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Blooms of *Karenia brevis* (Davis) G. Hansen & Ø. Moestrup on the West Florida Shelf: Nutrient sources and potential management strategies based on a multi-year regional study

Cynthia A. Heil [a,c,*], L. Kellie Dixon [b], Emily Hall [b], Matthew Garrett [c], Jason M. Lenes [d], Judith M. O'Neil [e], Brianne M. Walsh [e], Deborah A. Bronk [f], Lynn Killberg-Thoreson [f], Gary L. Hitchcock [g], Kevin A. Meyer [e], Margaret R. Mulholland [h], Leo Procise [h], Gary J. Kirkpatrick [b], John J. Walsh [d], Robert W. Weisberg [d]

[a] *Bigelow Laboratory for Ocean Sciences, 60 Bigelow Road, East Boothbay Harbor, ME 04544, USA*
[b] *Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA*
[c] *Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 800 1st Avenue South, St. Petersburg, FL 33701, USA*
[d] *College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, FL 33701, USA*
[e] *University of Maryland, Center for Environmental Science, Horn Point Laboratory, 2020 Horn Point Road, Cambridge, MD 21613, USA*
[f] *Department of Physical Sciences, Virginia Institute of Marine Science, The College of William & Mary, Gloucester Point, VA 23062, USA*
[g] *Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA*
[h] *Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Norfolk, VA 23529, USA*

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ABSTRACT

Identification and quantification of the nutrient sources supporting large, extended duration *Karenia brevis* blooms on the West Florida Shelf (WFS) in the eastern Gulf of Mexico are critical steps for effective bloom management and mitigation. Previous research had identified multiple (>12) potential nutrient sources available to *K. brevis* blooms on the WFS, which vary with bloom stage, location, biomass and bloom toxicity. This current study newly identified and quantified additional nitrogen (N) sources including water column nitrification, photochemical nutrient production, pelagic unicell N_2 fixation by diazotrophs other than the colonial cyanobacterium *Trichodesmium*, and remineralization from seasonal *Trichodesmium* biomass decay and microzooplankton grazing (and estimated regeneration). Newly identified phosphorus (P) sources include remineralization from *Trichodesmium* biomass decay and microzooplankton grazing. In estuarine environments, benthic nutrient flux, mixotrophic consumption of picoplankton, nutrient release from zooplankton and microzooplankton grazing, photochemical nutrient production, and nitrification all can contribute up to 100% of the N and/or P requirements of small ($<10^5$ cells L^{-1}) *K. brevis* blooms. During average estuarine flow years, combined estuarine sources contribute up to 17 and 69% of the N and P needs of these blooms, however local estuarine contribution can increase to 100% for exceptional, high flow years. In coastal and offshore environments, regenerated nutrient sources become increasingly important to blooms, with zooplankton excretion, nitrification, decay and regeneration of nutrients from dead fish and pelagic N_2 fixation potentially providing 100% of bloom N and P needs. During the largest observed coastal blooms (14.0×10^6 cells L^{-1}) N_2 fixation and release and decay of seasonal *Trichodesmium* bloom biomass were the only sources of N and P that were completely sufficient to support blooms of that magnitude.

Given the complexity of *K. brevis* bloom dynamics, the multiple available nutrient sources on the WFS and the importance of regenerated N forms in supporting blooms, efforts to reduce potentially controllable nearshore nutrient inputs should be undertaken with the understanding that while they may lead to enhanced coastal water quality, they may not have an immediate impact on the frequency or magnitude of nearshore *K. brevis* blooms. Additionally, time lags in ecosystem responses or differences in the time scales on which various process operate may require multi-year assessments to determine how

* Corresponding author at: Laboratory for Ocean Sciences, 60 Bigelow Drive, East Boothbay, ME 04544, USA. Tel.: +1 207 315 2567x304; fax: +1 207 315 2329.
E-mail address: cheil@bigelow.org (C.A. Heil).

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effective management practices are in relation to *K. brevis* blooms. Timely red tide related monitoring products that allow for effective focusing of monitoring needs for short-term prediction of impacts and targeted communication of scientific results to the public and stakeholders, remains the most effective means of *K. brevis* management.

1. Introduction

Harmful algal blooms (HABs) have been recognized to be increasing in frequency, extent and durations in recent decades (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993; Anderson et al., 2002). Coastal eutrophication is one of the factors likely responsible for this increase, but this relationship is complex, species specific, and poorly understood (Anderson et al., 2002, 2008; Glibert et al., 2005). Although *Karenia brevis* blooms are usually not included in a list of HAB species specifically linked with eutrophication (e.g. Anderson et al., 2008), Brand and Compton (2007) have suggested that *K. brevis* blooms in the eastern Gulf of Mexico are linked to increases in coastal nutrient inputs. Correlation analyses between historical bloom occurrence (using the same HAB historical database as Brand and Compton (2007)) and a variety of proxies of estuarine inputs (e.g. river flow, precipitation) reported weak correlations with combined river flow from the Peace and Apalachicola Rivers (the primary freshwater inputs), precipitation data from the entire state (Feinstein et al., 1956), or rainfall levels the month prior to the bloom (Dixon and Steidinger, 2002) in the central FL region however. Additionally no significant relationships were found between *K. brevis* blooms and either the largest freshwater discharge to the West Florida Shelf (WFS) (Apalachicola River), nor for highly managed systems such as the Caloosahatchee River (Dixon and Steidinger, 2002). Coastal eutrophication is undoubtedly occurring in this region, however. Turner et al. (2006) documented a 3-fold increase in the nitrogen (N) loading to the Charlotte Harbor region alone since the 1880s. Forecasts for future rapid population growth in the region (Crosset, 2005; Smith and Nogle, 2000) suggest that this loading trend will likely continue. Further complicating potential relationships between anthropogenic nutrient sources and *K. brevis* blooms is that fact that these blooms predate the extensive development, anthropogenic nutrient loading, and eutrophication that characterize present day southwest Florida. Evidence supporting this claim is based on numerous reports of extensive fish kills in the same WFS region since the 1500s, descriptions of human illnesses related to shellfish consumption that are clearly and uniquely symptomatic of Neurologic Shellfish Poisoning (NSP) (which is specific to brevetoxin exposure) and descriptions of human respiratory related impacts that clearly describe the symptomology of *K. brevis* associated respiratory distress (Steidinger, 2009; Lasker and Walton Smith, 1954; Walsh et al., 2006, 2009; Magaña and Villareal, 2006).

Karenia brevis blooms initiate offshore in oligotrophic shelf waters and are transported shoreward to nutrient-enriched, coastal and estuarine waters during development and maintenance stages. A specific example of how *K. brevis* cells are transported and distributed along the near shore region is provided by Weisberg et al. (2009) for the protracted bloom of 2005. Using a combination of observations with numerical circulation model simulations they demonstrated that the mode of transport is the bottom Ekman layer via an upwelling circulation. This work built upon earlier upwelling discussions by Weisberg et al. (2000, 2001) and Weisberg and He (2003) which further argued for the region from Tampa Bay to Charlotte Harbor being the epicenter for *K. brevis* blooms owing to the demonstrated upwelling circulation pathways. A consequence of this transport is that blooms occur across onshore–offshore nutrient gradients on the WFS. Given that *K. brevis* can thrive on many different forms of nitrogen (N) and phosphorus (P) (Vargo, 2009), and that the sources of nutrients vary between nearshore and offshore environments (Heil et al., 2014b, in this issue), linking *K. brevis* blooms to a single source of N or P has proven impossible. In offshore waters, *Trichodesmium* N_2 fixation and regeneration (Mulholland et al., 2004, 2006, 2014, in this issue) and zooplankton excretion (Lester et al., 2008; Vargo et al., 2008) can be significant sources of N and P. In the nearshore region, nutrients fueling bloom expansion and intensification also include anthropogenic nutrient inputs (Yentsch et al., 2008; Vargo et al., 2008; Uhlenbrock, 2009), benthic nutrient flux (Vargo et al., 2008; Dixon et al., 2014b, in this issue) as well as atmospheric inputs (Vargo et al., 2008). Although these many different potential nutrient sources available to *K. brevis* blooms have been identified, none have been temporally or spatially quantified over the geographic range of bloom environments.

Blooms also occur over a large latitudinal range on the WFS between the cities of Clearwater and Sanibel Island (Steidinger et al., 1998) that is characterized by disparate terrestrial nutrient inputs (Heil et al., 2007). Phosphorus (P) mining has occurred in the central Florida region since the late 19th century (Pittman, 1990), and coastal areas in this region are characterized by elevated inorganic and organic P coastal inputs and severe N limitation (McPherson and Miller, 1990). The more southerly region of the WFS receives gated flows from the heavily managed Caloosahatchee River, which is characterized by elevated DON and NH_4^+ concentrations (Heil et al., 2007; Lapointe and Bedford 2007; Uhlenbrock, 2009). Further south, inputs to the coastal region from the P-limited Everglades system have elevated dissolved organic nitrogen (DON) concentrations but little dissolved P (Heil et al., 2007).

How *Karenia brevis* blooms initiate and persist under such a wide range of disparate nutrient conditions has been the subject of much debate over the years. The first studies of nutrient controls on *K. brevis* blooms focused on riverine nutrient inputs, specifically from the Caloosahatchee River, with the goal of identifying the causes of the seemingly 'sudden' appearance of blooms in the nearshore region (Ketchum and Keen, 1948; Graham et al., 1954). Early explanations for the rapid appearance of blooms also included physical factors (e.g. the concentration of cells at fronts (Chew, 1953, 1955)) and the presence of trace metal chelators derived from riverine sources (Martin et al., 1971). In 1975, the sudden appearance of elevated *K. brevis* cell concentrations nearshore was explained by the recognition of offshore initiation of blooms and their subsequent transport, expansion and physical concentration over an extended period to the nearshore environment (Steidinger, 1975a,b). Despite this extensive early literature on the complex relationship between nutrient sources and *K. brevis* blooms (see Steidinger, 2009; Vargo, 2009; Brand et al., 2012 for reviews), nearshore estuarine and riverine nutrient inputs linked with coastal eutrophication have become a primary public and management focus in southwest Florida (Badrazzaman et al., 2012). Relating nutrient concentrations and *K. brevis* blooms is problematic however, because of the diversity and variability in nutrient supplies along the WFS, described above, and the lack of direct relationships between nutrient and *K. brevis* concentrations *in situ* (Vargo et al., 2008).

The NOAA supported ECOHAB: *Karenia* program (2006–2012) had the goals of (1) examining how the carbon (C), N and P physiology of *Karenia brevis* contributes to its local dominance and (2) identifying and quantifying the multiple nutrient sources available to *K. brevis* blooms over the spatial and temporal gradients which characterize these blooms. Here, we use the results from this and previous studies to develop recommendations in support of effective management of *K. brevis* blooms in southwest Florida.

2. Methods and results

2.1. Karenia brevis *blooms, 2007–2010*

The ECOHAB: *Karenia* field program conducted a 4-year sampling program on the WFS from 2007 to 2010 which consisted of research cruises in October of each year, the period when *Karenia brevis* blooms are most frequently observed (Table 1 in Heil et al., 2014a, in this issue). The program also included analysis and modeling of historical field data from both NOAA and NSF funded research on the 2001 *K. brevis* bloom. These cruises, aboard the Louisiana University Marine Consortium's (LUMCON) R/V *Pelican* sampled (1) a nearshore, high-biomass bloom (14.0×10^6 cells L^{-1}) with a large areal extent that was associated with fish kills in 2007 and had a nine and a half month duration; (2) a newly initiated, low biomass bloom (7.6×10^5 cells L^{-1}) in 2008; (3) an offshore, high biomass bloom (1.5×10^5 cells L^{-1}) that had a duration of six and a half months in 2009; and (4) a year (2010) during which there was no bloom ($<5.0 \times 10^3$ cells L^{-1}). A detailed history of each individual bloom is given in Supplemental Material.

During all three years (2007–2009), blooms were first detected and sampled in an area to the west/southwest of Sanibel Island (see Heil et al., 2014a, in this issue, Fig. 2). These blooms differed in their areal extent and cell concentrations despite their similar location, however (see Heil et al., 2014a, in this issue). Based on microscope observations of cells, cell concentrations, ancillary measurements and monitoring data (summarized in Table 1), it was determined that the 2001 and 2007 blooms were in maintenance phase at the time of sampling, the 2008 *Karenia* bloom sampled was in its initiation phase, and the 2009 bloom was in a late maintenance phase. Dixon et al. (2014a, in this issue) classified hydrological conditions during these blooms based on average salinity across the shelf throughout the sampling period and determined that drought conditions were present in 2007 (median salinity 37), with estuarine flows increasing and median coastal salinities declining each year through 2010 (median salinity 35).

2.2. Nutrient source estimates

Measurements of individual nutrient sources and uptake rates were made during cruises and field efforts as well as in dedicated laboratory experiments. During the project seven stations representative of the region (i.e. estuarine, coastal, and offshore) were sampled on each cruise (Table 2) with additional stations added in the areas of blooms when *Karenia brevis* was detected (O'Neil and Heil 2014, in this issue). Relevant results are reported in other papers in this volume and summarized here.

2.2.1. Previously identified nutrient sources

Methods for quantifying previously identified nutrient sources potentially fueling *Karenia brevis* blooms on the WFS are provided in detail, and data used for flux calculations in this paper, are found in the following references: benthic nutrient fluxes (Dixon et al., 2014b, in this issue), N_2 fixation and release by the cyanobacteria *Trichodesmium* spp. (Mulholland et al., 2014, in this issue), regeneration by zooplankton (Walsh and O'Neil, 2014, in this issue) and release from dying fish (Killberg-Thoreson et al., 2014b, in this issue).

Benthic fluxes ranged from 600 to 1600 μM m^{-2} d^{-1} for NH_4^+ and 100 to 250 μM m^{-2} d^{-1} for PO_4 (Dixon et al., 2014b, in this issue). Benthic flux estimates of N and P were elevated (Dixon et al., 2014b, in this issue) compared with prior estimates (Darrow et al., 2003; Vargo et al., 2008), especially for P (Table 4). Measurements of N_2-fixation by the colony forming marine cyanobacteria *Trichodesmium* on the WFS were made between 2001 and 2003 (Mulholland et al., 2006) and between 2007 and 2010 (Mulholland et al., 2014), in this issue), using either the acetylene reduction method or the $^{15}N_2$ gas method (Mulholland et al., 2006). Rates ranged from 0 to 13.6 nmol N L^{-1} d^{-1} and were comparable with prior estimates for the eastern Gulf of Mexico (Vargo et al., 2008). Nitrogen and P regeneration from zooplankton excretion were calculated by Walsh (2012) using zooplankton abundance, *Karenia brevis* cell concentrations, and copepod grazing rates measured during the project cruises (Walsh and O'Neil, 2014, in this issue) and zooplankton excretion rates from the southwest Florida shelf (Lester, 2005). These ranged from 1.7039 to 14.8078 μmol N $L^{-1}d^{-1}$ and 0.0004 to 0.2825 μmol P L^{-1} d^{-1}. An average zooplankton N regeneration rate based on 11 stations with *K. brevis* present sampled in the 2007, 2008 and 2009 blooms

Table 1
ECOHAB: *Karenia* bloom stages sampled and the criteria used to identify each bloom stage.

Year	Bloom stage during sampling	Bloom stage criteria
2007	Maintenance	(1) Monitoring history prior to sampling (2) Very high bloom biomass (3) Nearshore location (4) Bloom growth rates of 1 day^{-1}[a] (5) SiO_4 concentrations of 22.9 (±6.4) μmol L^{-1}[b]
2008	Initiation	(1) Low bloom biomass (2) Tracking of bloom movements from offshore to nearshore during the first week of the cruise coincident with increasing cell concentrations (3) Lack of detection of any *K. brevis* cells in the region by FWRI monitoring program prior to sampling
2009	Maintenance/Stationary	(1) Offshore location of bloom (2) Low to moderate concentrations of *K. brevis* cells present (3) *K. brevis* cells within the bloom contained unusual amounts of lipid bodies, indicative of older cells in stationary phase[c] (4) Lower SiO_4 concentrations of 2.7 ± 0.8 μmol L^{-1}[c]

[a] Sipler et al. (2013).
[b] Dixon et al. (2014a, in this issue).
[c] Steidinger (1979a,b).

Table 2
Locations of the stations sampled during week #1 of each research cruise during the study and their characterization as estuarine (E), coastal (C) or offshore (O) in analyses. Sites with *K. brevis* present were sampled during week #2 and followed with a surface drogue so there location varied between cruises.

Station	Type	Location	Latitude	Longitude
1	O	Offshore	27.1050° N	83.0580° W
2	C	Outside Charlotte Harbor	26.7157° N	82.2686° W
3	C	Mudhole Spring,[a] Caloosahatchee-River Mouth, San Carlos Bay	26.2595° N	82.0092° W
4	E	Inside Charlotte Harbor	26.7268° N	82.1902° W
5	C	Outside Sarasota Bay	27.2675° N	82.5900° W
6	E	Inside Tampa Bay	27.6835° N	82.5925° W
7	C	Outside Tampa Bay	27.5680° N	82.8050° W

[a] Station was located at the site of Mudhole Spring, one of the largest freshwater spring off the southwest Florida coast (Fanning et al., 1981).

(Walsh, 2012) was 4.99 μmol N L^{-1} d^{-1}, seven times greater than prior estimates (Vargo et al., 2008) (Table 3).

Release of nutrients from fish decay ranged from 37.7 (±12.6) to 102.3 (±47.0) mmol N L^{-1} g^{-1} wet weight d^{-1} and from 13.9 (±2.8) to 21.4 (±2.9) mmol P L^{-1} g^{-1} wet weight d^{-1} (Killberg-Thoreson et al., 2014b, in this issue). These rates were used in conjunction with *Karenia*-related fish kill data from the Texas Parks and Recreation red tide Pollution Response and Species Mortality (PRISM) fish kill database to estimate N and P inputs from *Karenia brevis* associated fish kills. Four representative *K. brevis* associated fish kill events along the Texas coastline were used to derive a potential range of nutrient inputs. Length-width regressions (provided by Texas Parks and Wildlife) were applied to length distribution data for each fish species from each kill event to provide a total weight for each species involved in the kill. These Texas data, in combination with N and P release data (Killberg-Thoreson et al., 2014b, in this issue), were used to calculate the range of N and P potentially produced per fish kill event. The resulting N and P amounts were divided by the ECOHAB: Florida receiving volume calculated from the distance between TB and CH out to the 10 m isobath (7.642×10^{12} L, Vargo et al., 2008) to achieve N and P flux rates to the coastal region. This volume, described by the distance between Tampa Bay and Charlotte Harbor out to the 10 m isobaths (Vargo et al., 2008), is based on the location of salinity fronts and elevated *K. brevis* cell concentrations that occur between the shore and the 10–12 m isobath described by Vargo et al. (2001) and the isothermal and isohaline nature of the water column out to this depth when blooms are in their early stages (Vargo et al., 2001; Dixon et al., 2014a, in this issue). Updated estimates of N and P flux from dead fish were approximately equivalent or an order of magnitude less than prior estimates depending upon the volume used in calculations. Use of the ECOHAB control volume resulted in N and P flux rates approximately 20% less than that of Vargo et al. (2008), while use of the areal extent of the four Texas *K. brevis* related fish kills resulted in one to three orders of magnitude reductions in both N and P fluxes.

Table 3
Average nutrient loads (mol day^{-1}) from the three main estuarine regions bordering southwest Florida.

	NH_4^+	NO_{3+2}	DIN ($NO_{3+2} + NH_4^+$)	PO_4^{-2}
Tampa Bay				
2000–2007[a]	6677	3418		7461
2007	187	917		1334
2008	1480	2131		2931
2009	436	720		2062
2010	769	2705		5406
Charlotte Harbor				
2000–2007[a]	21,960	12,268		17,229
2007[a]	1178	107		42
2008	8823	1818		946
2009[c]	8984	1827		990
2010	1634	302		2234
Caloosahatchee River				
2000–2007[a]	16,776	36,599		7834
2005[b]			198,442	23,032
2007	355	30		34
2008	3962	1286		626
2009	8940	297		1136
2010	16,355	713		854

[a] Estimate is based on summed flow and nutrient data from Dixon (2008) for the 2000–2007 period.
[b] Data from Uhlenbrock (2009) for the period from April 2005 to December 2005.
[c] Flow data was missing from 6 months of the year so was interpolated from an average of the remaining years for each of the missing months.

Riverine nutrient loading to the estuaries were calculated for the three main estuarine systems that influence the WFS, the Tampa Bay estuary, the Charlotte Harbor estuary and the Caloosahatchee River/San Carlos Bay estuary complex, for the combined 2001–2007 period (Dixon, 2008) and 2005 (Uhlenbrock, 2009) and 2007, 2008, 2009 and 2010 using the methodology of Dixon (2008). Nutrient loads of NH_4^+, NO_3^{2+}, and PO_4^{-2} for the major estuarine systems impacting the WFS over the period covered by the ECOHAB: *Karenia* Program are given in Table 3. These numbers represent minimum N and P fluxes as they do not include DON and DOP; the bioavailability of which is unknown in these systems. A high degree of both interannual and source variability are evident in load values. Loads of PO_4^{-2} were generally greatest in the Tampa Bay estuary, while NH_4^+ loads were generally highest in Charlotte Harbor. The loads of NH_4^+, NO_3^{+2}, and PO_4^{-2} from the Caloosahatchee River were lowest in 2007, a dry year (Dixon et al., 2014a, in this issue), but in general were highly variable depending upon upriver management needs and the subsequent highly variable Lake Okeechobee water releases. Of note are the 2005 Caloosahatchee River DIN and PO_4^{-2} loads, the highest observed from 2000 to 2010, which included the extraordinarily high Lake Okeechobee releases to the Caloosahatchee River in 2005 to manage elevated Lake Okeechobee water levels during one of the wettest years on record (Uhlenbrock, 2009; Dixon et al., 2014a, in this issue). The 2000–2007 estimated average loads of both N and P are elevated compared with the 2007–2010 period due to inclusion of this 2005 data. To calculate riverine flux to the estuaries, the combined loads from Tampa Bay, Charlotte Harbor and the Caloosahatchee River were summed and then diluted into the combined estuarine volumes of Tampa Bay and Charlotte Harbor calculated from surface area and average depths (obtained from Gulfbase.org). Caloosahatchee River N and P loading data from 2005 (Uhlenbrock, 2009), combined with the Charlotte Harbor estuarine volume, were used to calculate estuarine N and P fluxes due solely to the Caloosahatchee River. To calculate riverine flux to the coastal zone, loads (both for the 2007–2010 study period for and the 2005 Caloosahatchee River loads) were diluted into the ECOHAB: Florida receiving volume (7.642×10^{12} L, Vargo et al., 2008). Estuarine N and P flux to the coastal zone during 2007–2010 was an order of magnitude less that that calculated by Vargo et al. (2008) for the 1998–2001 period or for the flux to the coastal zone from the Caloosahatchee River in 2005 (Table 4). Riverine flows in advance of the 2007 cruise were minimal, amongst the lowest measured since 1970 (Dixon et al., 2014a, in this issue), and the estuarine N and P flux for the individual estuaries were the lowest of those measured that year. Flow in subsequent years gradually increased until 2010 (Dixon et al., 2014a, in this issue), which is reflected in increases in both N and P flux data.

Atmospheric areal deposition rates were calculated according to the method of Vargo et al. (2008) for 2007 through 2010 based on N deposition data available for National Atmospheric Deposition Program (NADP) National Trends Network (NTN) monitoring

Table 4
Comparison of nutrient flux rates to the southwest Florida coastal zone from established and newly identified west Florida shelf nutrient sources with previous estimates of nutrient sources supporting *K. brevis* blooms.

Nutrient source	Flux rate ($\mu mol\ L^{-1}\ d^{-1}$)	
	N	P
Established nutrient sources		
Benthic flux		
Vargo et al. (2008)	0.0016–0.100	0.000
Dixon et al. (2014b, in this issue)[a]	0.110	0.017
Decaying fish		
Vargo et al. (2008)	5.33	0.17
Killberg-Thoreson et al. (2014b, in this issue)[b]	4.20	0.10
Killberg-Thoreson et al. (2014b, in this issue)[c]	0.003–0.411	0.001–0.086
Zooplankton excretion		
Vargo et al. (2008)	0.71 (0.01–6.8)	0.49 (0.006–3.1)
This study Walsh (2012)	4.99 (1.7039–14.8078)	0.19 (0.0004–0.2825)
Estuarine flux		
Vargo et al. (2008)	0.035 (0.016–0.062)	0.0016 (0.0008–0.002)
Dixon et al. (2014b, in this issue)	0.002 (0.0004–0.0029)	0.0006 (0.0002–0.0011)
This study (Caloosahatchee River only, 2005)	0.026	0.003
Atmospheric deposition		
Vargo et al. (2008)	0.008 (0.0016–0.00046)	6.4×10^{-6} (1.5×10^{-5}–2.7×10^{-6})
This study	0.0103 (0.0099–0.0196)	N.D.
Trichodesmium N_2 fixation and N regeneration		
Vargo et al. (2008)	0.032–0.164	N.D.
Mulholland et al. (2014, in this issue)	0.176	N.D.
Newly identified sources		
Mixotrophic consumption of picoplankton		
Procise (2011)[d]	0.004–0.554	0.0003–0.0349
Jeong et al. (2005)[d]	0.0198–2.7720	0.0013–0.0198
Glibert et al. (2009)[d]	0.0038–46.4587	0.0002–2.9283
Pelagic N_2 fixation by N_2 fixers other than *Trichodesmium*		
Mulholland et al. (2014, in this issue)	0.006	N.D.
Trichodesmium N_2 fixation, N regeneration and seasonal biomass decay		
Lenes and Heil (2010)	1.7–788.3	0.2–39.7
Nitrification		
Bronk et al. (2014, in this issue)	0.059–0.218	N.D.
Photochemical nutrient production		
Bronk et al. (2014, in this issue)[e]	0.072–0.288	N.D.
Microzooplankton Grazing		
Walsh (2012)	0.721 (±0.350)	0.020 (0.010)

[a] Assumes a 10 m water column.
[b] Based on the biomass of dead fish for 4 *K. brevis* blooms from Texas Parks and Wildlife PRISM database diluted into the same volume used by Vargo et al. (2008).
[c] Used in this issue the average areal extent of these four Texas *K. brevis* related fish kills.
[d] Range is based on *K. brevis* concentrations of 0.1×10^6 cells L^{-1} and 14.0×10^6 cells L^{-1} and N and P quotas for *Synechococcus* of 1.65 fmol N $cell^{-1}$ (Richardson, 2004) and 0.104 fmol P $cell^{-1}$ (Bertilsson et al., 2003).
[e] Assumed that photoproduction occurred for 12 h per day. To calculate the range of daily photoproduction rates, rates of NH_4^+ and amino acid photoproduction were added and then multiplied by 12. Rates of NH_4^+ photoproduction were averaged for the three stations over the period from July through December when *K. brevis* blooms are most common over the two years. Note values for NO_3^- were not statistically different from 0.
N.D., not determined.

stations in central Florida (Table 5). As the NADP station used for previous estimates of areal deposition rates of N and P, Station FL19 in Hillsborough County, FL (Vargo et al., 2008), ceased collection in 2006, an adjacent station (Station FL41 in Sarasota County, FL) was used for current estimates. Station FL41 is characterized by a higher elevation than Station F19 (25 m vs 2 m) and is located within an area of the watershed characterized by different land use than Station F19. In addition, no P data are available for Station F41 so Vargo et al.'s (2008) estimate of areal P deposition was used for the current study. A cumulative areal deposition rate was calculated for estuarine areas using the same volumes calculated above for estuarine and coastal areas. Estimates of N atmospheric deposition rates to the coastal region made for both the ECOHAB: Florida program (1998–2002) and this study (Table 5) were comparable despite the change in monitoring stations. A high degree of interannual variation was evident in the data from the Sarasota County monitoring stations. Highest deposition rates occurred in 2007, more than double all the remaining years investigated, except 2001.

Phosphorus flux rates from nutrient sources in the current study displayed no consistent pattern when compared with prior estimates of Vargo et al. (2008) (see Table 4). Phosphorus flux rates for riverine sources were similar to prior estimates, despite the high variability observed in flows (Dixon et al., 2014a, in this issue) and loads (Table 4) between project years. Benthic P flux measurements made during the current study (Dixon et al., 2014b, in this issue) were more than 2500 times greater than previous estimates for southwest Florida coastal waters, which were based upon model derived benthic nutrient flux rates (Darrow et al., 2003) derived from South Atlantic Bight measurements off of Savannah, Georgia (Marinelli et al., 1998). Revised estimates of P flux from dead fish based upon direct measurements

Table 5
Comparison of ECOHAB: Florida and ECOHAB: *Karenia* mean annual atmospheric deposition rates. NM: not measured.

Source	Year	(μmol L^{-1} d^{-1})	
		N flux	P flux
Vargo et al. (2008)	1998–2001	0.008	6.4×10^{-6}
		(0.0016–0.00046)	(1.5×10^{-4} to 2.7×10^{-6})
This study	2007	0.019628	N.M.
	2008	0.008175	N.M.
	2009	0.005499	N.M.
	2010	0.008167	N.M.

The deposition site (Hillsborough County, FL19) used by Vargo et al. (2008) ceased data collection in 2006 so the nearest relevant site (Sarasota County, FL41) was substituted for subsequent calculations. FL41 collects only N data. N.M.: not measured.

of P production from decaying fish in laboratory experiments (Killberg-Thoreson et al., 2014b, in this issue) and Texas fish kill data, were similar to prior estimates when the same ECOHAB: Florida shelf volume (Vargo et al., 2008) was used in calculations (Walsh et al., 2006, 2009), but greatly reduced when the areal extent of the Texas fish kills were used in calculations. Zooplankton excretion rates of P were 0.19 μmol P L^{-1} d^{-1}, less than half that of prior estimates (Vargo et al., 2008).

2.2.2. Newly identified nutrient sources

Previously unquantified or unrecognized nutrient sources for *Karenia brevis* blooms identified and quantified in the field during the ECOHAB: *Karenia* project include: mixotrophic consumption of *Synechococcus* spp. by *K. brevis* (Procise, 2011; Jeong et al., 2005; Glibert et al., 2009), N_2 fixation by diazotrophs other than *Trichodesmium* (Mulholland et al., 2014, in this issue), nutrients contributed by *Trichodesmium* bloom biomass decay (Lenes and Heil, 2010), nitrification and photochemical nutrient production (Bronk et al., 2014, in this issue) and nutrient regeneration from microzooplankton grazing determined using the dilution method of Landry and Hassett (1982) (Walsh, 2012). Methods are described in detail in each reference. An average value for microzooplankton grazing rates (as μg Chl *a* day^{-1}) was taken for the four stations that were measured during 2008 and 2009 cruises where *K. brevis* concentrations were greater than 0.1×10^6 cells L^{-1} (Walsh, 2012). A C:Chl *a* ratio of 48.5 was assumed based on previous measurements made in eastern Gulf of Mexico shelf waters (Dagg, 1995), and the Redfield molar ratio of 106:16:1 was used to derive potential N and P availability within blooms from microzooplankton grazing. Rates ranged from 0.429 to 1.180 μM L^{-1} d^{-1} for N and 0.012 to 0.034 μM L^{-1} d^{-1} for P.

Rates of pelagic N_2 fixation by non-*Trichodesmium* diazotrophs ranged from non-detectable to 13.6 nmol N L^{-1} d^{-1}, several orders of magnitude less than the 1.7–788.3 μmol N $L^{-1}d^{-1}$ N flux calculated by Lenes and Heil (2010) for combined *Trichodesmium* N_2 fixation, N regeneration and seasonal biomass decay. Previous estimates of the contribution of newly fixed N from *Trichodesmium* blooms in support of *Karenia brevis* bloom biomass (Mulholland et al., 2004, 2006; Vargo et al., 2008) are based solely on measured rates of *Trichodesmium* N_2 fixation and release of recently fixed N_2 (as NH_4^+ and DON). *Trichodesmium* blooms annually on the WFS (Lenes et al., 2001) and its seasonal biomass constitutes a large nutrient pool of both N and P as these blooms decay (Lenes and Heil, 2010). Phosphorus flux from this source ranged from 0.2 to 39.7 μmol P L^{-1} d^{-1}. Rates of nitrification ranged from 0.059 to 0.218 μM L^{-1} d^{-1} while photochemical nutrient production ranged from 0.072 to 0.288, assuming 12 h of daylight to convert hourly rates to daily rates (Bronk et al., 2014, in this issue).

Nutrient flux rates in the coastal zone for several previously unquantified sources (e.g. nitrification, photochemical nutrient production) were the same order of magnitude as several prior estimates (Table 4). For N, flux rates for previously unquantified sources ranged from a low of 0.004 and 0.006 μmol N L^{-1} d^{-1} for mixotrophic consumption of *Synechococcus* spp. and pelagic N_2 fixation respectively, to a high of 788 μmol L^{-1} d^{-1} for combined *Trichodesmium* N_2 fixation and biomass decay. Greatest variability was associated with N flux rates derived from mixotrophic consumption of *Synechococcus* spp., which ranged from 0.004 μmol N L^{-1} d^{-1} (Procise, 2011) to 46.459 87 μmol N L^{-1} d^{-1} −1 (Glibert et al., 2009) and combined *Trichodesmium* N_2 fixation, N regeneration and seasonal biomass decay, which ranged from 1.7 to 788.3 μmol N L^{-1} d^{-1} (Lenes and Heil, 2010). The high N flux rates associated with grazing on *Synechococcus* spp. (Glibert et al., 2009) may be the result of *Synechococcus* cell lysis during experiments (Sipler et al., 2013), especially as Glibert et al. (2009) noted that only 2–3 *Synechococcus* cells were observed within *Karenia brevis* cells by confocal microscopy at any given time. This is considerably fewer cells than would be present if *K. brevis* was grazing at the maximum ingestion rate of 83.8 *Synechococcus* cells *K. brevis* $cell^{-1}$ h^{-1} reported by Glibert et al. (2009).

Phosphorus flux rates in the coastal zone from new measured sources ranged from 0.0003 μmol P L^{-1} d^{-1} for mixotrophic consumption of *Synechococcus* spp. (Procise, 2011) to 39.7 μmol P L^{-1} d^{-1} for *Trichodesmium* seasonal biomass decay (Lenes and Heil, 2010). Both N and P flux rates from microzooplankton regeneration were approximately 10 fold less than rates for macrozooplankton regeneration. The large range associated with several of the N and P flux rates (e.g. combined *Trichodesmium* N_2 fixation and biomass decay and photochemical nutrient production within blooms) suggests that a high amount of temporal and/or spatial variability is associated with these sources.

2.3. Estimation of Karenia brevis nutrient requirements and needs met by nutrient sources

Since bloom concentrations observed over the ECOHAB: *Karenia* program ranged from background concentrations (0.001×10^6 cells L^{-1}) to 1.4×10^7 cells L^{-1}, the N and P required to support a bloom was calculated for three bloom cases: a small (1×10^5 cells L^{-1}) bloom, a medium (1.0×10^6 cells L^{-1}) bloom, and a high (1.4×10^7 cells L^{-1}) *Karenia brevis* bloom to provide a representative range of bloom conditions. The defined small bloom *K. brevis* cell concentrations are those at which bloom related fish kills first occur (Steidinger, 2009). The maximum concentration used was based upon the highest cell concentration observed during the ECOHAB: *Karenia* program during the 2007 bloom (Heil et al., 2014b, in this issue). Calculations utilized these cell concentrations, a growth rate of 0.2 divisions d^{-1} and N and P cell content of 1.08×10^{-5} μg $cell^{-1}$ and 4.88×10^{-7} μg $cell^{-1}$, respectively (Heil, 1986; Shanley, 1985; Van Dolah and Leighfield, 1999). A Redfield molar ratio of 106:16:1 was assumed along with a 12 h photoperiod for uptake to calculate N demand.

The extent to which individual nutrient sources in the offshore, coastal and estuarine environments met the calculated N and P needs of the three representative *Karenia brevis* blooms of differing cell concentrations is given in Fig. 1. Calculations are based on assumed depths of 5, 10 and 45 m respectively for the three environments. Flux rates of both N and P sources were generally higher than previous estimates (Vargo et al., 2008). At small (1.0×10^5 cells L^{-1}) *Karenia brevis* bloom concentrations, sufficient N and P is available from multiple nutrient sources to meet biomass needs in all three environments. These sources include macrozooplankton excretion, microzooplankton grazing, decay of

dead fish, mixotrophic picoplankton consumption and *Trichodesmium* N_2 fixation, release and biomass decay. Combined riverine sources contributed 17 and 69% of *K. brevis* N and P requirements respectively to small blooms in estuaries during the study period, however these percentages increased to 100% of both N and P needs for Caloosahatchee River flux in 2005. For the coastal zone, combined estuarine contributions decreased to 1 and 6% of N and P needs for the study period, and 13 and 30% of needs for Caloosahatchee River contributions in 2005. *Trichodesmium* N_2 fixation and N regeneration can contribute up to 88% of small bloom N requirements, while several sources, including nitrification and photochemical nutrient production, can meet 100% of N requirements, but do not contribute to P requirements. At the medium bloom concentration (1.0×10^6 cells L^{-1}) however, only combined *Trichodesmium* N_2 fixation, release and biomass decay and zooplankton have flux rates sufficient to meet bloom N and P requirements. Decay of dead fish and nitrification combined can contribute up to 100% of N requirements of at this larger bloom concentration while combined riverine sources contribute 0 and 1% of N and P requirements during the 2007–2010 period, and 1 and 3% of needs for Caloosahatchee River flux in 2005. At the high bloom concentration (1.4×10^7 cells L^{-1}) only combined *Trichodesmium* N_2 fixation, release and biomass decay alone can contribute 100% of the N required by cells.

3. Discussion

3.1. Nutrient sources supporting Karenia brevis blooms

The variety of available nutrient sources on the WFS and the timing and location at which they are supplied relative to emerging and existing *Karenia brevis* populations complicates management or mitigation efforts targeting nutrient source reduction. For example, the large and varied geographical area and environments (i.e. estuaries, coastal and offshore regions) over which blooms can occur on the WFS, the variety of chemical forms of nutrients available at any one time, the seasonal variations in freshwater inputs (e.g. affecting estuarine outflows), and temperature all influence the location and magnitude of available nutrients at any one time. Combined, the four bloom periods sampled during the ECOHAB: *Karenia* program and the 2001 bloom data provide an unprecedented database for analyzing the impacts of nutrient inputs and sources during different bloom stages when the physiological state of bloom populations likely varied. This study includes data from: (1) two large, well-developed blooms in maintenance stage (2001 and 2007); (2) an emerging bloom (2008); (3) an older offshore bloom (2009); and (4) a period when there was no bloom (2010). This range of bloom stages, conditions and locations allowed us to assess how the nutrient sources available to *K. brevis* blooms vary over time and influence bloom development, expansion and duration. A variety of N sources appear to be important in fueling or sustaining *K. brevis* blooms and the timing of these inputs appears crucial to determining the impact of blooms. Sources of allochthonous N include: N_2 fixation, estuarine inputs, atmospheric deposition and photochemical nutrient production. Several of these sources of N, particularly N_2 fixation, are especially important during bloom initiation and development due to their magnitude and location relative to bloom initiation. Autochthonous, or regenerated N sources, sustain populations and control the expansion and duration of blooms once they have begun. The validity of the application of the concept of 'new' and 'regenerated' N (*sensu stricto* Dugdale and Goering, 1967) to some systems has been questioned (Jacques, 1991) however. Brand et al. (2012) point out that the concept may need to be revised for coastal areas where both new and regenerated N occur in myriad chemical forms (e.g. Killberg-Thoreson et al., 2014b, in this issue) and for blooms of *K. brevis*, whose populations are concentrated in such a way that regenerated nutrients produced outside of the bloom area are being supplied to the cells in a manner that could fit the description of "new nutrients" i.e. transported allochthonously into the bloom area.

Blooms of the marine diazotroph, *Trichodesmium*, occur annually on the WFS, and high abundances often co-occur with *Karenia brevis* blooms (Walsh and Steidinger, 2001; Lenes et al., 2001; Lenes and Heil, 2010). Fixation of N_2 and subsequent release of fixed N by *Trichodesmium* is a significant N source in the eastern Gulf of Mexico (Mulholland et al., 2002, 2004, 2014, in this issue); up to 47% of this recently fixed N_2 is bioavailable and transferred to co-occurring plankton (Mulholland et al., 2014, in this issue; Sipler et al., 2013). N_2 fixation by *Trichodesmium* plays a significant role in supplying N to *K. brevis* blooms, provided *Trichodesmium* is present at high enough concentrations (Mulholland et al., 2002, 2004; Vargo et al., 2008). Comparison of the N supplied from *Trichodesmium* N_2 fixation and regeneration with *K. brevis* N demands (Fig. 1) demonstrates that this source can supply 88% of *K. brevis* N demands of small blooms for coastal and offshore blooms, and 91% when pelagic N_2 fixation from N_2 fixers other than *Trichodesmium* is included. Although we have assumed N flux from N_2 fixation by *Trichodesmium* and pelagic unicellular cyanobacteria is insignificant in estuaries, N_2 fixation from other estuarine cyanobacteria, including *Lyngbya* spp. (Paerl et al., 2008), may contribute significantly to bloom support in estuaries during certain time periods. *Trichodesmium* blooms also represent a large seasonal particulate nutrient pool on the WFS (Lenes and Heil, 2010). Although regeneration of seasonal *Trichodesmium* bloom biomass occurs at different time scales than N_2 fixation, including N release from *Trichodesmium* upon cell death (Lenes and Heil, 2010), demand calculations demonstrate that *Trichodesmium* could satisfy up to 85% of N and 100% of P requirements of large (1.0×10^6 cells L^{-1}) *K. brevis* blooms in coastal and offshore environments. Additional support of the hypothesis of the significance of *Trichodesmium* derived nutrients to blooms is provided by Harmful Algal Bloom (HAB) SIMulations (HABSIM) model results for the large 2001 *K. brevis* bloom (Lenes et al., 2012). Lenes and co-authors (2012) identified *Trichodesmium* related N_2 fixation, release and biomass decay and nutrients from dead fish decay as the most significant nutrient sources supporting sustained, high biomass blooms. N_2 fixation thus represents both a short-term N supply from direct excretion as NH_4^+ or DON (Mulholland et al., 2004, 2006, 2014, in this issue; Sipler et al., 2013) as well as a longer-term nutrient source as *Trichodesmium* biomass decays and is regenerated on a seasonal basis (Lenes and Heil, 2010).

In addition to N_2 fixation, allochthonous nutrient sources on the WFS include riverine and estuarine inputs. Comparison of estuarine nutrient fluxes with calculated bloom requirements from 1998 to 2001 (Vargo et al., 2008) showed that these sources were, on average, only sufficient to support small ($<1.0 \times 10^5$ *Karenia brevis* cells L^{-1}) *K. brevis* blooms. Further, estuarine contributions diminished in importance relative to other nutrient sources, such as zooplankton excretion and dead fish decay, when extended to the entire coastal region. Estimates of N and P from estuarine sources during the time period of the present study were less than that of the individual contributions of macro and microzooplankton excretion (Walsh, 2012), benthic fluxes (Dixon et al., 2014b, in this issue), nitrification and photochemical nutrient production (Bronk et al., 2014, in this issue), *K. brevis* grazing (Jeong et al., 2005; Glibert et al., 2009; Procise, 2011), regeneration from dead fish (Killberg-Thoreson et al., 2014b, in this issue), N_2 fixation (Mulholland et al., 2004, 2006, 2014, in this issue), and regeneration over the larger coastal region (Lenes and Heil, 2010). They were however, sufficient to support up to 17 and 69%

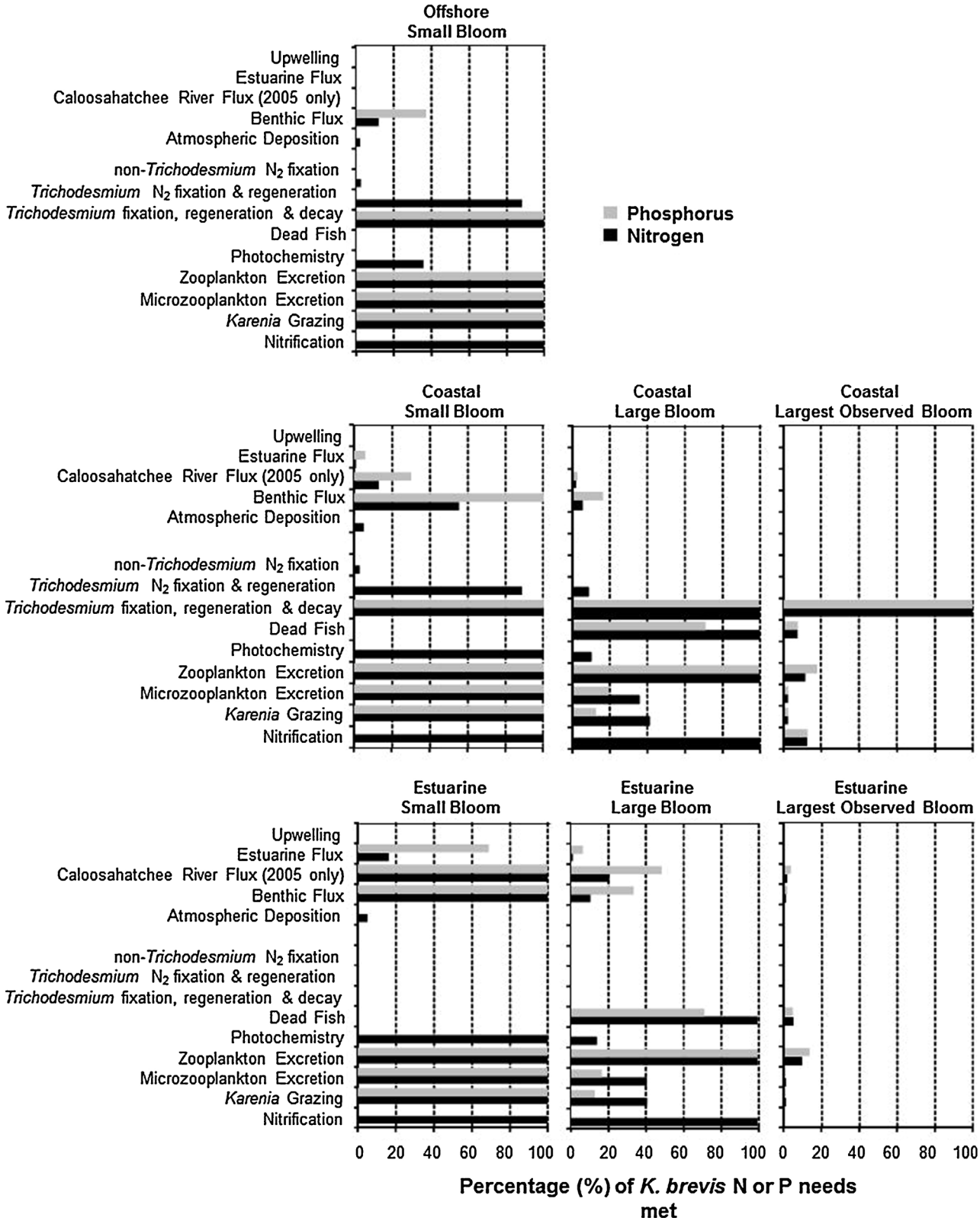

Fig. 1. Comparison of the percentage of nitrogen (N) and phosphorus (P) needs of small (10^5 cells L^{-1}), large (10^6 cells L^{-1}, when fish kills occur) and very large *K. brevis* blooms (10^7 cells L^{-1}) met by each nutrient source for blooms located in estuarine, coastal and offshore environments. Offshore upwelling was assumed not to contribute N or P. Estuarine flux and decay from dead fish contributions were assumed to be 0 for offshore blooms, while N_2 fixation, associated regeneration and seasonal decay were assumed to be 0 for estuaries. Fluxes related to excretion were scaled for estuarine and offshore environments based on NH_4^+ remineralization measurements of Harrison (1978) along an onshore–offshore gradient, assuming prey abundance was not limiting.

of the N and P needs of small *K. brevis* blooms (1.0×10^5 cells L^{-1}) in estuaries. River nutrient loads were low in 2007 as this was second driest year since 1970, and gradually increased through 2010 (Dixon et al., 2014a, in this issue). Estimated nutrient loads for 2007–2010 were up to an order of magnitude lower than the average annual estuarine load estimates for the 2000–2007 period (Table 3), which likely reflects both the drier years from 2007 to 2010 and the impact of elevated natural and managed nutrient loads and flows in the 2003–2005 period, which were wetter than 90% of years since 1970 (Dixon et al., 2014a, in this issue). The 17 and 69% of *K. brevis* N and P needs met by estuarine sources thus represent contributions during relatively dry years. Only in 2005, when Caloosahatchee River releases were elevated due to both high rainfall and management actions (see Table 3, Fig. 1), did estuarine nutrient sources contribute up to 100% of N and P requirements of small estuarine blooms. Estuarine sources of nutrients are clearly important for sustaining small, local bloom populations, but flows are highly variable due to natural interannual variability in rainfall and management of flows in systems such as the Caloosahatchee River (Heil et al., 2007; Uhlenbrock, 2009; Yentsch et al., 2008). The magnitude of N regeneration from multiple sources (e.g. zooplankton excretion, nutrients derived from decay of dead *Trichodesmium* and fish) and *K. brevis's* preference for NH_4^+ over NO_3^- (Killberg-Thoreson, 2011; Killberg-Thoreson et al., 2014a, in this issue; Bronk et al., 2014, in this issue) suggests that any allochthanous N inputs to this system could impact *K. brevis* blooms on longer time scales because ultimately, the N will be recycled, likely in a form that is utilizable by *K. brevis*. Consequently, management activities should be aimed at total N loads.

Nutrients derived from benthic flux are likely available to all stages of *Karenia brevis* blooms given the shallow, well mixed nature of the WFS. Measurements of benthic nutrient fluxes (Dixon et al., 2014b, in this issue) were considerably larger than previous estimates for the WFS based on modeling results using measurements from the South Atlantic Bight (Darrow et al., 2003; Vargo et al., 2008). Flux rates of NH_4^+ from sediments were within ranges previously reported for Gulf coast estuaries, but were less than isotope based groundwater discharge rates of NH_4^+ estimated for Tampa Bay (Swarzenski et al., 2007) and the southeastern Gulf coastal region in general (Hu et al., 2006). Although the extent to which benthic microalgal populations present in WFS sediments (Okey et al., 2004) may modify this flux to overlying waters is unknown, *K. brevis's* migratory ability (Heil, 1986; Kamykowski et al., 1998), which has been shown to include the ability to directly migrate into sediment pores (Sinclair and Kamykowski, 2008), allows direct access to this N and P source for all bloom stages.

Prior theoretical estimates suggested that dead fish can be a substantial nutrient source to development and maintenance stages of *Karenia brevis* blooms (Vargo et al., 2008; Walsh et al., 2006, 2009) once *K. brevis* blooms exceed cell concentrations (1.0×10^5 cells L^{-1}, Steidinger, 2009) sufficient to kill fish. However, these estimates did not consider the fish species killed as a result of *K. brevis* blooms, or their size ranges and abundances. Based on experimental data, we now have a better estimate of concentrations of N, P and carbon (C) produced by decaying fish (Killberg-Thoreson et al., 2014b, in this issue), which includes both inorganic and organic N, P and C forms. Dead fish aggregate along frontal features on the WFS (Walsh et al., 2009) and thus may represent more of a nutrient point source, and a variable one at that, than the estimates indicate.

Zooplankton excretion also represents a potentially large source of both N and P fueling *Karenia brevis* blooms (Lester, 2005; Vargo et al., 2008). Similar values were reported during the current study (Walsh, 2012). It is likely that these fluxes are overestimates based on the use of data from laboratory experiments with zooplankton in calculations as well as both direct and indirect impacts of brevetoxins on zooplankton grazing within blooms. Zooplankton N excretion is highly variable *in situ*, depending on time of day as well as a variety of other physiological and nutritional factors including the quality of N ingested, the biochemical composition of ingested N compounds, and the N:C ratio of food relative to the copepod and its C and N assimilation and growth efficiencies (Tang and Dam, 1999; O'Neil, 1999; Miller and Roman, 2008). Moreover, all of these calculations were based on previously published literature values, most of which were estimated from measured rates of zooplankton of similar size or feeding habits. Zooplankton N and P excretion may also be reduced through lethal and sublethal impacts of brevetoxins. *K. brevis* has been demonstrated to be directly rejected as a food source by copepods (Huntley et al., 1986; Turner and Tester, 1989), or has led to elevated heart rates and loss of motor function in copepods if consumed (Sykes and Huntley, 1987). It is also an inadequate food source for juvenile copepod stages (Huntley et al., 1987) and can lead to decreased ingestion rates and egg production in adult copepods (Waggett et al., 2012). The estimated contribution of zooplankton excretion to bloom support, up to 100% of both N and P for all but the largest blooms in estuarine, coastal and offshore environments, is thus likely an overestimation.

Several previously unrecognized N and P sources may contribute significantly to bloom support (Fig. 1), including regeneration from microzooplankton grazing (Walsh, 2012), and photochemical nutrient supply and nitrification within blooms (Bronk et al., 2014, in this issue, N only). Examination of the role of microzooplankton grazing in HABs, particularly *Karenia brevis* blooms, has lagged behind those of other portions of the microbial loop in marine ecosystems (Azam et al., 1983) especially in subtropical waters (Caron, 1984). An average microzooplankton grazing rate of 1.38 (0.67) μg Chl *a* d^{-1} was measured within the 2008 and 2009 *K. brevis* blooms, which corresponded to flux rates of 0.721 μM L^{-1} d^{-1} N and 0.020 μM L^{-1} d^{-1} of P. Both rates were less than macrozooplankton excretion rates, suggesting that while important to N and P supply, microzooplankton grazing may be more affected by *K. brevis* presence than that of copepods, although further study is needed. *K. brevis* has been shown to have an allelopathic impact on co-occurring phytoplankton species (Kubanek et al., 2005; Prince et al., 2008) which may also potentially act as a defense against microzooplankton predation. This species produces multiple allelopathic compounds distinct from brevetoxins (Prince et al., 2010) that may act by lowering photosynthetic efficiency and decreasing competitor membrane permeability of competitors (Prince et al., 2008).

An important factor influencing nutrient supply to blooms on both a spatial and temporal basis is salinity. The salinity tolerances of *Karenia brevis* play a key role in its distribution (Steidinger, 2009) and consequently its access to nearshore nutrient sources. A salinity barrier of 24 (Finucane, 1960, 1964; Steidinger, 2009; Maier-Brown et al., 2006), below which *K. brevis* does not thrive, has been well established for this species based on extensive historical records. *K. brevis* is thus excluded from riverine and estuarine areas with salinities < 24, allowing more euryhaline phytoplankton species access to riverine and estuarine N and P sources at these lower salinities. An exception to this spatial restriction occurs in times of drought, however, when estuarine salinities are elevated, allowing *K. brevis* blooms to penetrate further spatially into estuaries (Steidinger and Ingle, 1972a,b; Landsberg and Steidinger, 1998).

The cell concentrations within a *Karenia brevis* bloom can also influence available nutrient sources. As blooms age, increases in the amount of dissolved brevetoxins due to cellular lysis over time lead to the transfer of brevetoxins from the particulate to the dissolved pools (see Fig. 2) (Pierce et al., 2000; Lenes et al., 2013).

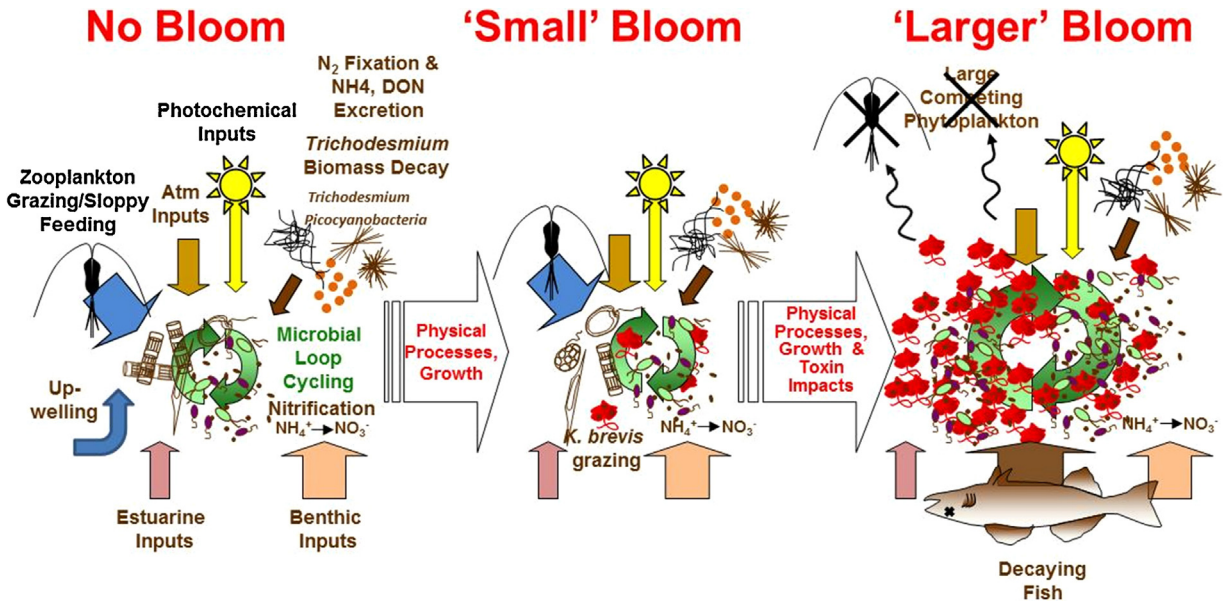

Fig. 2. Schematic model of how nutrient sources and cycling change from a 'no bloom' situation to a small bloom ($<0.1 \times 10^6$ cells L^{-1}) to large fish killing bloom conditions.

Brevetoxins are potent ichthyotoxins and impact food webs in both particulate and dissolved forms (Landsberg et al., 2009). Both zooplankton grazing (Walsh and O'Neil, 2014, in this issue) and excretion, which can contribute significantly to both N and P supporting blooms (Lester et al., 2008; Vargo et al., 2008; Walsh, 2012), are negatively impacted by exposure to brevetoxin via consumption of cells (Huntley et al., 1986; Tester et al., 2000; Lester et al., 2001; Walsh, 2012). Based on estimated regeneration values, microzooplankton may be more significantly impacted than mesozooplankton however. Nutrient regeneration from decaying fish also is likely to become a significant source once bloom concentrations exceed 10^5 cells L^{-1} (Heil and Steidinger, 2009).

Although ambient dissolved inorganic N and P concentrations are generally low on the WFS (Vargo et al., 2008; Dixon et al., 2014a, in this issue), N and P flux rates, either from selected sole sources or from combined sources (Table 4), are sufficient to supply *Karenia brevis* blooms with required nutrients to at least 10^6 cells L^{-1} bloom densities in both estuarine and coastal environments.

Different regions of the country, even different regions within a state, have different HAB management needs. While all local, state and federal environmental managers have the goals of protecting human and environmental health and minimizing the economic impacts of HABs, how this is best accomplished varies with the region, local economies, resources at risk, stakeholders impacted, and the particular HAB species involved. Development and application of effective nutrient management strategies in the eastern Gulf of Mexico requires knowledge of both the nutrient sources available to *Karenia brevis* during all bloom stages and locations and the three-dimensional bloom pathways. While reduction of selected nutrient sources may be possible, the approach should be undertaken with an understanding that, given the multiple nutrient sources available to blooms, reduction of a single source may not lead to an immediate decrease in *K. brevis* bloom severity, occurrence, concentration or impacts. Alterations in nutrient inputs which alter the N:P ratio of delivery to receiving waters may also potentially increase *K. brevis* bloom toxicity via induction of P limitation (Hardison et al., 2013). Some NO_3^- sources are obviously not amendable to direct control, e.g. upwelling, although they may be predicted and monitored (Weisberg et al., 2014, in this issue) in support of forecasting conditions favoring, or equally important suppressing, bloom initiation. Other sources (e.g. atmospheric deposition) are the result of complex processes which make mitigation equally complex and difficult. What is clear is that estuarine nutrient sources contribute both N and P to estuarine and coastal *K. brevis* blooms, and management efforts should be undertaken to minimize these sources. This is especially applicable to nearshore sources of localized importance, such as the Calooshatchee River under conditions of high managed flow which occurred in 2005, which could undoubtedly be managed to reduce nutrient delivery to adjacent *K. brevis* blooms.

Targeting bloom initiation for mitigation efforts and bloom prediction on a seasonal time scale is one feasible management option in the eastern Gulf of Mexico. Monitoring for bloom initiation, while scientifically, technologically, and logistically challenging, has made huge progress with the identification of potential conditions responsible for bloom initiation (Weisberg et al., 2014, in this issue). Whereas Weisberg et al. (2009) demonstrated that upwelling is a necessary condition for bloom formation near shore, too much upwelling can inhibit bloom development by introducing elevated concentrations of inorganic nutrients to the WFS by upwelling of deeper Gulf of Mexico water onto the shelf. Such occurred to some extent in 1998 (Weisberg and He, 2003; Walsh et al., 2003) and resulted in near bottom diatom blooms at the 45 m isobath (Heil et al., 2001). More recently 2010 was a year of prolonged and intensified upwelling caused by interactions of the Gulf of Mexico Loop Current with the shelf slope near the Dry Tortugas. Weisberg et al. (2014, in this issue) utilized data from 2010, a rare year when no *Karenia brevis* bloom initiated in the eastern Gulf, to further develop a hypothesis of *K. brevis* bloom initiation in which both the dynamics of the ocean circulation and the biology of the organism are each necessary conditions, but neither alone are sufficient conditions for bloom development. Given these findings Weisberg (2011) advances a multidisciplinary monitoring strategy for the WFS, which now may potentially allow prediction of bloom initiation as well as provide the opportunity to target bloom formation for early mitigation efforts.

Seasonal predictions of other HAB occurrence and severity, e.g. *Alexandrium* in New England (McGillicuddy et al., 2011; Anderson

et al., 2014) has been facilitated by the presence of identifiable cyst beds that can be mapped and predicable coastal current system. The lack of identified *Karenia brevis* cyst beds and/or refuge offshore populations, combined with the large areal extent of potential bloom initiation zones and the seasonally unpredictable nature of coastal currents and physical forcing mechanisms on the WFS, complicates seasonal prediction for *K. brevis* blooms. Prediction of potential *K. brevis* bloom magnitude and severity on longer term seasonal and annual scales is potentially possible based on knowledge of primary nutrient sources available to blooms. Quantification of the magnitude and severity of blooms may be interpreted in a number of different ways: as high cell concentrations, extended bloom durations, large spatial coverages, significant economic impacts, and/or specificity and severity of environmental impacts. If extended duration and high biomass are the qualifying criteria, then sensitivity analyses conducted for bloom modeling efforts (Lenes et al., 2012) suggest that the three most important nutrient sources to the WFS coastal area for sustaining blooms of higher magnitude are *Trichodesmium* spp. N_2 fixation, N release and biomass decay, nutrient inputs from decay of dead fish, and to a lesser extent riverine nutrient inputs. All three sources are, or can be, monitored directly or by proxy. *Trichodesmium* spp. abundance and distribution can be monitored either directly via MODIS satellite algorithms (Cannizzaro et al., 2008) in near real-time or indirectly via monitoring of dust transport to and deposition in the eastern Gulf of Mexico in spring through early summer (April–June). Dead fish are monitored by the Florida Fish and Wildlife Conservation Commission through the FWC Fish Kill Hotline. Riverine nutrient inputs as well as subsequent estuarine processing of riverine nutrient pools can be calculated from USGS flow data and local and county nutrient monitoring programs or *in situ* observing system arrays that include nutrient sensors (e.g. the Sanibel Captiva Conservation Foundations River Estuary Coastal Observing Network). The complex interactions between multiple nutrient sources and the inherent physical unpredictability of the system on time scales greater than a week however suggests that such a prediction would be qualitative at best. At worst, a seasonal prediction would be detrimental to stakeholders reliant on the tourism industry and could potentially incur significant economic losses via the effects of negative publicity on tourism and associated industries.

The environmental and human health impacts of *K. brevis* blooms are unique, and these impacts occur in a state with a heavy economic reliance on both tourism and its natural resources. Bloom impacts on tourism occur on relatively short (e.g. day) time scales while natural resource impacts involve both short and longer (e.g. month) time scales. Part of successful *K. brevis* management in Florida has been the focus on monitoring and reducing these shorter-term impacts. This has led to a heavy reliance on the development of shorter-term, i.e. 5 day, monitoring and predictive products such as the HAB particle trajectory tools, developed by USF in collaboration with FWRI and NOAA's Harmful Algal Bloom Operational Forecast System (HAB-OFS). Both rely heavily on local cell monitoring data and a physical model which effectively and reliably predicts local water movements. This relatively narrow window of prediction has allowed for targeted monitoring of specific areas where impacts are likely to occur (i.e. respiratory irritation or dead fish at specific beaches), or areas with vulnerable marine mammal populations.

Stakeholder and public education and communication remains a primary management tool for *Karenia brevis* blooms in the Gulf of Mexico (Stevely et al., 2008). Knowledge of current and forecasted bloom conditions and impacts allows stakeholder planning to minimize or avoid these impacts (Morgan et al., 2010; Nierenberg et al., 2010). A critical component of education is effective communication of both research results and monitoring efforts, especially predictive efforts (e.g. particle trajectory nowcast-forecasts). Communications should target audience and stakeholder interests, whether a specific stakeholder group, environmental managers or the general public. During this study, newsletters targeting both managers and the general public were created in collaboration with science communicators http://ian.umces.edu/press/newsletters/publication/394/red_tides_of_the_west_florida_shelf_science_and_management_2013-02-04/. Communications should also provide sufficient background information to allow understanding of the issue and the data/results. The time scale of communication should be considered as well: time sensitive monitoring results may require a different form of dissemination than research results

4. Conclusions

Multiple (>12) nutrient sources of varying magnitude support different *Karenia brevis* bloom stages in estuarine, coastal and offshore regions of the WFS. These sources vary with bloom location, biomass and toxicity. In estuarine and coastal environments, combined N_2 fixation (includes *Trichodesmium*, pelagic N_2 fixation by other N_2 fixers, the release of newly fixed N and the decay and recycling of *Trichodesmium* bloom biomass), mixotrophic consumption of picoplankton, nutrient release from zooplankton grazing, decay of red tide related dead fish and microzooplankton grazing, photochemical nutrient production, nitrification and benthic flux all can contribute up to 100% of the N and/or P requirements of small (10^5 cells L^{-1}) *K. brevis* blooms. During average estuarine flow years, combined estuarine sources contribute up to 17 and 69% of the N and P needs of these blooms in estuaries, however this can increase to 100% during exceptional, high flow (e.g. 2005) years. For larger (1.0×10^6 cells L^{-1}) blooms in coastal environments, combined N_2 fixation, mixotrophic consumption of picoplankton, nutrient release from zooplankton grazing and decay of red tide related dead fish dominate nutrient supply dominate nutrient supply. Only N and P supplied by N_2 fixation and release and decay of seasonal *Trichodesmium* bloom biomass were sufficient to support the largest (14.0×10^6 cells L^{-1}) observed bloom. Reduced nitrogen forms dominated the majority of the N sources, suggesting that blooms are primarily supported via regenerated nutrients. However all possible allochthanous N and P inputs to the system should be limited where possible.

5. Recommendation summary

Management of *Karenia brevis* blooms in the eastern Gulf of Mexico is challenging. The following are recommendations based on ECOHAB: *Karenia* Program research results.

(1) Efforts should be made to reduce nutrient inputs as much as possible because all nutrient inputs eventually are incorporated into the bioavailable pool due to recycling processes.
(2) Effective targeted communication of scientific results to the public and stakeholders remains a challenge for all aspects of *K. brevis* science, including nutrient research. Outreach efforts should focus on communication and presentation of timely, accurate science to targeted audiences using professional communicators and appropriate media where possible.
(3) Effective HAB monitoring efforts at both state and federal levels must include monitoring for known physical conditions that favor or suppress the initiation, transport and export of *K. brevis* blooms in the southwest Florida region in addition to *K. brevis* cell concentrations.
(4) Identification and provision of the funding necessary to maintain the southwest Florida coastal observing system

infrastructure on an operational basis is critical to effective HAB management in the region, including monitoring for potential offshore conditions favoring *K. brevis* bloom initiation (Weisberg et al., 2014, in this issue). This system has shown its utility for the provision of red tide related monitoring and forecasting products as well as event response applications (i.e. forecast models for oil transport and impacts of the Deepwater Horizon oil spill).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.hal.2014.07.016.

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