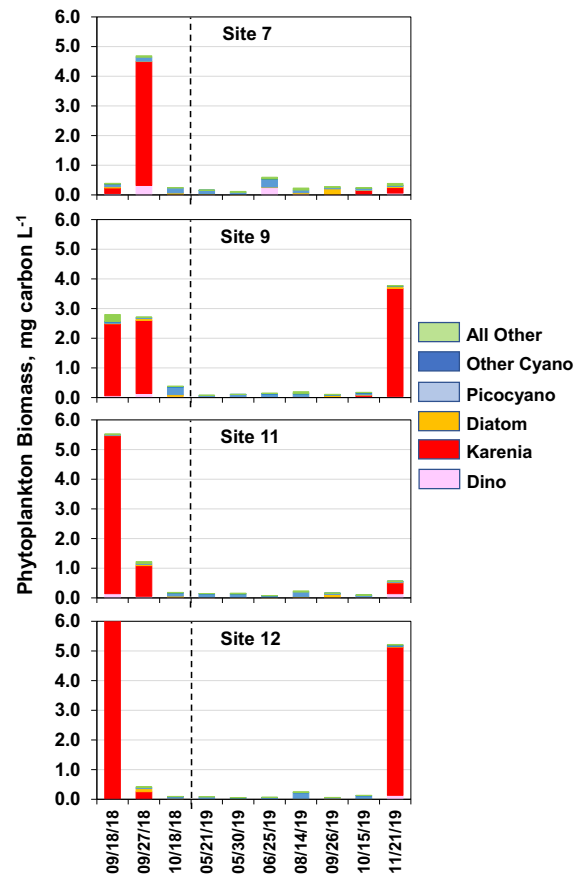

Fig. 9 Biomass (mg carbon l^{-1}) of phytoplankton by group in samples from four selected sites in the nearshore study regions, i.e., pink—dinoflagellates (excluding Karenia brevis); red—*Karenia brevis*; orange—diatoms; light blue—picocyanobacteria; blue—other cyanobacteria; green—all other phytoplankton taxa

reflecting the disproportionate effect of large *A. sanguinea* blooms in the early months of the wet season on seasonal mean values. During the wet period of the 2018–2019 study period, mean biomass levels in the upper estuary were lower than in 2009–2010, while the reverse was true in the lower estuary, largely reflecting differences in the distribution of *A. sanguinea* between the two study periods. In San Carlos Bay, mean and median biomass levels were similar in the wet periods of both study periods. In the nearshore region outside of the Bay mean wet season, total biomass levels were higher than in either the estuary or Bay, reflecting the presence of an intense red tide dominated by *K. brevis*.

Table 2 Mean and median total phytoplankton biomass (mg carbon l^{-1}) during the dry (November–April) and wet periods (May–October) in the Caloosahatchee Estuary (Sites 1–3) and nearshore regions of San Carlos (Sites 4 and 7)

Site	2009–2010				2018–2019	
	Dry		Wet		Wet	
	Mean	Median	Mean	Median	Mean	Median
1	0.61 (1.03)	0.12	1.07 (1.49)	0.22	0.32 (0.24)	0.16
2	0.34 (0.43)	0.14	0.88 (1.64)	0.16	0.29 (0.14)	0.24
3	0.04 (0.01)	0.04	0.29 (0.36)	0.11	0.18 (0.07)	0.18
4	0.05 (0.03)	0.03	0.16 (0.08)	0.15	0.16 (0.05)	0.16
7	NA	NA	NA	NA	0.77 (1.39)	0.25

Standard deviations are shown in parentheses

Dominant phytoplankton taxa in different salinity regimes

Oligohaline (≤5 psu) conditions in surface water were primarily limited to Sites 1 and 2 in the upper estuary during the wet season (May–October), with the exception of two months at Site 3 in the lower estuary, i.e., July and August of 2009 and 2019 (Figs. 4, 5). The Top-50 list was predominantly populated by freshwater taxa during oligohaline conditions, including cyanobacteria, dinoflagellates, euglenophytes, diatoms, and chlorophytes (Table 3). Also prominently represented on the list is the marine diatom *Skeletonema costatum* (Greville) Cleve, which is known for its tolerance to low salinities (Brand 1984). Several euryhaline dinoflagellates were also on the list, including *A. sanguinea, Peridinium quinquecorne* T. H. Abé, *Karlodinium veneficum* (D. Ballantine) J. Larsen, and *Scrippsiella* sp. In the cases involving the latter taxa, water below the pycnocline fell within the lower mesohaline range, suggesting that the prominent presence of these taxa may have in part been based on the presence of higher salinities below the pycnocline.

During mesohaline conditions (salinities > 5–18 psu), the Top-50 list was dominated by the HAB dinoflagellate *A. sanguinea* and the diatom *S. costatum*, both of which are euryhaline species (Table 3). Several other marine dinoflagellates were well represented, including the mixotrophic/heterotrophic species *Polykrikos schwartzii* Bütschli, *Protoperidinium* pp., and *Scrippsiella trochoidea* (F. Stein) A. R.

Loeblich III. The diatom genus *Chaetoceros* was also present under mesohaline conditions.

During polyhaline conditions (salinities > 18–30 psu), the Top-50 list was dominated by the HAB dinoflagellates *A. sanguinea* and *K. brevis*, both of which were observed at major bloom levels of biomass (i.e., > 4 mg carbon l^{-1}) (Table 3). The list also contained several euryhaline diatoms, including *S. costatum, Rhizosolenia setigera* Brightwell and *Coscinodiscus*, as well as six mixotrophic/heterotrophic species of dinoflagellates; *Gyrodinium spirale* (Bergh) Kofoid & Swezy, *P. quinquecorne, Gonyaulax, Gyrodinium* cf. *pingue* (F. Schütt) Kofoid & Swezy, and the toxic HAB species *K. veneficum*.

Under euhaline conditions (salinities > 30 psu), the Top-50 list was dominated by the HAB dinoflagellate *K. brevis*, with numerous observations of major bloom biomass levels (Table 3). The other noteworthy taxa on the list were dinoflagellates, including *P. quinquecorne, Protoperidinium*, and HAB species *A. sanguinea* and *Prorocentrum minimum* (Pavillard) J. Schiller.

Picocyanobacteria were an important component of the phytoplankton communities of the Caloosahatchee Estuary, San Carlos Bay and the nearshore community, as highlighted by the large presence of the group in the Top-50 list of individual taxa biomass contributions across all salinity regimes from oligohaline (salinities ≤ 5 psu) to euhaline (salinities > 30 psu) (Table 3). While picocyanobacteria were not observed at major bloom biomass levels (i.e., > 2 mg carbon l^{-1}), as some other species were,

Table 3 Top-50 list of the highest individual taxa biomass (μg carbon l^{-1}) values in four salinity regime groups: oligohaline (≤ 5 psu), mesohaline (> 5–18 psu), polyhaline (> 18–30 psu), and euhaline (> 30 psu)

Genera/Species	Group	Frequency in Top-50	Biomass Range mg l^{-1}	Peak density cells ml^{-1}
Oligohaline				
Spherical Picocyano*	Cyanobacteria	13	0.04–0.14	612,833
*Peridinium**	Dinoflagellate	6	0.03–0.35	393
Skeletonema costatum	Diatom	5	0.04–0.82	74,476
*Euglena**	Euglenophyte	4	0.05–0.80	482
Scrippsiella trochoidea	Dinoflagellate	3	0.06–0.08	60
*Synechococcus**	Cyanobacteria	3	0.04–0.14	333,978
Akashiwo sanguinea	**Dinoflagellate**	**2**	**0.60–1.60**	**217**
Cryptophyte*	Cryptophyte	2	0.04–0.05	4,847
*Limnothrix redekei**	Cyanobacteria	2	0.04–0.07	1,042
Peridinium quinquecorne	**Dinoflagellate**	**1**	**0.10**	**91**
*Pleodorina**	Chlorophyte	1	0.09	437
*Coelomoran tropicalis**	Cyanobacteria	1	0.08	6,931
*Cuspidothrix**	Cyanobacteria	1	0.06	272
*Aulacosiera granulata**	Diatom	1	0.06	1,948
*Oscillatoria**	Cyanobacteria	1	0.06	45
*Aphanacapsa incerta**	Cyanobacteria	1	0.05	137,803
Karlodinium veneficum	**Dinoflagellate**	**1**	**0.04**	**295**
*Gonium pectorale**	Chlorophyte	1	0.04	473
Aulacoseira ambigua*	Diatom	1	0.03	3,579
Mesohaline				
Spherical Picocyano	Cyanobacteria	21	0.06–0.36	1,582,291
Akashiwo sanguinea	**Dinoflagellate**	**10**	**0.07–7.29**	**855**
Skeletonema costatum	Diatom	7	0.06–0.44	19,421
Polykrikos schwartzii	Dinoflagellate	3	0.06–0.92	52
Protoperidinium	Dinoflagellate	3	0.05–0.06	9
Scrippsiella trochoidea	Dinoflagellate	2	0.06–0.16	121
Chaetoceros	Diatom	1	0.36	783
Pyrophacus	Dinoflagellate	1	0.25	53
Dactyliosolen fragilissimus	Diatom	1	0.11	250
Synechococcus	Cyanobacteria	1	0.07	155,640
Polyhaline				
Spherical Picocyano	Cyanobacteria	24	0.04–0.16	705,176
Karenia brevis	**Dinoflagellate**	**4**	**0.06–4.17**	**3,642**
Akashiwo sanguinea	**Dinoflagellate**	**3**	**0.09–4.05**	**568**
Cryptophyte spp.	Cryptophyte	3	0.04–0.05	5,663
2-5μ nanoplankton	Eukaryote	3	0.04–0.05	5,663
Skeletonema costatum	Diatom	2	0.15–9.12	9,119
Rhizosolenia setigera	Diatom	2	0.12–0.13	2,429
Gyrodinium spirale	Dinoflagellate	2	0.04–0.08	10
Coscinodiscus	Diatom	2	0.03–0.04	5
Peridinium quinquecorne	**Dinoflagellate**	**1**	**0.18**	**136**
Gonyaulax	Dinoflagellate	1	0.07	30
Protoperidinium	Dinoflagellate	1	0.07	91
Gyrodinium cf. pingue	Dinoflagellate	1	0.05	91

Table 3 (continued)

Genera/Species	Group	Frequency in Top-50	Biomass Range mg l^{-1}	Peak density cells ml^{-1}
Karlodinium veneficum	**Dinoflagellate**	**1**	**0.04**	**263**
Euhaline				
Karenia brevis	**Dinoflagellate**	**23**	**0.12–6.03**	**5,265**
Spherical Picocyano	Cyanobacteria	18	0.08–0.22	966,266
2-5μ nannoplankton	Eukaryote	3	0.09–0.13	14,059
Peridinium quinquecorne	Dinoflagellate	1	1.29	994
Protoperidinium	Dinoflagellate	1	0.24	94
Cryptophyte	Cryptophyte	1	0.19	19,683
Rhizosolenia setigera	Eukaryote	1	0.17	1,010
Akashiwo sanguinea	**Dinoflagellate**	**1**	**0.12**	**10**
Prorocentrum minimum	**Dinoflagellate**	**1**	**0.1**	**244**

Taxa in bold text are listed on the IOC-UNESCO list of harmful algal bloom species (Lundholm et al., 2009-onward). Taxa with "*" are/or include likely freshwater forms

they were generally the most numerically abundant photoautotrophic taxa in all salinity regimes (Table 3) and sampling sites, reaching cell densities of up to 1.6×10^9 cells l^{-1}.

Relationships between environmental factors and phytoplankton biomass

Overall, the two taxa that reached the highest biomass levels over the study period were *A. sanguinea* and *K. brevis* (Figs. 8, 9; Table 3). From a salinity perspective, they dominated two ends of the spectrum with some overlap, with major blooms of *A. sanguinea* primarily observed under mesohaline conditions in the Caloosahatchee Estuary, and *K. brevis* under euhaline conditions in nearshore regions outside of the estuary (Fig. 10). In terms of potential drivers of blooms of the two taxa, differences were also observed in the relationships with discharge rates from the S-79 into the estuary. The major blooms in the estuary (i.e., carbon biomass values > 2 mg l^{-1}) were dominated by *A. sanguinea* and limited to periods of low to moderate S-79 discharge (i.e., < 2000 cfs) (Fig. 11).

The negative relationship between S-79 discharge and *A. sanguinea* was further indicated by the results of Spearman's correlation analysis, which revealed a negative correlation between mean discharge
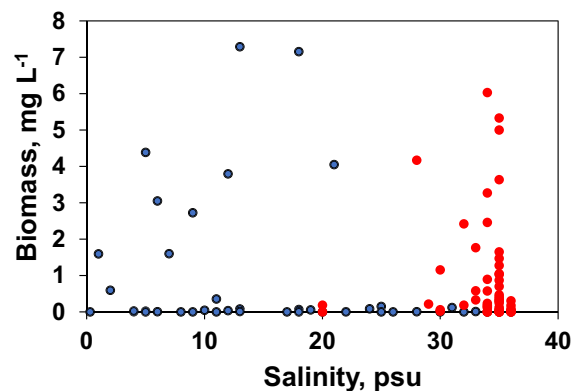

Fig. 10 Surface water salinity versus biomass (mg carbon l^{-1}) observations of *Akashiwo sanguinea* (blue circles) and *Karenia brevis* (red circles) biomass observations, for the 13 sampling sites over the study period

and dinoflagellate biomass for the estuarine region (Fig. 12a). For cyanobacteria, biomass was positively correlated with temperature, discharge, and TN concentrations in the estuary. In the case of the nearshore region, where blooms of *K. brevis* were the major feature, dinoflagellate biomass was positively correlated to TN concentrations (Fig. 12b). No significant correlations were observed for cyanobacteria or diatoms for the nearshore region, but "All Other" group
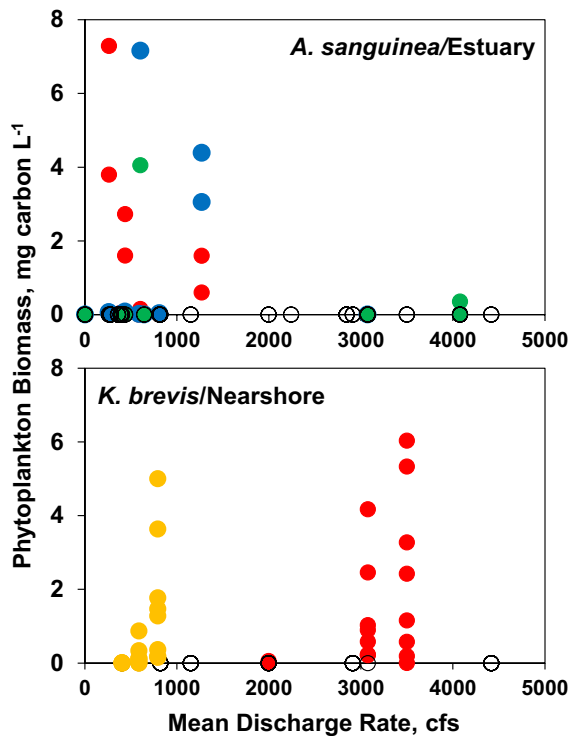
Fig. 11 The relationships between mean discharge rates prior to the sampling dates and biomass of *Akashiwo sanguinea* in the Caloosahatchee Estuary and *Karenia brevis* in the nearshore region. In the top panel (*A. sanguinea*), colored circles represent the Site 1 (red), Site 2 (blue), and Site 3 (green). In the bottom panel (*K. brevis*), colored circles represent 2018 blooms (red) and 2019 blooms (orange). Open circles represent dates in each region when neither species was observed

biomass, which was dominated by nanoplanktonic eukaryotes (such as cryptophytes), was positively correlated to TN and TP concentrations (Fig. 12b).

NMDS analysis was used to further examine potential environmental predictors of phytoplankton taxa/species biomass patterns in the two major regions of the study, i.e., Caloosahatchee Estuary and adjacent nearshore region (Fig. 13).

The ordination of samples by NMDS for the Caloosahatchee Estuary revealed that higher *A. sanguinea*, dinoflagellates, and cryptophytes biomass were associated with higher TP concentrations, but lower TN concentration, TN:TP ratio, mean discharge, and temperature (Fig. 13a, b). By contrast, higher picocyanobacteria and other cyanobacteria (predominantly filamentous forms) biomass were associated with higher TN:TP ratio and temperature.

The ordination of samples by NMDS for the nearshore region revealed that *K. brevis* and dinoflagellates biomass were higher for higher discharge, salinity, and TN:TP ratio (Fig. 13c, d). Picocyanobacteria and other cyanobacteria biomass were higher for higher temperature, while *Rhizosolenia* spp. and other diatoms (including *Pseudo-Nitzschia* spp. and *Chaetoceros* spp.) biomass were higher for higher TP. However, the length of other diatom vectors was less important than the vector for *Rhizosolenia*.

Discussion

The relationships between environmental variables and phytoplankton biomass and composition were different in the Caloosahatchee Estuary and the adjacent nearshore environment. In the Caloosahatchee Estuary, salinity regimes are affected by micro-tidal mixing with the Gulf of Mexico, and inflows from local watersheds, of which discharges from the S-79 control structure are major components. Low rainfall and low discharge levels generally coincide with the dry season, during which mesohaline (salinities 5–18 psu) or polyhaline (18–30 psu) conditions prevail through most of the estuary. From the perspective of the phytoplankton community, this is reflected in the major roles that euryhaline marine dinoflagellates, diatoms and picocyanobacteria play in phytoplankton community biomass under these salinity regimes. The highest phytoplankton biomass levels in the estuary were observed in the periods of transition from low to high discharge, suggesting a dual influence of inputs of nutrients in early stages of elevated rainfall, and sufficient water residence time to allow for biomass accumulation. In another study of an estuary associated with a water control structure in South Korea, peak phytoplankton biomass levels were also correlated to periods of low discharge (Sin et al. 2013).

By contrast, during periods of high S-79 discharge into the Caloosahatchee estuary oligohaline (≤5 psu) and lower mesohaline (>5–10 psu), conditions are common in the estuary, as reflected by the frequent presence of freshwater taxa and some exceptionally low salinity tolerant marine taxa, such as *Skeletonema costatum* (Brand, 1984). The prominence of cyanobacteria during the summer season is also reflected in the positive correlation with temperature, which comports with the preference of
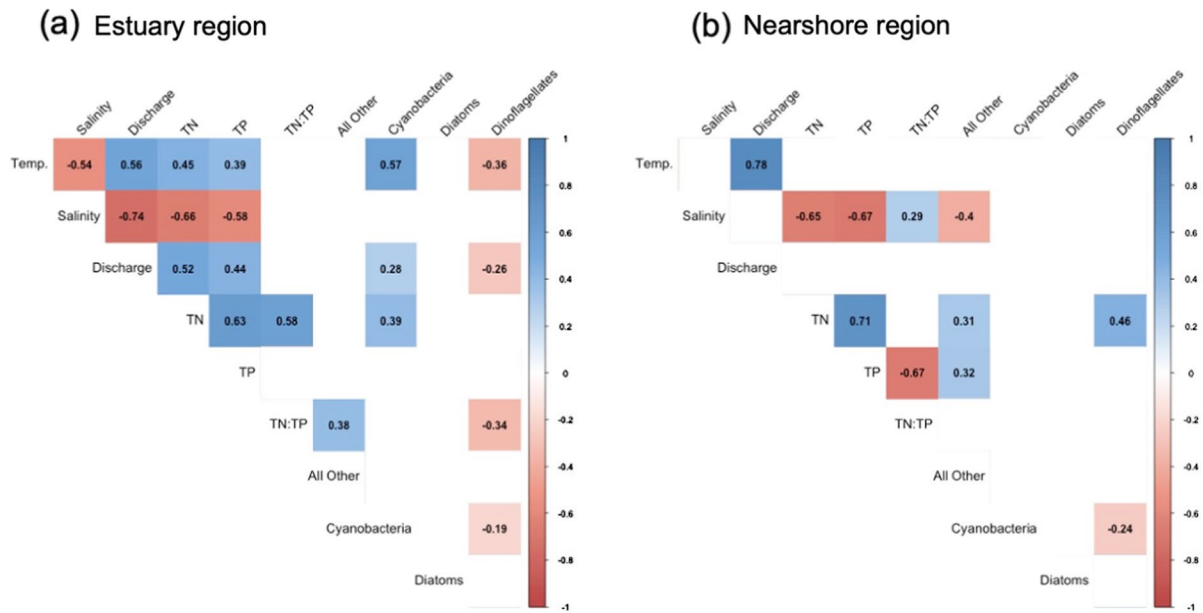
(a) Estuary region

(b) Nearshore region

Fig. 12 Correlation matrix of Spearman's correlation coefficients between phytoplankton biomass and environmental variables for the estuary (**a**) and nearshore (**b**) regions. Blue squares correspond to positive correlations and red squares to negative correlations. The color intensity is proportional to the correlation coefficient value. When a correlation coefficient was not significantly different from '0,' the corresponding cell in the matrix was left blank

many bloom-forming cyanobacteria for high temperatures (Paerl et al., 2016). While high discharge periods during this study were generally associated with relatively low phytoplankton biomass in the estuary, high biomass of cyanobacteria can be periodically introduced to the estuary via regulated discharges of water into the C-43 Canal from Lake Okeechobee, which is regularly subject to intense cyanobacteria blooms (Rosen et al., 2018; Phlips et al., 2020; Metcalf et al., 2021).

In the nearshore region outside of the Caloosahatchee Estuary, euhaline (> 30 psu) conditions predominate, although near the mouth of the estuary in San Carlos Bay, strong estuarine outflows can result in polyhaline conditions, particularly in the wet season. Euhaline conditions in the nearshore regions are favorable for the influx of red tides dominated by the toxic dinoflagellate *Karenia brevis*, which is not tolerant of low salinities (Heil et al., 2014a; Steidinger, 2009; Vargo, 2009). During this study period, there were noteworthy differences in the dominant species in phytoplankton blooms in the Caloosahatchee Estuary compared to nearshore regions, as described below.

Phytoplankton blooms in the estuary

During the 2009–2010 study period, major blooms of the dinoflagellate *A. sanguinea* were observed in the upper Caloosahatchee Estuary during the periods of low to moderate discharges in the Spring. The highest TP concentrations in the upper estuary also occurred in the late Spring, indicating that the initial flushes from the watershed during the beginning of the wet season yielded high phosphorus concentrations. The importance of the period of elevated phosphorus levels is further indicated by the close proximity of TP and *A. sanguinea* biomass vectors on the NMDS analysis axes for the Caloosahatchee Estuary (Fig. 13a, b). The NMDS relationship suggests that high phosphorus levels in Spring S-79 discharges may have contributed to the intensity of *A. sanguinea* blooms. By contrast, sustained high S-79 discharge rates in the Summer shorten water residence times (WRT), which limit the time needed for substantial biomass accumulation of marine taxa, thereby limiting the potential for bloom formation. The importance of WRT is supported by the negative correlation between mean discharge rate and dinoflagellate biomass (Fig. 12a),
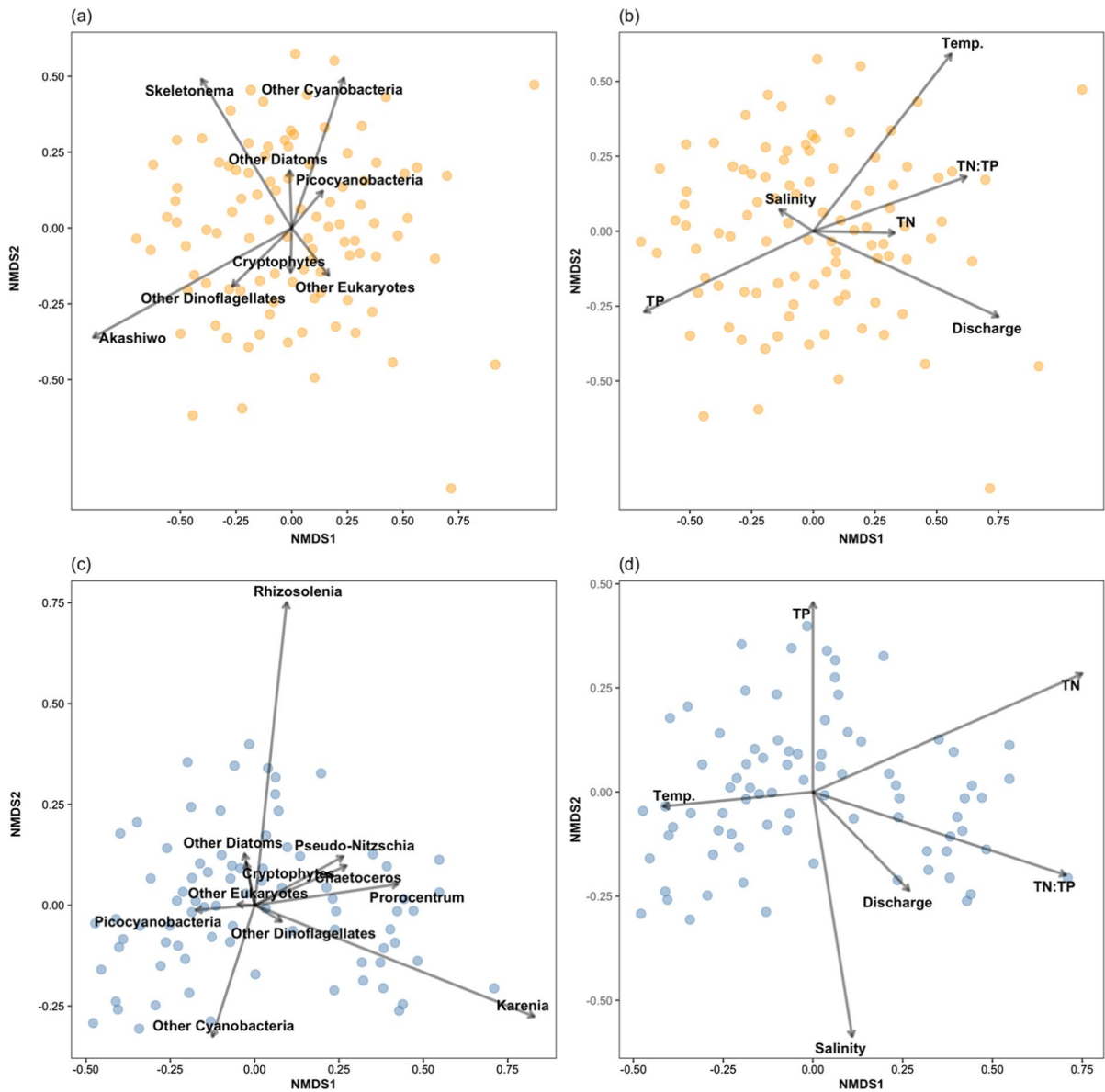
Fig. 13 Phytoplankton samples collected in the estuary (**a**, **b**) and nearshore (**c**, **d**) are visualized in NMDS ordination space (stress = 0.16). Vectors indicate strength and direction of phy-toplankton biomass taxa/species gradients (**a**, **c**) and environmental gradients (**b**, **d**)

as well as the opposing directions of the *A. sanguinea* biomass and mean discharge vectors on the NMDS analysis axis for the estuary (Fig. 13a, b). The effect of S-79 discharge on WRT in the Caloosahatchee Estuary has been investigated by Wan et al. (2013). In the upper estuary at the City of Ft. Myers (i.e., Site 2), their study estimated that at high discharge rates (i.e., 2000 to 6000 cfs), WRT values range from < 1

to 10 days. Given that maximum growth rates of most phytoplankton in the natural environment largely range between 0.1 and 1.0 doubling per day (Stolte & Garcés, 2006), the latter range of WRTs have the potential to limit the formation of major blooms. By contrast, periods of low discharge (i.e., 0 to 1,000 cfs) are associated with WRT from 30 to 80 days, a range suitable for potential bloom development, given

sufficient nutrient levels and acceptable salinity levels. Similar observations of the importance WRT in defining phytoplankton biomass were found during chlorophyll *a* modeling efforts by Sun et al. (2022).

During the spring of 2009, *A. sanguinea* blooms first appeared in the upper-most region of the estuary in February, then peaked in early May before substantial increases in S-79 discharges late in May. After May, lower levels of *A. sanguinea* were found further downstream in the estuary until July. These observations support the hypotheses of an earlier study of the estuary, which increases in water temperature in the spring enhance algal growth rates, allowing for increases in phytoplankton biomass in the estuary (Buzzelli et al., 2014). As observed in our study, increases in S-79 discharge rates in the summer push chlorophyll maxima progressively further downstream in the estuary, while sustained increases in S-79 discharge rates in summer lower the potential for blooms in the estuary, as reflected by the comparatively low phytoplankton biomass observed in the summer of both study periods. The latter observations highlight the major role water residence time plays in defining the potential for HAB development of marine taxa in the estuary. The importance of water residence times and flushing rates in defining bloom potential has been documented for other Florida ecosystems (Murrell et al., 2007; Phlips et al., 2004, 2012, 2020, 2021; Dix et al., 2013; Hart et al., 2015), and estuaries around the world (Knoppers et al., 1991; Monbet, 1992; Eyre, 2000). In the Caloosahatchee Estuary, low discharge levels from the S-79 provide both the time and salinity levels needed for accumulation of marine phytoplankton biomass. Blooms of *A. sanguinea*, and other marine taxa, are supported by the nutrient levels sustained in the upper estuary, even under comparatively low rainfall and S-79 discharges, manifesting the nutrient-enriched character of watershed inputs to the Caloosahatchee Estuary (Doering & Chamberlain, 1999; Doering et al., 2006; Medina et al., 2021, 2022). *A. sanguinea* also benefits from its mixotrophic capabilities, which allows it to acquire organic carbon and nutrients through phagotrophy (i.e., consumption of particulates) (Burkholder et al., 2008).

The major role played by *A. sanguinea* in blooms observed in the Caloosahatchee Estuary during this study period is in part related to the euryhaline nature of the species (Matsubara et al., 2007; Badylak et al.,

2014a), which is an advantage in the upper estuary where salinities generally vary within the mesohaline to lower polyhaline range (5–25 psu). Another key factor in the prominence of *A. sanguinea* is the apparent presence of a seed bank of resting cysts in the upper estuary, as evidenced by previous observations of all major developmental life stages of the species during the beginning of the 2009 bloom in the upper estuary (Badylak et al., 2017). *A. sanguinea* is a globally important HAB species (Hallegraeff et al., 2003; Lassus et al., 2016) and common to many Florida estuaries (Badylak et al., 2007, 2014b, 2017; Quinlan & Phlips, 2007; Phlips et al., 2010; Hart et al., 2015). While *A. sanguinea* is not known to be toxic, it has been associated with mass mortality events of marine animals (Harper & Guillen, 1989; Shumway 1990; Kahru et al., 2004), in part due to the potential for the development of hypoxic conditions during intense blooms (Hallegraeff et al., 2003). *A. sanguinea* is also known to produce large quantities extracellular carbohydrate polymer (Badylak et al., 2014b), as shown for other taxa linked to ecosystem disruption associated with polymer production (Phlips et al., 1989, 1999; Liu & Buskey, 2000; Botes et al., 2002; Smayda, 2002; Gobler & Sunda, 2012).

Nearshore blooms

In the nearshore environment outside the Caloosahatchee Estuary, major phytoplankton blooms observed in 2018–2019 involved the toxic red tide dinoflagellate *Karenia brevis*. *K. brevis* red tides occur on a regular basis in the Gulf of Mexico (Tester et al., 1991; Steidinger, 2009; Vargo, 2009; Brand et al., 2012; Heil et al. 2014a). The blooms have been the subject of extensive research, in part because of threats to human health and marine resources (Kirkpatrick et al., 2004; Fleming et al., 2005; Landsberg et al., 2009; Heil & Muni-Morgan, 2021). Unlike *A. sanguinea* blooms, *K. brevis* blooms generally start offshore on the continental shelf of the Gulf and then are distributed along the west coast of Florida by prevailing circulation patterns (Weisberg et al., 2019). The more stenohaline character of *K. brevis* than *A. sanguinea* is reflected in its preference for salinities above 30 psu (i.e., euhaline conditions), although historical observations of blooms frequently extend to salinities down to 20 psu (Steidinger, 2009). The

latter trends are supported by the relationship between salinity and *K. brevis* biomass observed in this study.

The potential influence of outflows from the Caloosahatchee Estuary on the intensity of red tide events has been an important topic of concern for decades, particularly as it relates to effects of nutrient loads from the estuary (Steidinger, 2009; Vargo, 2009; Heil et al., 2014b; Medina et al., 2021, 2022). The Caloosahatchee Estuary is one of a number of major sources of nutrient inputs to the southwest coast of Florida (Heil et al., 2014b). However, quantifying the specific role of these inputs in red tide events has been challenging because of the multitude of potential sources of nutrients for red tides, such as deep-water upwelling from the edge of the shelf (Walsh et al., 2003), nutrient inputs from shelf sediments (Dixon et al., 2014b), nitrogen fixation by diazotrophic cyanobacteria (e.g., *Trichodesmium*) (Mulholland et al., 2014), and re-cycling of nutrients from decomposing biomass (Bronk et al., 2014), including the decomposition of biomass from animal mortality caused by the toxic red tides (Killberg-Thoreson et al., 2014b). Much of the focus has been on nitrogen because phytoplankton production in the coastal environment near the Caloosahatchee Estuary is most often nitrogen limited (Dixon et al 2014a; Heil et al. 2014b; Killberg-Thoreson et al., 2014a), as reported for several other coastal regions along the west and east coast of Florida (Bledsoe & Phlips, 2000; Phlips et al., 2002; Bledsoe et al., 2004). In terms of the general role of nitrogen availability for blooms, the positive correlation between TN concentration and dinoflagellate biomass observed in our study (Fig. 12b) further supports the importance of nitrogen supply. The potential importance of nitrogen from Caloosahatchee estuary discharges in terms of red tide intensity is also suggested by the close proximity of *K. brevis* and both mean discharge and TN:TP ratio vectors on the NMDS analysis axes for the Caloosahatchee Estuary (Fig. 13c, d).

Medina et al., (2021) used non-linear time series analyses to show a relationship between the seasonal timing of peaks of NO_x (nitrate + nitrite) concentrations in water entering the Caloosahatchee Estuary from the S-79 control structure, and peak seasons of *K. brevis* blooms in the coastal region near the Caloosahatchee Estuary, between 26° and 27°N latitude, and up to 16 km from the shore. While this is a valuable observation about the seasonality of the

two variables, many questions remain about the specific causal linkages. For example, how much is the temporal pattern of *K. brevis* biomass in the region related to autochthonous primary production versus allochthonous introductions of biomass from outside the region? In addition, what is the relative role of NO_x in supporting *K. brevis* blooms, by comparison to other soluble and particulate, inorganic and organic source of nitrogen? The latter question is complicated by the mixotrophic character of the species, and by the potential contribution of other nitrogen forms, including dissolved organic nitrogen (DON) (Pisani et al., 2017), as observed in many other ecosystems (Glibert et al., 2006; Bronk et al., 2007; Ivey et al., 2020; Kang & Kang, 2022). It is noteworthy that the peak *K. brevis* biomass values observed in this study were 6 mg carbon l^{-1}, which equates to roughly 1 mg nitrogen l^{-1}, based on estimates using the Redfield ratio, or five times higher than peak NO_x values reported by Medina et al., (2021), further suggesting that a range of different nitrogen types and sources help drive blooms.

During this study, major *K. brevis* blooms (i.e., $> 10^6$ cells l^{-1}) were observed in the latter half of the 2018 wet season and the end of the 2019 wet season. The period was associated with mean wet season total nitrogen concentrations in the Caloosahatchee Estuary approximately twice as high as in the wet season of 2009–2010. The high TN:TP ratios observed during the wet seasons of 2018–2019 further suggest a role for nitrogen inputs from the estuary in the high intensity of red tides in 2018 and 2019, compared to 2009–2010. The Bloom Severity Index (BSI) recently developed by Stumpf et al. (2022) reported a moderate to high index value of 8 for 2018 (i.e., the highest value over the 1953–2019 recording period) and 2.2 for 2019. By comparison, the 2009 BSI was 0.4, and < 0.1 for 2010, both among the lowest values for the period of record. Differences in TN concentrations in the S-79 outflow may in part reflect temporal variability in the relative volumes of water from Lake Okeechobee versus other tributaries that introduce water to the C-43 Canal from watersheds downstream of Lake Okeechobee, as well as inputs from watersheds in the immediate vicinity of the estuary. It is known that specific sources of nutrient inputs differ in nutrient composition and concentrations spatially and temporally (Heil et al., 2014b; SFWMD, 2014; Medina et al., 2021, 2022). Similar observations have

been made for discharges from Lake Okeechobee and regional watersheds associated with the St. Lucie estuary on the east coast of Florida (Moncada et al., 2021). The character and quantity of these external inputs to the Caloosahatchee and St. Lucie estuaries also vary in relation to the timing and levels of rainfall levels and changes in regulatory discharges from water control structures (e.g., S-77, S-79). The difference in TN concentrations in the Caloosahatchee Estuary between the 2009–2010 and 2018–2019 time periods could also be partially attributable to changes in watershed characteristics over the ten-year gap between the 2009–2010 and 2018–2019. The 10-year period was subject to substantial economic growth in the region after the depressing effects of the"Great Recession" of 2007–2009. World-wide development in coastal watersheds has accelerated cultural eutrophication and increased the potential for algal blooms (Glibert et al., 2005; Paerl et al., 2006, 2008; Heisler et al., 2008; Moore et al., 2020; Anderson et al., 2021).

Other important taxa

Outside of time periods dominated by *A. sanguinea* blooms in the Caloosahatchee Estuary and *K. brevis* blooms in the nearshore environment, one characteristic shared by all regions and salinity regimes observed in this study was the importance of picoplanktonic cyanobacteria. Although biomass levels for picocyanobacteria did not reach the peaks encountered for the former two taxa, they were generally the most numerically abundant phytoplankton group, reflecting their importance in microbial loop processes (Stockner, 1988; Pomeroy et al., 2007; Fenchel, 2008). For example, the mixotrophic red tide species *K. brevis* has been shown to graze on the picoplanktonic cyanobacteria (Glibert et al., 2009). Similarly, the strong presence of other mixotrophic and heterotrophic dinoflagellates on the Top-50 list of major biomass contributors to phytoplankton biomass in our study highlights the potential importance of the microbial loop in the dynamics of phytoplankton in the Caloosahatchee Estuary and nearshore environment. The opposing direction of vectors for picoplanktonic cyanobacteria and *K. brevis*/dinoflagellates biomass in the NMDS plots for the nearshore region (Fig. 13c, d) seems to support this observation.

Another noteworthy feature of the Top-50 list during oligohaline conditions in the Caloosahatchee Estuary is the prominence of a wide range of freshwater taxa that are introduced to the estuary from the C-43 Canal via the S-79. These include a number of cyanobacteria genera that contain potentially toxic species. Previous studies of HAB cyanobacteria in the canal systems emanating from Lake Okeechobee that flow into the Caloosahatchee and St. Lucie estuaries have observed a number of toxin-producing filamentous cyanobacteria, such as *Raphidiopsis raciborskii* (Woloszynska) Aguilera & al. and *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) Wacklin, Hoffmann & Komárek (Rosen et al., 2018; Metcalf et al., 2021). These taxa were observed during our study, but at low levels of biomass. Another potentially toxic freshwater taxa observed the Top-50 list was the dinoflagellate genus *Peridinium* (Adachi, 1965; Rengefors & Legrand, 2001; Carty, 2014). *Peridinium* species have been linked to ichthyotoxic events (Hashimoto et al., 1968), and algicidal effects against some other algal taxa (Wu et al., 1998). There is a range of potential allochthonous threats to the estuary from introductions of freshwater HAB species from the C-43 Canal, particularly given the role of inputs from Lake Okeechobee.

One important HAB phenomena in the Caloosahatchee Estuary not encountered during our study period was the introduction of intense toxic cyanobacteria blooms from Lake Okeechobee into the estuary via the C-43 Canal. Discharges of water containing high concentrations of toxic cyanobacteria have been observed in both the Caloosahatchee Estuary via C-43 Canal inputs (Metcalf et al., 2021) and the St. Lucie estuary on the east coast of Florida via inputs from the St. Lucie Canal (Phlips et al., 2012). One of the most common toxic species involved in the HAB events in both estuaries has been *Microcystis aeruginosa* (Kützing) Kützing (Phlips et al., 2012; Rosen et al., 2018; Metcalf et al., 2021), a prolific producer of the hepatotoxin microcystin (Black et al., 2011). Large volume discharges of bloom-laden water into the estuaries from Lake Okeechobee not only represent a risk of exposure to cyanobacterial toxins, but, as in the case of *K. brevis* blooms, these events also represent a risk for hypoxia and disruption of food web structure and function (Phlips et al., 2012; Milbrandt et al., 2021). The introductions can result in widespread surface scums of *M. aeruginosa* in the

estuaries, which can contain microcystin concentrations over 1000 μg l^{-1} (Phlips et al., 2012; Metcalf et al., 2021); very high considering WHO guideline for human recreational exposure is 24 μg l^{-1} (WHO, 2020).

Future directions

Local, state, and federal environmental organizations are actively pursuing research to find ways to control and/or mitigate exposure of the Caloosahatchee estuaries, and nearshore regions, to HAB events (Heil & Muni-Morgan, 2021; U.S.E.P.A., 2021). It is likely that future rates, duration, and timing of discharges from S-79 into the Caloosahatchee Estuary will play key roles in defining the character and impacts of freshwater and marine HABs on the estuary and associated nearshore environments. Predicting the future impacts of HABs on the Caloosahatchee Estuary (Buzzelli et al., 2014; Julien & Osborne, 2018), and other estuaries around the world, will be complicated by the effects of cultural eutrophication on nutrient loads and climate change on temperature, rainfall, and tropical storms (Howarth et al., 2000; Heisler et al., 2008; Glibert et al., 2014; Thompson et al., 2015; Paerl et al., 2016; Gobler, 2020; Phlips et al., 2020; Anderson et al., 2021). It has been predicted that there will be increased in all four factors in the near and long term, creating additional challenges for managing nutrient loads, hydrology, and biology of the estuary and nearshore regions. Efforts to mitigate the intensity and frequency of major HAB events in coastal ecosystems around the world have often focused on the control of nutrient availability (Nixon, 1995; Cloern, 2001; Heisler et al., 2008), which is certainly an important target for management. However, in many ecosystems like the Caloosahatchee Estuary, hydrologic considerations are also of central importance. The presence of water control structures in the Caloosahatchee ecosystem present challenges to satisfying multiple and sometimes conflicting objectives, including the control of Lake Okeechobee water levels to avoid catastrophic flooding, providing water for agriculture activities, maintaining navigational needs, and preserving the health of the lake, estuary, and adjacent coastal regions (SFWMD, 2014). At the same time, the presence of these structures provides opportunities to manage HABs through targeted regulation of discharge regimes,

ideally with recognition of future changes in climatic conditions and cultural eutrophication. As additional temporally intensive data on the dynamics of phytoplankton communities and nutrient concentrations in the Caloosahatchee ecosystem become available, it should be possible to develop quantitative models that help to define optimal discharge regimes to minimize ecosystem disruptions from HABs.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors do not have any potential conflict of interest.

Ethical approval The research did not involve human participants and/or animals.

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