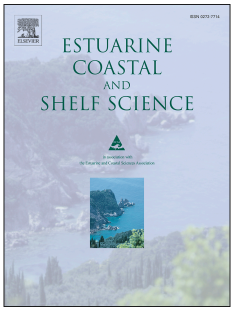

Isotopic Insights into Nutrient Contributions and Transport Pathways in North-Central Florida Bay

Joshua O. Linenfelser, W.Ryan James, Ryan J. Rezek, Rolando O. Santos, Tom A. Frankovich, Christopher J. Madden, James A. Nelson, Justin Lesser, Jennifer S. Rehage

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Author names: Joshua O. Linenfelser, jline006@fiu.edu, Florida International University (Department of Earth and Environment), Miami (FL), USA, ORCID ID: 0009-0001-7169-5271 (Corresponding Author); **W. Ryan James**, wjames@fiu.edu, Florida International University (Institute of Environment), Miami (FL) ORCID ID: 0000-0002-4829-7742, USA; **Ryan J. Rezek**, rrezek@coastal.edu, Coastal Carolina University, South Carolina, USA, ORCID ID: 0000-0002-3054-6384; **Rolando O. Santos**, rsantosc@fiu.edu, Florida International University (Department of Biological Sciences), Miami (FL), USA, ORCID ID: 0000-0002-3885-9406; **Tom A. Frankovich**, tfrankov@fiu.edu, Florida International University,Miami (FL), USA; **Christopher J. Madden**, cmadden@sfwmd.gov, South Florida Water Management District, Miami (FL), USA; **James A. Nelson**, Jimmy.Nelson@uga.edu, University of Georgia, Athens (GA), USA, ORCID ID: 0000-0003-4607-3953; **Justin Lesser**, jlesser@fiu.edu, Florida International University, ORCID ID: 0000-0003-0262-2607 (Corresponding Author); **Jennifer S. Rehage**, rehagej@fiu.edu, Florida International University, Miami (FL), USA, ORCID ID: 0000-0003-0009-6906

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Abstract: Coastal nutrient loading is a primary driver of changes to habitat structure, species diversity, and increases in hypoxia and harmful algal blooms in estuaries worldwide. Understanding both nutrient source contributions and transport mechanisms is critical for effective management and mitigation. Using stable isotopes, this study aimed to evaluate relative contributions of nutrient sources influencing the coastal Everglades, specifically lake systems along the north-central boundary of Florida Bay. Carbon, nitrogen, and sulfur stable isotope values of algae grown *in situ* were used as a natural tracer of nutrient sources across hydrologic seasons (wet and dry seasons of 2021-2022), and across a north to south estuarine gradient in these coastal lake systems. Mean δ15N values (+2 to +3‰), consistent across both space and time, provided evidence that allochthonous N may primarily influence this region. Spatial variation in isotopic values across the study system provided evidence of a dynamic nutrient regime with varying sources across space. Bayesian mixing models identified a mixture of upstream marsh and downstream bay derived nutrients influencing the nutrient regime of the system. Seasonally, we observed higher marsh derived nutrient inputs in the upper portion of the system during the wet season, and greater nutrient inputs from Florida bay during the dry season, primarily in the lower portion of the estuary. This study highlights how freshwater flows, influenced by restoration efforts, and marine inputs, predicted to increase with accelerating sea level rise, can influence nutrient inputs to coastal regions.

Key Words: nutrients, nitrogen, stable isotopes, estuary, Florida Bay, algae

Introduction

Excess nutrient loading leading to eutrophy and poor water quality are a widespread concern facing coastal regions worldwide (Nixon et al., 1986; Valiela, 2006; Todd et al., 2019; Malone & Newton, 2020). Globally, estuarine and coastal waters have shifted from balanced and productive ecosystems to ones experiencing sudden trophic changes, biogeochemical alterations, and deteriorating habitat quality that can be difficult to revert and thus can persist for decades, despite restoration efforts (Pinckney et al., 2001; McCrackin et al., 2017). In the United States and European Union, eutrophic conditions occur in more than half of coastal waters (Breitburg et al., 2018; Malone & Newton, 2020). Global impacts of coastal nutrient pollution include changes to habitat structure, decreased biodiversity, and increased hypoxic events and harmful algal blooms (Cloern, 2001; Glibert et al. 2005a, 2005b; Melesse et al., 2008).

Sources of nutrients entering estuarine ecosystems can vary as they are derived from a variety of anthropogenic and natural processes (Munn et al., 2018; Todd et al., 2019). Estuarine ecosystems are strongly regulated by hydrologic processes, which control nutrient loading from both marine and terrestrial influences (Koch et al., 2012). For instance, river runoff and atmospheric deposition contribute to the majority of anthropogenic N in coastal ecosystems (Howarth et al., 1996; Green et al., 2004; Howarth, 2008; Jickells et al., 2017). Worldwide, over half of the dissolved inorganic nitrogen (DIN) entering coastal ecosystems is derived from anthropogenic sources (Galloway et al., 2004; Howarth, 2008; Lee et al., 2016). Natural inputs of nitrogen to coastal systems include organic matter recycling, riverine inputs, atmospheric deposition, biological N fixation, and onshore transport from the open ocean (Voss et al., 2011). Globally, 95% of nutrients enter coastal systems through nonpoint (diffuse) source inputs (Malone & Newton, 2020) and identifying their origin can be challenging.

Stable isotopes have proven to be a powerful tool to identify nutrient sources. Several studies have shown nitrogen (N), carbon (C), and sulfur (S) stable isotopes to act as useful tracers of nutrients entering coastal estuarine systems (Kendall et al., 2008; Bruland & Mackenzie, 2010; Swart et al., 2013; Jones et al., 2018; Murphy et al., 2022). For instance, $\delta^{15}N$ ($^{15}N/^{14}N$ isotopic ratio) values have been used to distinguish between anthropogenically-derived and natural N sources (McClelland & Valiela, 1997; Costanzo et al., 1988; Bannon et al., 2008; Bruland & Mackenzie, 2010; Swart et al., 2013). $\delta^{13}C$ ($^{13}C/^{12}C$ isotopic ratio) values often produce strong spatial gradients in estuaries, with more enriched $\delta^{13}C$ in marine areas and more depleted values in freshwater reaches, where greater respiration depletes δ^{13}C (O'Leary, 1988; Mook and Tan, 1991; Chanton and Lewis, 1999; Hellings et al., 1999). Furthermore, variation in δ^{13}C values can be used to determine rates of production, indicating nutrient availability and temperature (Fry & Wainwright, 1991; Swart et al., 2013). Differences in δ^{34}S (δ^{34}S/δ^{32}S isotopic ratio) values can distinguish among sources of S derived from porewater vs. rainwater sulfates, sedimentary sulfides, and S present in the water column, with a gradient of greater enrichment from fresh to marine waters (Fry et al., 1982; Peterson & Fry, 1987; Connolly et al., 2004).

Florida Bay is historically characterized as a highly oligotrophic system containing low surface water nitrogen and phosphorus (P) concentrations (Rudnick et al., 1999; Glibert et al., 2009). However, as a product of decades of chronic deficits in freshwater inflows and resulting hypersalinity, Florida Bay has experienced recurring seagrass die-offs, increased nutrient concentrations, and persistent algal blooms (Philips & Badylak, 1996; Zieman et al., 1999; Hall et al., 2016; Glibert et al. 2021). The central region of Florida Bay is where N from Everglades freshwater inputs converges with P derived from the Gulf of Mexico, making it the region of Florida Bay with the highest chlorophyll-a concentrations and water column productivity (Fourqurean and Robblee 1999; Madden, 2010). Furthermore, this region of Florida Bay most prominently displays ecosystem shifts, as it has the lowest freshwater inputs and highest water residence times, and thus the greatest degree of hydrologic isolation (Glibert et al., 2009; Madden, 2010). Current efforts to restore the Everglades ecosystem aim to improve the quantity, quality, distribution, and timing of water flows into Florida Bay (USACE and SFWMD, 2011a; National Academies of Sciences, Engineering, and Medicine, 2022). Following restoration efforts, studies have shown continued shifts toward greater DIN loading in the mangrove transition zone at the wetland-bay interface of north-central Florida Bay with subsequent increases in phytoplankton blooms, primarily consisting of the picocyanobacterium *Synechococcus* spp., that extend out into central Florida Bay (Shangguan et al., 2017a; Glibert et al., 2021). Although P is the limiting nutrient throughout the Everglades ecosystem (Rudnick et al., 1999), the *Synecchochus* spp. has numerous physiological mechanisms which allow for its sustained growth in low phosphate and high ammonium conditions that characterize this region (Shangguan et al., 2017b; Glibert et al. 2021). Given the role of nutrients in fueling these blooms, it is important to understand both the sources of nutrient inputs and the pathways through which they are transported into and within the system.

In this study, we used natural abundance stable isotope tracers ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$) from algae, collected using *in situ* artificial substrate samplers, to identify the seasonal contributions of nutrients entering the north-central Florida Bay region. This study traces nutrient dynamics by analyzing isotopic patterns aligned with known hydrologic and ecological gradients, enabling us to infer the relative contributions of nitrogen inputs and their transport through the system. Specifically, our study asked two questions: (1) What are the primary contributions and associated transport mechanisms of nutrient input to the systems? (2) and how do nutrient contributions and transport dynamics vary spatially across water bodies and temporally between hydrologic seasons? Given the geographic isolation, regional hydrology, and previous research findings of the study site, expected potential nutrient inputs include atmospheric deposition, surface and groundwater flows, N_2 biological fixation, internal recycling processes, and legacy nutrients from historic wading bird rookeries and waterfowl populations (Corbett et al., 1999; Sutula et al., 2001; Inglett et al., 2011; Eggenberger et al. 2019; Owens et al., 2021). We hypothesized that upstream nutrient inputs would dominate during the wet season as greater freshwater flow has the potential to transport nutrients to the system, while the dry season would primarily be influenced by either nutrients derived from downstream reverse flow into the system or greater internal recycling processes (Sutula et al., 2001; Frankovich et al., 2011; Glibert et al., 2021; Shangguan et al. 2017a; Owens et al., 2021). We expected spatial and temporal gradients to follow a trend such that northern areas would experience greater influence from freshwater marsh-derived nutrients, primarily in the wet season, while the southern portions of the system would be more influenced by Florida Bay associated nutrient sources, primarily in the dry season (Sutula et al., 2001; Frankovich et al., 2011).

2. Materials and Methods

2.1 Study system

We focused our examination of nutrient sources in two subestuaries at the northern rim of north-central Florida Bay: the Alligator Creek subestuary (hereafter Alligator) and the McCormick Creek subestuary (hereafter McCormick), referred to together as the Everglades Coastal Lake Systems (ECLS, Fig. 1). These two lake chains act as buffers between the Taylor slough drainage and Florida Bay and are considered a main transport vector of nutrients and chlorophyll *a* to Florida Bay (Shannguan et al., 2017; Glibert et al., 2021). The Alligator lake chain consists of West Lake, Cuthbert Lake, Long Lake, and the Lungs which leads through Alligator creek into Garfield

Bight, while McCormick contains Seven Palm, Middle Lake, and Monroe Lake, which leads through McCormick creek into Terrapin Bay (Fig. 1). The northern lakes in each system receive greater freshwater inputs, derived from both Taylor Slough ground and surface water, while the southern waterbodies are more marine-dominated due to proximity to Florida Bay (Frankovich et al., 2011; Frankovich et al. 2012; Price et al., 2021). The greatest

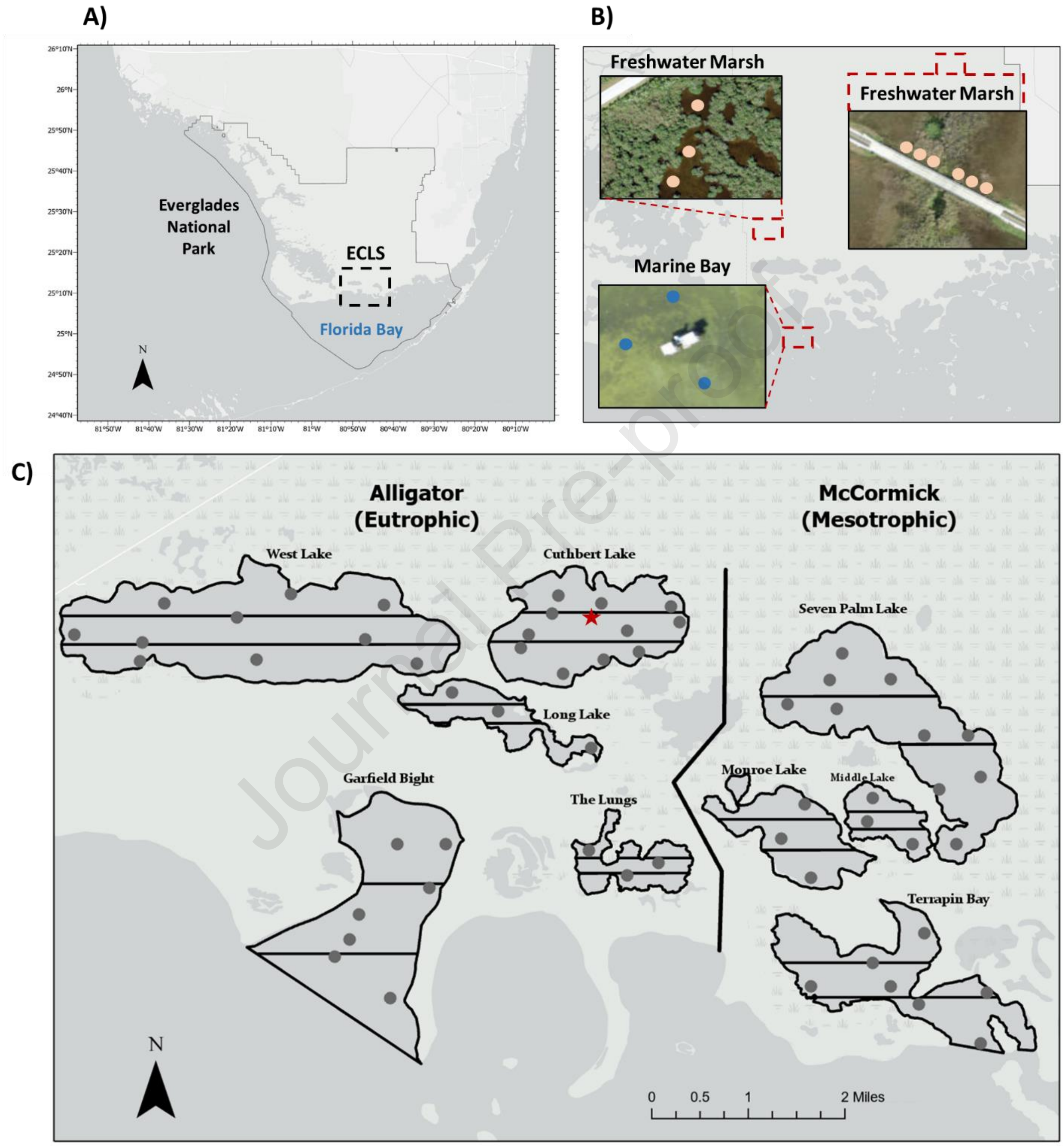


Fig. 1 A) Location of the Everglades Coastal Lake Systems (ECLS) at the northern rim of north central Florida Bay, Everglades National Park. B) Locations of sampling sites for the freshwater marsh and Florida Bay endmembers used in mixing models. C) Map of all sampling sites in the Alligator and McCormick lake systems. Horizontal lines show strata used for randomization of sites. Red star shows location of the Cuthbert Rookery.

contribution of water to both systems is direct precipitation, particularly in the wet season (June-October), with peak values exceeding 0.3 m/month of rainfall, followed by groundwater, and surface water inputs (Price et al., 2021). Groundwater discharge is highest in McCormick (2.25 m/yr) compared to only 0.65 m/yr in Alligator (Price et al., 2021). Both systems exhibit positive groundwater input throughout all months except April in McCormick and April-May in Alligator (peak of the dry season, Price et al., 2021), when instead, there is a net recharge of groundwater from the lakes. The semi-enclosed nature of the lake systems makes them susceptible to eutrophic conditions as they have limited flushing capabilities, allowing for the buildup of nutrients, particularly in Alligator (Frankovich et al., 2011; Frankovich et al., 2012).

The limited freshwater inflow and structural connectivity of Alligator results in seasonally high salinity levels (>50 ppt) and high-water residence times (estimated at ~ 6 months; Price, 2021; Shangguan et al. 2017a). Additionally, Alligator contains a bird rookery on a mangrove island in the center of Cuthbert Lake (Ogden et al. 2014, Fig. 1). The rookery was the site of a historical bird megacolony (Ogden et al. 2014), with wading birds estimated around 6,000 individuals annually (Job, 1905; Chapman, 1908), and today acts as a colony for a much lower number of wading birds consisting primarily of Wood Storks and Great Egrets that have an annual abundance of approximately 400 individuals (Cook & Baranski, 2019; Cook & Baranski, 2021; M.C. Cook, personal communication, 2024). Similar bird rookeries have been shown to result in increased nutrient concentrations (Wainright et al., 2008). Alligator has trophic state index values ranging from 54 to 64 characterizing it as a eutrophic system that exhibits high water column nutrient concentrations, persistent algal blooms, low light availability to the benthos, and less diverse submerged aquatic vegetation (SAV) cover (Frankovich et al., 2012; Frankovich et al., 2017; Eggenberger et al., 2019). In contrast, McCormick is closer to the flow path of Taylor Slough and is comprised of relatively short, open, channels connecting the lake chain to Florida Bay. These topographic characteristics make for greater hydrologic connectivity to both freshwater inflow and Florida Bay. Consequently, McCormick has lower salinity levels, lower water residence times (4-5 months; Price, 2021; Price, 2022), and trophic state index values ranging from 40 to 44, classifying it as mesotrophic with lower nutrients, salinities, and phytoplankton concentrations (Frankovich et al., 2017, Eggenberger et al., 2019).

2.2 Seasonal Sampling

Microalgae samples were collected from 71 samplers deployed seasonally across the ECLS (Fig. 1), at two freshwater endmember sites located in the marsh upstream of the ECLS (n=9 samples), and at a marine endmember site located just south of Garfield Bight (n=3 samples, Fig. 1B). The respective endmember sites provided isotopic values indicative of freshwater and marine nutrients. The two marsh sites were located in freshwater marshes at the Taylor Slough bridge and upstream of Alligator (685 m from West Lake). Within the ECLS, we used a stratified random sampling design for site selection, which allowed for a fine-scale spatial variation gradient (Fig. 1C). Each waterbody was delineated into three equally-sized strata, and sites were randomly selected within each stratum (with a 600 m buffer around each site). The upstream lakes (West, Cuthbert, Seven Palm) and the downstream bays (Garfield Bight and Terrapin Bay) had a greater number of sites (10-12 sites) to account for both their larger surface area and for sites of interest, while the middle lakes had fewer sites due to their relatively small size (< 2 km^2; 3 sites each, Fig. 1C). A site of interest in Alligator was the Cuthbert Rookery. Samplers were randomly distributed within a 50 m radius around the rookery (n=3 sites). Bird excrement was also sampled from the Cuthbert Rookery (n=4 replicates). This sampling scheme totaled 79 samples per season.

Sampling was conducted in the hydrologic wet (Sept-Feb) and dry (Mar-Aug) seasons of 2020-2021, at times matching the maximum and minimum freshwater inflows (Appendix 1). During both seasons, samplers were deployed for 3-week sampling periods, where algae grew in situ. Wet season sampling took place from late October through early November 2021. Due to sampling failure stemming from a portion of algal samplers tipping over with high winds, 16 of the 71 sites were redeployed in mid-November to early December, a time frame still within the yearly salinity minimum (Appendix 1). Dry season sampling took place in the 3-week period of late April into early May 2022. At all sites, salinity, temperature, and dissolved oxygen (DO) measurements were taken using a YSI probe, while turbidity was measured with a Secchi disk at both sampler deployment and pickup (Appendix 2).

In aquatic environments, artificial substrate sampling is a useful technique for assessing water quality (Biggs & Kilroy, 2000). Artificial substrate provides homogeneous conditions in size, handling and age of algal growth surfaces, removing unwanted substratum and temporal variability associated with naturally collected samples (Biggs & Kilroy, 2000; Costanzo et al., 2001; Francoeur et al., 2013). In our study, we deployed an artificial substrate sampler at each of the 71 sampling sites. We constructed algal samplers using 2 plates of plexiglass and a PVC stake (Appendix 3A-B). Samplers were deployed with the top plate at 30 cm from the surface to ensure a consistent supply of light and free-flowing water across the substrate surface which are the preferred conditions for algal growth (Biggs & Kilroy, 2001). Following the three-week soak period in both seasons, microalgae growth was scraped from plexiglass slides upon collection, and stored in a sampling bag on ice. Upon return to the lab, samples were frozen at -20° C until processed.

Bird excrement was hand collected from the Cuthbert rookery in Cuthbert Lake. Excrement was scraped from mangrove leaves on the rookery, and birds were identified at the time of sampling. This included Cormorants and White Egrets (n = < 10) in the wet season, and White Egrets (n = ~100), Wood Storks (n = ~50), and Cormorants (n = ~ 20) in the dry season. Samples were stored in whirl-paks on ice, then frozen at -20° C until processed.

2.3 Laboratory analyses

The samples were defrosted and run through a 100-micron sieve into a 1000 ml beaker using deionized water to separate any detritus or shell material from microalgae. A subsample of microalgae was then vacuum filtered through a pre-combusted 47 mm Whatman GF/F filter. The filtered samples were then dried at 55 °C in a drying oven for 48 h and then scraped before weighing them. Samples were weighed out between 2.0 to 3.0 mg and placed in 5 x 9 mm silver capsules for $\delta^{13}C$ analysis and in 5 x 9 mm tin capsules for $\delta^{15}N$ and $\delta^{34}S$ analysis. Samples in silver capsules used for $\delta^{13}C$ analysis were decarbonated using 5% hydrochloric acid to remove ambient calcium carbonate (Chanton & Lewis 1999). Cuthbert rookery samples were carefully scraped to separate excrement from mangrove leaf and branch material. Samples were then dried at 55 °C for 48 h and ground to a powder using a ceramic mortar and pestle. Powdered samples were then weighed out between 0.5 to 0.7 mg and placed in 5 x 9 mm tin cups for $\delta^{13}C$ and $\delta^{15}N$ analysis.

$\delta^{13}C$ and $\delta^{15}N$ isotopic abundances were determined using elemental combustion analysis on a Carlo Erba 1108 coupled to a DeltaV Plus isotope ratio mass spectrometer via a Conflo IV interface at Florida International University. A total of 150 samples were analyzed for C and N including 118 algal samples from the ECLS, 24 algal samples from the marsh and bay end members, and 8 bird excrement samples. $\delta^{34}S$ was analyzed at the Woods Hole Marine Biological Stable Isotope Laboratory using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. Due to logistical constraints, only a subset of samples were analyzed for S totaling 70 samples including 58 algal samples from the ECLS and 12 samples from the upland marsh endmember. The analytical precision based on replicate analyses of isotopically homogeneous international standards is +/- 0.3 ‰ for the $\delta^{34}S$ measurement. The results are presented in standard delta notation as parts per thousand (‰), using international standards of atmospheric nitrogen (air, N2), Vienna PeeDee Belemnite (V-PDB) for carbon, and Vienna-Canyon Diablo Troilite for sulfur.

2.5 Data Analyses

Statistical tests were run using Bayesian mixing models in R version 4.2.1 (R Core Team, 2021) using the package MixSIAR (v3.1.1, Stock et al., 2018) to determine the relative nutrient source contributions to algal samples at each site sampled throughout the ECLS. MixSIAR utilizes a Markov Chain Monte Carlo (MCMC) Bayesian framework to sample from the posterior distribution of the model parameters, which in this case is the nutrient source contributions (Stock et al. 2018). Models were run for each season with $\delta^{15}N$ and $\delta^{13}C$ values of algae from each site set as a fixed factor and the source values derived from the mean $\delta^{15}N$ and $\delta^{13}C$ of the marsh and bay endmembers combined between seasons. Although Lamb (2007) reports a 2–4‰ fractionation factor for nitrogen assimilation in algae, and $\delta^{13}C$ and $\delta^{34}S$ also exhibit minimal fractionation during uptake (Glibert et al., 2019), no fractionation corrections were applied. All samples used in the model represent primary producers, primarily composed of diatoms (microscope observation, Appendix 4), collected in situ using standardized methods and thus are assumed to have experienced similar fractionation processes. This consistency across endmembers and mixture sites allows for direct comparison of isotopic values without the need for applying correction factors. Ordinary kriging was used to interpolate the geographic distribution of the mean $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ patterns across both seasons throughout the ECLS for all 118 algal samples collected. Furthermore, ordinary kriging interpolations were used to present the mean proportion of freshwater marsh vs marine bay contributions to each site across both seasons within the ECLS. The ordinary kriging method of interpolation uses a weighted moving average that's informed by a semivariogram output to produce interpolations. Ordinary kriging estimates unknown cell values across space by using spatial correlation such that cells near each known point are given more weight than those farther away (Fletcher & Fortin, 2018).

3. Results

3.1 Environmental Conditions

Environmental conditions during sampling varied seasonally and across the ECLS, with salinity and dissolved oxygen showing the highest variation (Appendix 2). Salinity in Alligator (dry = 21.80‰ and wet = 15.94‰) and McCormick (dry = 26.02‰ and wet = 19.02‰) varied similarly between seasons with higher salinity in the dry compared to the wet season. As expected, salinity was higher in bays and lowest in upstream lakes. DO levels varied slightly between seasons, and instead varied strongly across systems (Alligator = 5.73 mg/L and McCormick = 8.08 mg/L), with the lowest DO observed in the Lungs in the dry season (3.2 mg/L). McCormick had higher light transparency than Alligator (0.62 m vs. 0.49 m), with the lowest transparency also at the Lungs (0.35 m). Temperatures stayed relatively consistent across systems (Alligator = 27.14°C vs. McCormick = 26.77°C), with little seasonal variation.

3.2 Bulk Isotopic Composition and Spatiotemporal Variation

Microalgal δ^{15}N, δ^{13}C, and δ^{34}S values varied across systems and seasons but remained in the expected isotopic range for estuarine primary producers (Fry et al., 2006; Bouillon et al., 2011; Peterson et al., 1986) (Table 1; Appendix 5). The overall mean across all samples was –23.28‰ for δ^{13}C, and 3.10‰ for δ^{15}N (Table 1). There was greater variation in δ^{13}C than δ^{15}N, with δ^{13}C ranging between -15.55‰ to –34.20‰ across all microalgae samples, whereas δ^{15}N values ranged between 0.05‰ and 6.41‰. Mean δ^{34}S values for algae ranged from -10.9‰ to 19.6‰, with the overall mean value at 7.27‰. Across systems, isotopic values were similar with Alligator being more depleted than McCormick in δ^{15}N (+2.90‰ vs. +3.22‰), and more enriched than McCormick

Table 1. δ^{15}N, δ^{13}C, and δ^{34}S values (means ± SD) for all algal samples in both seasons throughout the study system, along with mixing model results using the δ^{15}N and δ^{13}C values to determine the proportion of bay and marsh source nutrient inputs to the ECLS.

	Stable Isotope Results				**Mixing Model Results**	
Season	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$	Lake	Bay	Marsh
				Taylor Slough Endmember		
Wet	3.32 ± 0.89	-30.67 ± 2.09	14.32 ± 2.81	Marsh		
Dry	4.05 ± 1.09	-30.49 ± 2.55	11.50 ± 4.18	Marsh		
				Alligator		
Wet	2.97 ± 0.87	-21.86 ± 1.75	7.00 ± 7.91	West Lake	0.60 ± 0.14	0.40 ± 0.14
Dry	2.16 ± 1.11	-20.78 ± 1.93	6.75 ± 4.34	West Lake	0.73 ± 0.11	0.27 ± 0.11
Wet	3.61 ± 1.65	-24.92 ± 0.31	6.70 ± 1.65	Cuthbert Lake	0.37 ± 0.03	0.63 ± 0.03
Dry	2.88 ± 0.63	-24.12 ± 1.08	13.47 ± 0.84	Cuthbert Lake	0.49 ± 0.09	0.51 ± 0.09
Wet	3.22 ± 0.92	-24.67 ± 0.74	3.53 ± 2.46	Long Lake	0.39 ± 0.06	0.61 ± 0.06
Dry	2.45 ± 0.45	-23.73 ± 1.08	2.27 ± 1.53	Long Lake	0.53 ± 0.09	0.47 ± 0.09
Wet	2.64 ± 0.30	-24.67 ± 0.62	1.17 ± 0.75	The Lungs	0.40 ± 0.04	0.60 ± 0.04
Dry	2.41 ± 0.12	-22.64 ± 0.07	0.97 ± 2.34	The Lungs	0.61 ± 0.01	0.39 ± 0.01
Wet	2.98 ± 0.34	-18.39 ± 2.33	2.17 ± 3.26	Garfield Bight	0.78 ± 0.18	0.22 ± 0.18
Dry	3.10 ± 0.50	-16.94 ± 1.11	0.97 ± 1.76	Garfield Bight	0.87 ± 0.07	0.13 ± 0.07
				Cuthbert Rookery		
Wet	2.69 ± 0.49	-23.34 ± 1.15	NA	Microalgae		
Dry	3.22 ± 0.71	-22.58 ± 1.24	NA	Microalgae		
Wet	7.36 ± 0.53	-25.67 ± 4.37	NA	Bird Excrement		
Dry	6.63 ± 0.73	-28.12 ± 3.34	NA	Bird Excrement		
				McCormick		
Wet	3.07 ± 0.51	-25.19 ± 0.54	-4.97 ± 2.68	Seven Palm	0.36 ± 0.04	0.64 ± 0.04
Dry	2.62 ± 0.65	-24.02 ± 1.17	-2.60 ± 6.07	Seven Palm	0.51 ± 0.10	0.49 ± 0.10
Wet	2.39 ± 0.27	-24.26 ± 0.14	-5.67 ± 4.27	Middle Lake	0.44 ± 0.01	0.56 ± 0.01
Dry	2.44 ± 0.41	-22.54 ± 0.35	-7.53 ± 3.17	Middle Lake	0.62 ± 0.03	0.38 ± 0.03
Wet	3.40 ± 0.25	-25.73 ± 0.02	6.17 ± 1.47	Monroe Lake	0.32 ± 0.04	0.68 ± 0.04
Dry	2.02 ± 0.29	-21.23 ± 0.98	-1.03 ± 2.68	Monroe Lake	0.72 ± 0.06	0.28 ± 0.06
Wet	4.15 ± 0.82	-21.05 ± 1.86	-1.13 ± 5.44	Terrapin Bay	0.66 ± 0.14	0.34 ± 0.14
Dry	4.52 ± 1.07	-18.56 ± 1.09	-3.97 ± 5.33	Terrapin Bay	0.86 ± 0.04	0.14 ± 0.04
				Florida Bay Endmember		
Wet	2.81 ± 0.24	-17.26 ± 0.84	NA	Bay		
Dry	3.05 ± 0.13	-16.16 ± 0.10	NA	Bay		

in both δ^{13}C (-22.12‰ vs. –22.83‰) and δ^{34}S (+4.65‰ vs. -2.60‰). Wading Bird excrement collected as a potential N source ranged in δ^{15}N values between 6.63‰ to 10.15‰ and in δ^{13}C between –22.06‰ and –31.20‰.

Throughout the ECLS, algal δ^{13}C values showed different spatial patterns between the two lake systems but were consistently enriched in the dry season (Fig. 2B, Table 1). In Alligator, δ^{13}C values were on average -23.87‰ in the wet and -21.80‰ in the dry season, while in McCormick, δ^{13}C values averaged -22.75‰ in the wet and -21.51‰ in the dry season. In McCormick, we also saw a strong pattern of increasing δ^{13}C enrichment from the northern fresher waterbody (Seven Palm) to the southern saltier bay (Terrapin Bay) that was consistent seasonally, except for Monroe Lake in the wet season having the most depleted values (Fig. 2b). In Alligator, instead of

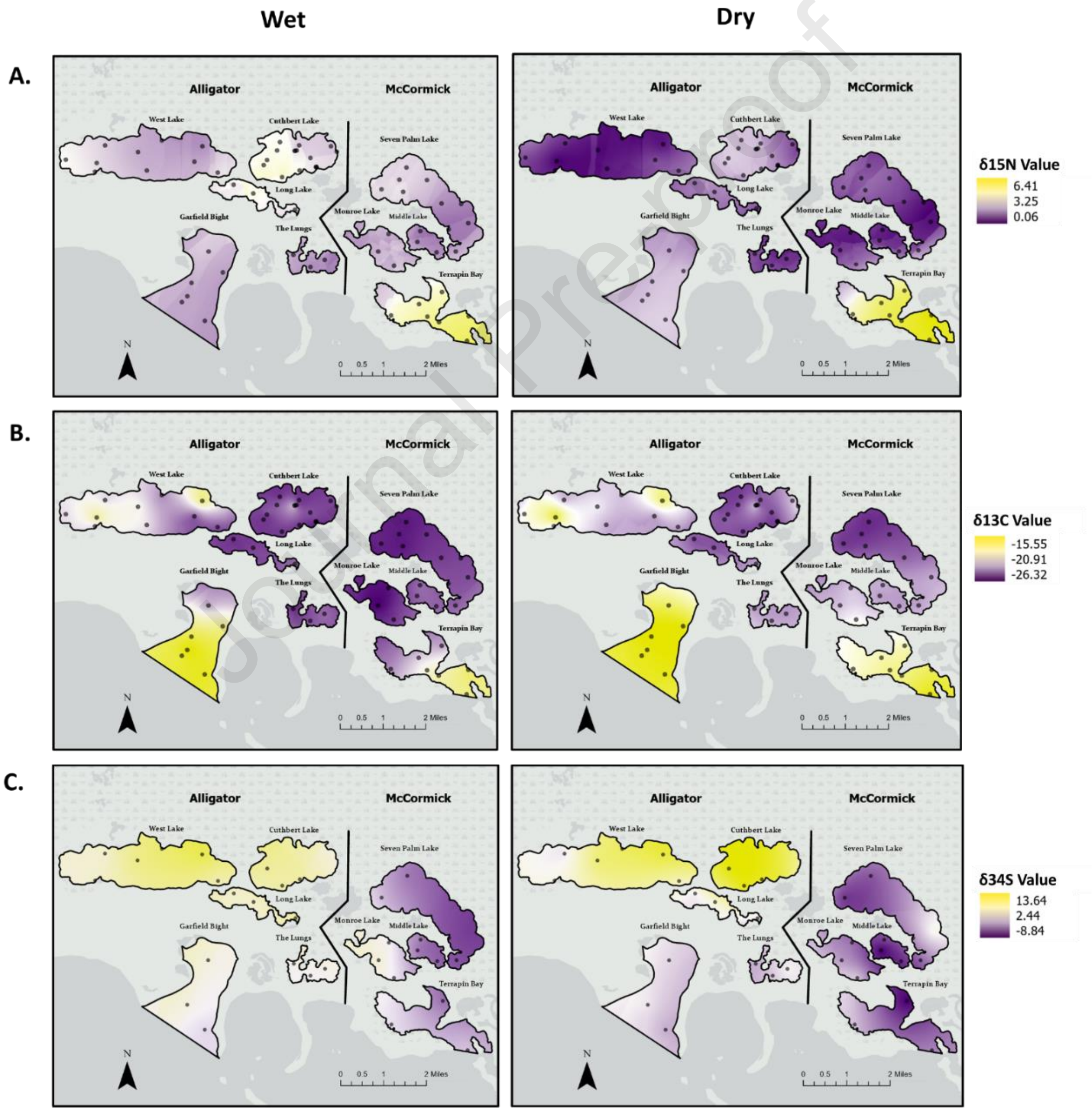


Fig. 2 Ordinary kriging interpolations showing spatial variation in a) δ^{15}N, b) δ^{13}C, and c) δ^{34}S algal values in the wet and dry seasons across the ECLS. Dots show all sampling sites used in interpolations

following a gradient, δ^{13}C values were enriched at the bay site and the uppermost lake (West Lake), with more depleted values elsewhere (Cuthbert, Long Lake, and The Lungs). Across waterbodies, δ^{13}C was most variable in West and Monroe Lake and the bay sites, Terrapin Bay and Garfield Bight (Fig. 2b).

In contrast to δ^{13}C, δ^{15}N showed low spatial variability, but a consistent, although less pronounced, seasonal pattern (Fig. 2a, Table 1). δ^{15}N values were slightly enriched in the wet season in both systems (Alligator wet = 3.12‰ and dry = 2.68‰; and McCormick wet = 3.35‰ and dry = 3.09‰). Across waterbodies, the most enriched δ^{15}N values were observed in Terrapin Bay, while the largest seasonal shift was seen in Monroe Lake (Table 1). δ^{15}N values were the most variable in West Lake and Terrapin Bay, particularly in the dry season (Fig. 2a).

For δ^{34}S, we observed contrasting patterns across the lake systems, and inconsistent seasonal differences (Fig. 2c, Table 1). On average, δ^{34}S values were more enriched in Alligator (wet = 4.29‰ vs. dry = 5.00‰) when compared to McCormick which displayed more depleted δ^{34}S values (wet = -1.40‰ vs. dry = -3.83‰). The biggest seasonal shifts were observed in Monroe and Cuthbert lakes and were relatively minor elsewhere. In Alligator, we observed a marked north-south gradient of enriched to depleted values, while McCormick generally had a more uniform distribution of relatively depleted values (Fig. 2c).

For the freshwater marsh and Florida Bay endmembers, δ^{15}N values were 3.69‰ and 2.93‰ respectively; which were similar to δ^{15}N values found throughout the ECLS (Table 1). Marsh and bay endmembers δ^{13}C values were –30.6‰ and –18.7‰ respectively, showing the expected marked variation between marsh and bay-derived C (Fig. 3). For δ^{34}S, marsh values were enriched, at 12.91‰. And while δ^{34}S at the Florida Bay endmember was not measured due to logistic constraints, mean δ^{34}S values taken from the combination of Garfield Bight and Terrapin Bay sites fell at -0.49‰. All three isotopes showed little variation in the endmember sites and were consistent between seasons.

Last, microalgae samples collected in the proximity of the Cuthbert Rookery had mean isotope values of +2.69‰ and +3.22‰ for δ^{15}N, and –23.34‰ to –22.57‰ for δ^{13}C in the wet and dry season, respectively (Table 1). These values did not differ from those of algae collected elsewhere in Cuthbert Lake (Fig. 2). In contrast, bird excrement samples taken from the rookery had more enriched δ^{15}N values (wet = +6.63‰ and dry = +7.36‰), and more depleted δ^{13}C values (wet = –28.12‰ and dry = –25.67‰).

3.3 Bulk mixing contributions and spatiotemporal variation

Mixing model results showed that the C and N nutrient load to the ECLS was derived from both marsh and bay sources, with a consistently higher contribution of bay nutrients (Table 1). In Alligator, the bay contribution was 59% relative to 41% marsh contribution, while in McCormick, bay contribution was 56% relative to 44% marsh contribution. In both systems, seasonal shifts caused a greater contribution of bay nutrients in the dry season, while the wet season had a close to equal mixture of bay and marsh influence. The increased marine contribution in the dry season resulted in a 66% bay contribution in both Alligator and McCormick, whereas in the wet season, the bay contribution was lower, at 52% in Alligator vs. 45% in McCormick. Spatially, nutrient contributions followed an expected trend of greater marsh contribution in the northern lakes to greater bay contribution in the southern waterbodies (Fig. 3). In Alligator, the greater wet season marsh influence was observed throughout the lakes, except in West Lake, which had a wet season bay contribution of 60%. In contrast, McCormick showed a pattern of greater marsh influence in the wet season in Seven Palm, which declined in Middle Lake, and then increased again in

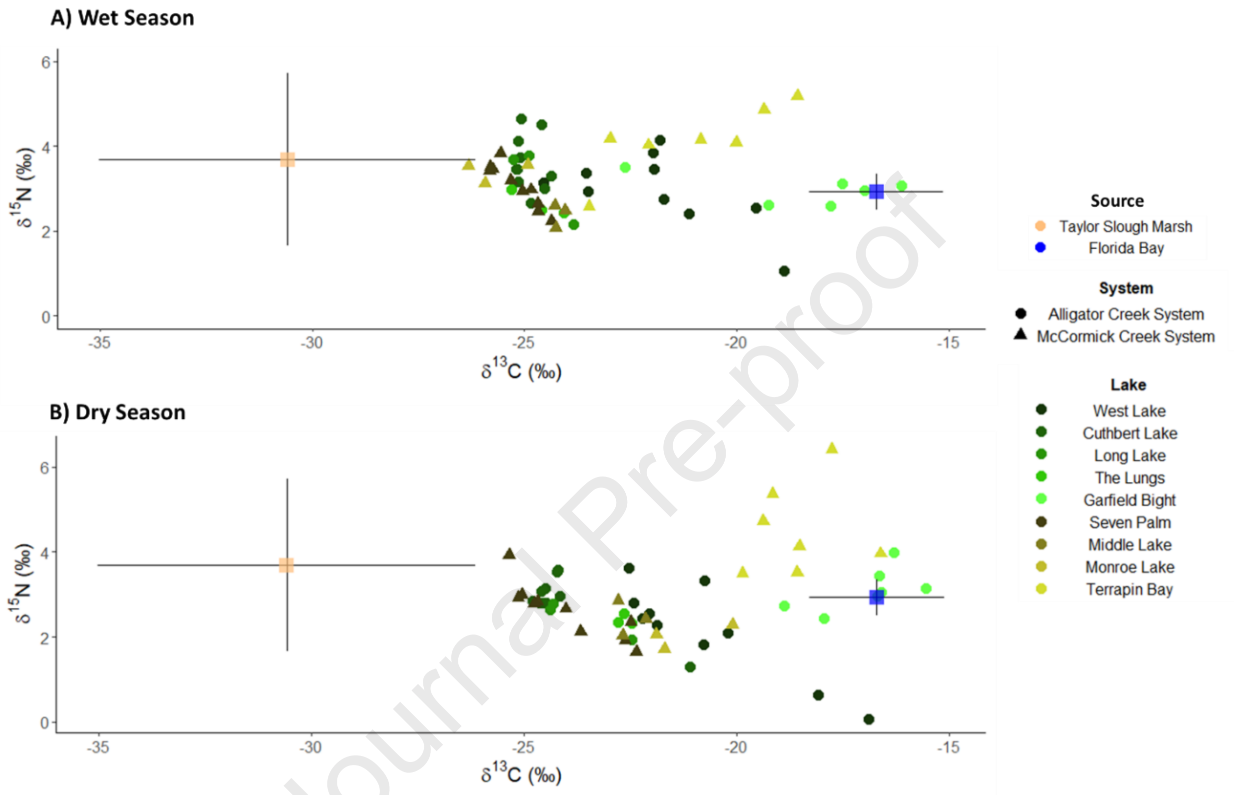


Fig. 3 Biplots of mean algal δ^{15}N and δ^{13}C values across the a) wet and b) dry seasons for each water body (shades of green are means for the Alligator waterbodies while shades of brown are mean values for the McCormick waterbodies). Shown are also mean (and SD) for the marsh and bay endmembers

Monroe Lake (Table 1, Fig. 3). In the dry season, McCormick showed equal bay and marsh contributions in Seven Palm Lake and an increasing pattern of bay influence downstream.

4. Discussion

Globally, coastal and estuarine ecosystems are experiencing increased nutrient loads that hinder ecosystem health and function (Paerl et al., 2014; Malone & Newton, 2020). The Florida Bay ecosystem faces these same threats as changing freshwater flow regimes have been implicated in driving increases in ammonium (NH4+) and dissolved organic nitrogen (DON) (Shangguan et al., 2017a; Glibert et al., 2021). Here, we used $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ to identify seasonal sources of nutrients driving production in the Alligator and McCormick systems, located at the northern rim of Florida Bay. We found that the nutrient load within both systems was seasonally driven by upstream and downstream sources. Mixing models showed that C and N loads were derived from both marsh and bay sources, with a greater contribution of marsh sources in the wet season and greater bay influence in the dry season. As anticipated, a pronounced marsh nutrient influence was observed in upstream water bodies, whereas a more substantial bay nutrient influence was evident downstream within these two lake chains, with the exception of West Lake.

4.1 Distinguishing Nutrient Contributions

Hydrologic inputs that can transport nutrient loads to this system include precipitation and surface and groundwater flows from both upstream (freshwater marshes in Taylor Slough) and downstream (Florida Bay) endmembers. Alternative processes of nutrient transport to this system include biological N_2 fixation, internal recycling processes, and legacy nutrients derived from bird excrement. These sources are all expected to vary in their isotopic values providing a useful proxy for source determination (Savoie et al. 1987; McClelland & Valiela, 1997; Bohlke et al., 2003; Corbett at al.,1999). This isotopic variation is the result of fractionation, which occurs as physical, chemical, and biological processes sort isotope species with varying nuclei mass (Sigman & Casciotti, 2001). This principle has been successfully applied in nearby Biscayne Bay, Florida, where Swart et al. (2013) identified land-derived anthropogenic inputs as the dominant nutrient source based on distinct isotopic signatures, while excluding fertilizers as a major contributor.

Identifying the unique isotopic values of different nutrient sources is a crucial first step in differentiating their contributions to estuarine environments. Source $\delta^{15}N$ values in South Florida for atmospheric deposition range between -7‰ to +1‰, whereas groundwater discharge values range from +2‰ to +8‰ and N_2 fixation values are close to 0‰ (Savoie et al. 1987; Macko & Ostrom, 1994; McClelland & Valiela, 1997; Katz & Bohlke, 2000; Bohlke et al., 2003; Bruland & Mackenzie, 2010). Isotopic values of animal waste and bird excrement have been reported to fall within a relatively enriched range of +10‰ to +20‰ (Wainright et al., 1998; McClelland & Valiela, 1998). However, excrement collected at the Cuthbert rookery had slightly lower $\delta^{15}N$ values, with means of +6.63‰ and +7.36‰ in the dry and wet seasons, respectively. Surface water $\delta^{15}N$ values were determined from assimilated $\delta^{15}N$ values taken from our algae samples at the endmember sites, which ranged between +3‰ to +4‰. Lastly, internal nitrogen recycling is hypothesized to be primarily driven by the seasonal decomposition of the dominant submerged aquatic vegetation (SAV) species, *Chara hornemannii* (Owens et al. 2021). An ongoing study has determined the mean nitrogen isotopic signatures of Chara within the ECLS to be between +1‰ and +2‰ (Santos et al., unpublished data), and the remineralization of organic nitrogen to ammonia generally has small fractionation affects around ~0‰ (Brandes and Devol, 2002; Kendall, 1998; Lamb, 2007) to -2.5‰ (Möbius, 2013).

The majority of samples in our study (94%, 132 out of a total of 141 samples) exhibited $\delta^{15}N$ values consistent with nitrogen originating from surface water and groundwater sources encompassing freshwater marshes and Florida Bay. The remaining 9 samples have values between 0‰ and 2‰ which is more consistent with N derived from N_2 fixation, atmospheric deposition, and organic matter decomposition. The N source that showed a strong deviation away from the expressed $\delta^{15}N$ values are legacy nutrients derived from excrement from the Cuthbert rookery, a historic wading bird megacolony (Ogden et al., 2014). Nesting bird colonies on island rookeries have been shown to produce high nutrient loads, through defecation, which can alter hydrochemical function in surrounding areas as far as 10 km away (Golovkin & Garkavaya 1975; Staunton Smith & Johnson 1995; Wainwright et al., 1998; Kolb et al., 2010). The collected excrement samples were more enriched than the algal values surrounding the rookery (+3.22‰ in the wet and +2.69‰ in the dry season). This deviation in values points to legacy nutrients not having a major influence on N loads to Alligator.

The majority of algal samples within the ECLS fell within the isotopic range of marsh and bay derived nutrient transport (Appendix 5). Although, N_2 fixation, atmospheric deposition, and internal recycling can also contribute to

δ^{15}N values that mix with surface and groundwater inputs, we posit that this is a less significant nutrient contribution. This conclusion is supported by the observed spatial variations in δ^{15}N and δ^{13}C, which exhibit a north-south gradient, suggesting that water flow is the primary mode of nutrient transport through the system. This aligns with the established principle that inputs of nutrients to coastal wetlands occur via biologic and geologic pathways, however, hydrologic input usually dominates (Likens et al., 2013). Although we expect multi-source nutrient inputs, this data highlights flow regimes as the principal mechanism transporting nutrients within the ECLS.

4.2 Origins of flow-derived nutrients

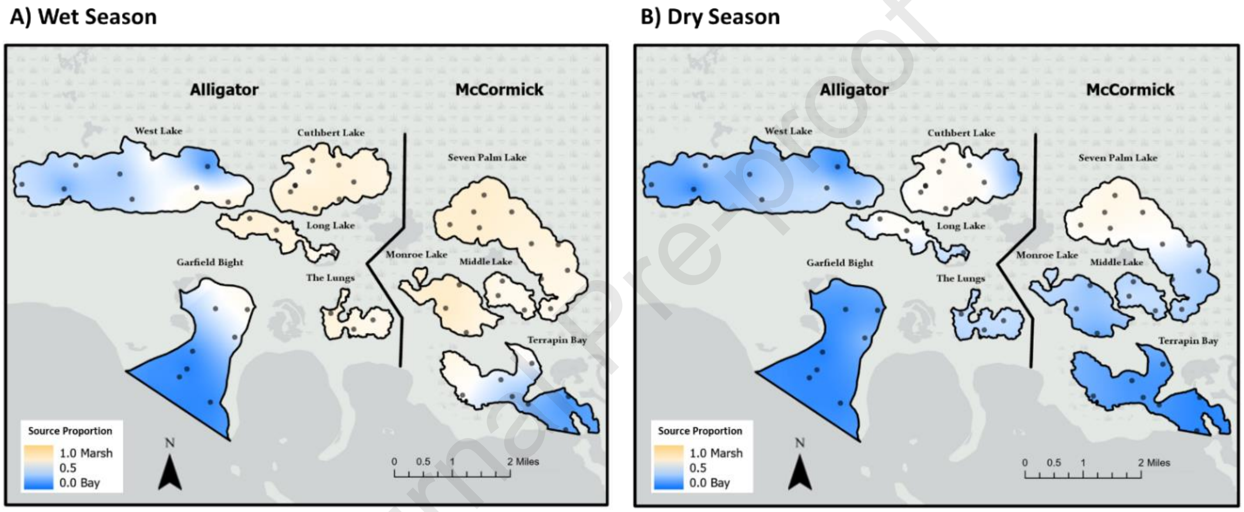
δ^{15}N values in our study indicate that the primary N inputs include surface and/or groundwater flow from both Florida Bay and freshwater marshes. However, δ^{15}N alone cannot definitively distinguish between these two sources. To achieve more precise source identification within estuarine environments, we can utilize additional stable isotope tracers like δ^{13}C and δ^{34}S. These tracers offer complementary information due to the distinct biogeochemical processes that influence their isotopic values in various source environments, particularly in estuaries (O'Leary, 1988; Chanton and Lewis, 1999; Connolly et al. 2004). δ^{13}C and δ^{34}S values have proven to be a valuable tool for tracking nutrient resources across the Everglades estuarine gradient (Fry and Smith, 2002; Rezek et al., 2020). This effectiveness stems from the significant isotopic variation that occurs along the salinity gradient, reflecting the transition from freshwater to marine ecosystems.

Studies of marsh hydrology have shown that surface water flow from west of ECLS is negligible (Sutula et al., 2001), leaving surface water inflows primarily driven by managed inputs from Taylor Slough and the L31W and C-111 canal basins to the east. Rudnick et al. (1999) showed that approximately 90% and 60% of N and P, respectively, are removed from surface water entering Taylor Slough. Thus, we expect that marsh surface flows primarily contain nutrients derived from the decomposition of autochthonous plant material in the marsh region closely preceding the ECLS. This is supported by the fact that freshwater entering Florida Bay tends to be rich in organic material, with high DON and DOC (Glibert et al. 2009; Shangguan et al. 2017a; Wilson et al., 2018). Our marsh algal samples were relatively depleted in δ^{13}C and enriched in δ^{34}S. This depletion in δ^{13}C is characteristic of an organic-rich environment, where respiration of organic material in the water column and sediments can cause significant ^{13}C-depletion of the DIC pool (Chanton and Lewis, 1999). In contrast, marsh algal δ^{34}S values were the most enriched samples collected, indicating the presence of sulfate derived from diffusion and oxidation of sulfide to sulfate in the sediment and shallow groundwater (Orem et al., 2011).

Groundwater flow of nutrients through the karst limestone underlying the Everglades has been shown to provide as much N and P as surface water to Florida Bay (Fennema et al., 1994; Corbett et al., 1999; Saha et al., 2011). In the ECLS, groundwater inputs are a greater contributor to water than surface flows (Price et al., 2021). N and C in groundwater are likely sourced from marsh nutrients, as there is presumably natural organic matter mineralization seeping through the immediately overlying sub-aqueous carbonate sediments (Shinn et al., 1994; Bohlke et al., 2003). Similarly, the enriched δ^{34}S values in our marsh algal samples are indicative of groundwater-sourced sulfate. Thus, although we did not collect N, C, and S isotopic samples from groundwater, we can assume that groundwater would fall into the δ^{15}N, δ^{13}C, and δ^{34}S isotopic range of the overlying marsh. Thus, we consider the upland marsh endmember values in our mixing models to represent both surface and groundwater flow of nutrients to the ECLS (Fig. 5).

On the other hand, the Florida Bay endmember has a direct connection with the ECLS at the southern extent of both Alligator and McCormick. Here, tidal flow is minimal, such that flow into the lakes from the bay is primarily wind-driven (Glibert et al., 2009; Eggenberger et al., 2019), and reverse flow of bay waters upstream has the potential to transport nutrient loads into the ECLS. Florida Bay endmember algal δ^{13}C values were enriched, possibly indicating DIC sourced from decomposing seagrass (typically between -10‰ to -15‰; Fourqurean et al., 2005). δ^{34}S values were not measured at the Florida Bay end member, but adjacent sites in Garfield Bight and Terrapin Bay were depleted, which may represent δ^{34}S of the bay environment. Depleted δ^{34}S values may be indicative of the assimilation of rainwater sulfate (+5‰; Orem et al., 2011) or benthic-pelagic coupling of sedimentary sulfides (~-24‰; Connolly et al., 2004).

ECLS nutrients could be differentiated as being derived from the freshwater marshes or Florida Bay using δ^{13}C and δ^{15}N in a two-endmember mixing model. However, δ^{34}S values displayed limitations as a tracer in this system. Unlike δ^{13}C and δ^{15}N, δ^{34}S values fell outside the range encompassed by the mixing model, particularly in the McCormick system, where they exhibited consistently depleted isotopic values throughout. This spatial uniformity suggests minimal influence from isotopically distinct sulfur sources and renders δ^{34}S an unsuitable tracer within the ECLS. Although upstream and downstream sources were not widely separated in δ^{15}N isotopic space, they were separated well in δ^{13}C space, allowing for the mixing model to differentiate primary nutrient inputs across both space and time. The observed mixing model results followed expected trends such that greater freshwater derived nutrients entered the ECLS in the wet season compared to marine-derived nutrients that primarily influenced the system in the dry season (Fig. 5). It is important to note that while the mixing model accounts for natural variation in source and mixture isotopic values through probabilistic frameworks, they do not provide definitive source identifications; our results offer a general understanding of dominant nutrient loading contributions rather than a complete accounting of all potential sources (Bedard-Haughn et al. 2003; Kendall et al., 2008).


A) Wet Season

B) Dry Season

Fig. 4 Spatial variation in the contribution of bay and marsh nutrients across the ECLS in the a) wet and b) dry seasons. Spatial variation is shown with ordinary kriging interpolations of the mixing model results.

4.3 Seasonal shifts and *spatial gradients*

While flow regimes appear to be the primary driver of isotopic patterns across space in our study, seasonal shifts in values suggest subtle changes in the nutrient regime that may reflect additional processes. Systemwide δ^{15}N values followed a trend of more depleted values in the dry season and enriched values in the wet season. This observed shift may be attributed to seasonal shifts in nutrient concentrations or seasonal mortality events of Chara. Unpublished data recorded from 2006-2018 from the ECLS showed higher TN concentrations in the dry season in Alligator (dry = 166.93 µM and wet = 132.83 µM) and in McCormick (dry = 80.24 µM and wet = 73.0 µM) (Frankovich, unpubl. data). The availability of N in the surrounding DIN pool can have a considerable influence on the fractionation of N assimilated in a producer, such as algae, affecting the overall interpretation of $\delta^{15}N$ measurements (Fourqurean et al., 2005). Greater N availability in the dry season allows for increased fractionation to occur, where algae preferentially assimilate ^{14}N over ^{15}N and inherently exhibit more depleted $\delta^{15}N$ values. Fourqurean et al. (2005) recorded a similar seasonal pattern of isotopically light values in turtle grass (*Thalassia testudinum*) in winter, when reduced plant growth requires lower N and there are higher concentrations of N available to producers. Seasonal variation in N concentrations may also result from shifts in submerged aquatic vegetation coverage. The dominant submerged aquatic vegetation in the ECLS is *Chara hornemannii*, whose abundance is strongly regulated by seasonal changes in salinity (particularly those above 25 psu; Frankovich et al., 2011; 2012). Dry season increases in salinity drive seasonal *Chara* die-off events that lead to elevated NH_4^+ effluxes and phytoplankton blooms (Owens et al., 2021). Decomposition of *Chara* and phytoplankton through microbial degradation causes $\delta^{15}N$ enrichment in the residual POM, and $\delta^{15}N$ depletion in the surrounding NH_4^+ pool (Liu et al., 1996). Most phytoplankton preferentially uptake NH_4^+ (Montoya et al., 1991; Velinsky & Fogel, 1999; Maguer et al., 2000), so it is possible that our samples show slightly depleted $\delta^{15}N$ values due to the assimilation of relatively depleted remineralized NH_4^+. In contrast, $\delta^{13}C$ values showed a trend of enrichment in the dry season relative to the wet, likely driven by a shift between marsh-derived carbon entering the ECLS in the wet season and greater marine-derived carbon during the dry season.

In contrast to the seasonality of $\delta^{13}C$ and $\delta^{15}N$, algal $\delta^{34}S$ did not vary between wet and dry seasons. Instead, $\delta^{34}S$ showed marked spatial variation. Although $\delta^{34}S$ has the capability to differentiate sources of water entering a system of interest (Connolly et al., 2004), it may prove an ineffective marker due to the greater influence of marine-sourced sulfate relative to freshwater sulfates in estuarine habitats (Fry et al., 2002). Pelagic microalgae favors the assimilation of seawater sulfate due to its greater availability at higher concentrations (generally ~ 28 mM; Fry et al., 2002), rather than the uptake of freshwater sulfate (often < 0.2 mM; Fry et al., 2002) in all but the lowest salinity estuarine waters (<1 PSU; Fry et al., 2002). $\delta^{34}S$ values have been shown to remain uniform across estuaries with open connection to marine waters, making it an unsuitable tracer of water entering these estuaries (Fry et al., 2002).

McCormick has relatively open, short channels connecting the lakes with Florida Bay (Frankovich et al., 2012; Eggenberger et al., 2019), and our results suggest that McCormick is functioning in this manner, with a uniform isotopic signal that is relatively depleted in $\delta^{34}S$, similar to values in the downstream bay. Ultimately, although McCormick receives greater freshwater from Taylor Slough compared to Alligator, it also has a greater connection to Florida Bay, presumably allowing for higher concentrations of seawater sulfate, which masks the ability of $\delta^{34}S$ to act as a tracer. In contrast, Alligator shows a gradient of more enriched $\delta^{34}S$ in upstream lakes to more depleted values downstream which may be a result of the long, slender creeks which can inhibit the mixing of water masses (Frankovich et al. 2011). The configuration of Alligator potentially allows for the separation of seawater and freshwater-derived sulfate across space, allowing producers to uptake and differentiate fresh vs. marine-derived sulfate.

Overall, both systems appear to receive nutrients from the same hydrological mechanisms, receiving greater marsh derived nutrient contributions in the wet season and greater bay contributions in the dry season (Fig. 6). We expect that the hydrologic characteristics of the lake systems directly regulate nutrient loading mechanisms across the north-to-south gradient in the lake chains. Both systems show a greater influence of marsh-derived C and N in the northern water bodies, while the southern waterbodies are more influenced by Florida Bay nutrients. This finding agrees with Price (2022), where surface waters in both McCormick and Alligator are described as a mixture of upland marsh and Florida Bay waters, further suggesting hydrologic processes as nutrient transport mechanisms to the ECLS. Yet, West Lake and Monroe Lake deviate from these gradients. West Lake exhibits greater bay influence than expected given its upstream placement. An ECLS water budget (Price, 2021) describes West Lake as having the least surface water and groundwater input, with main hydrologic inputs instead stemming from rainwater and Florida Bay surface water. These findings point to the possibility that an influx of bay nutrients into West Lake

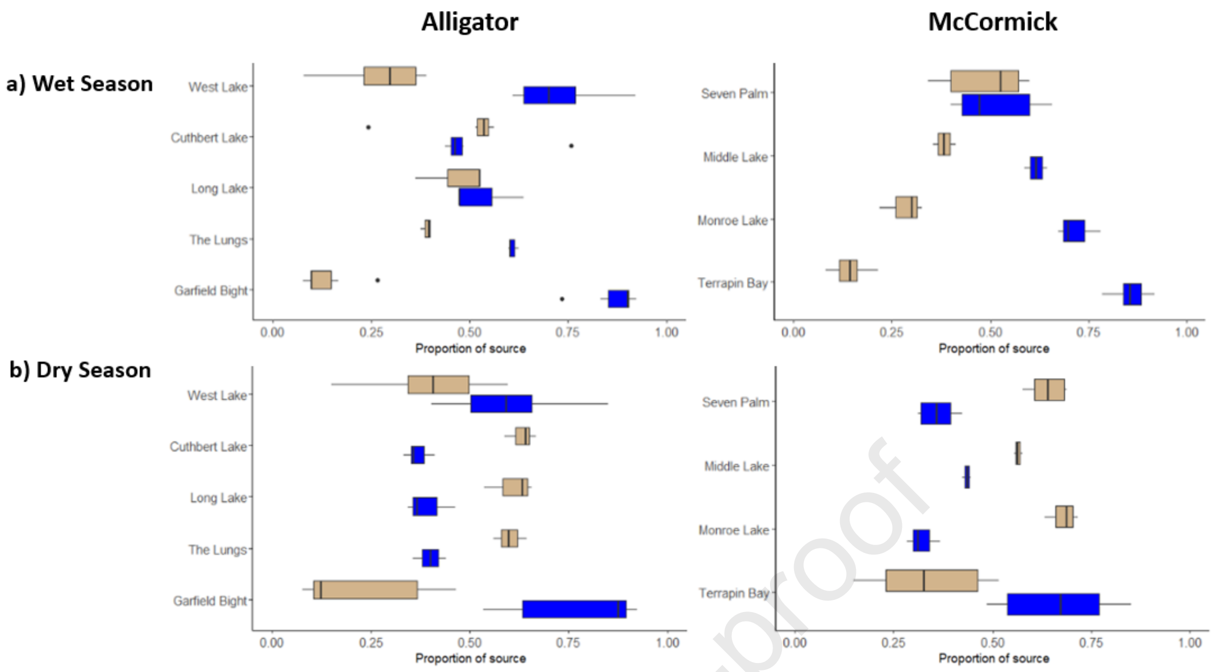


Fig. 5 Boxplot showing variation in the contribution of marsh and bay nutrient sources across waterbodies in Alligator and McCormick in the a) wet and b) dry seasons.

during the dry season is not flushed out by wet season freshwater influence due to its limited connectivity. Monroe Lake also stood out as an exception, showing a wet season increase in marsh nutrients despite its downstream placement. This matches water budget data where Monroe Lake has an unknown water source, suspected to be the result of either an influx of groundwater discharge or overland flow (Price, 2021).

5. Summary and implications

Ongoing hydrologic restoration efforts aim to increase freshwater flows to the coastal Everglades and Florida Bay (Sklar et al. 2019), and there is a need to understand the role of increased flows on nutrient regimes. The objective of this study was to determine the major nutrient contributions and pathways to the ECLS with the goal of gaining a better understanding of how changing freshwater flow dynamics will influence regional nutrient regimes. Our findings indicate nutrient inputs derived primarily from upstream marsh and downstream bay water sources. Upstream freshwater flow transports reactive forms of N that could increase N loading to the ECLS. Nutrient enrichment of these waters could increase general productivity of the system and enhance the possibility of phytoplankton blooms in north-central Florida Bay. At the same time, increased flows will also result in a variety of ecological changes that may act as a buffer to N loading into Florida Bay. For instance, increased freshwater flow has been shown to reduce the maximum dry season salinities (Sklar et al., 2019), promoting SAV diversity and community stability throughout the ECLS (National Academies of Sciences, Engineering, and Medicine, 2022). SAV is a keystone community in Florida Bay that provides many important physicochemical functions aiding in sediment stabilization, nutrient assimilation, and reducing benthic nutrient exchange to the water column (Yarbro and Carlson 2008; Glibert et al., 2009; Owens et al., 2021). The ability of SAV to sequester nutrients and counteract nutrient loading impacts with continued restoration initiatives needs further consideration.

This study highlights the vulnerability of estuaries worldwide to excess nutrient loading from land-based sources. Such inputs can significantly alter water quality and disrupt the stability of aquatic flora and fauna. Estimates suggest that nearly a quarter of human-driven nitrogen delivered to coastal watersheds ultimately reaches estuaries (Malone & Newton, 2020). Our findings further underscore the utility of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ as effective tracers for investigating nutrient sources, transport pathways, and the overall nutrient regime within coastal ecosystems. By elucidating these processes, this research provides valuable information that can inform targeted mitigation strategies and guide efforts to reduce nutrient loads, ultimately promoting the health and sustainability of our coastal environments.

Statements & Declarations

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Competing Interests

The authors have no conflict of interest to declare.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Joshua Linenfelser. Data analysis was performed by Joshua O. Linenfelser and W. Ryan James. The first draft was written by Joshua O. Linenfelser and all authors provided comments on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

Once accepted for publication, all data will be stored in a data repository.

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Appendices

Fig. A.1 Salinity conditions at the Garfield Bight hydrological station at the time of sampling in 2021-2022 (blue lines), relative to the 24-year mean (and 95% confidence interval in grey shading). In the ECLS, the wet season and corresponding minimum salinity lags behind rainfall, such that the lowest salinity is observed in October-December, while maximum salinity is observed in April-May. Highlighted areas correspond to periods of algal sampling, which targeted the yearly salinity maximum and minimum.

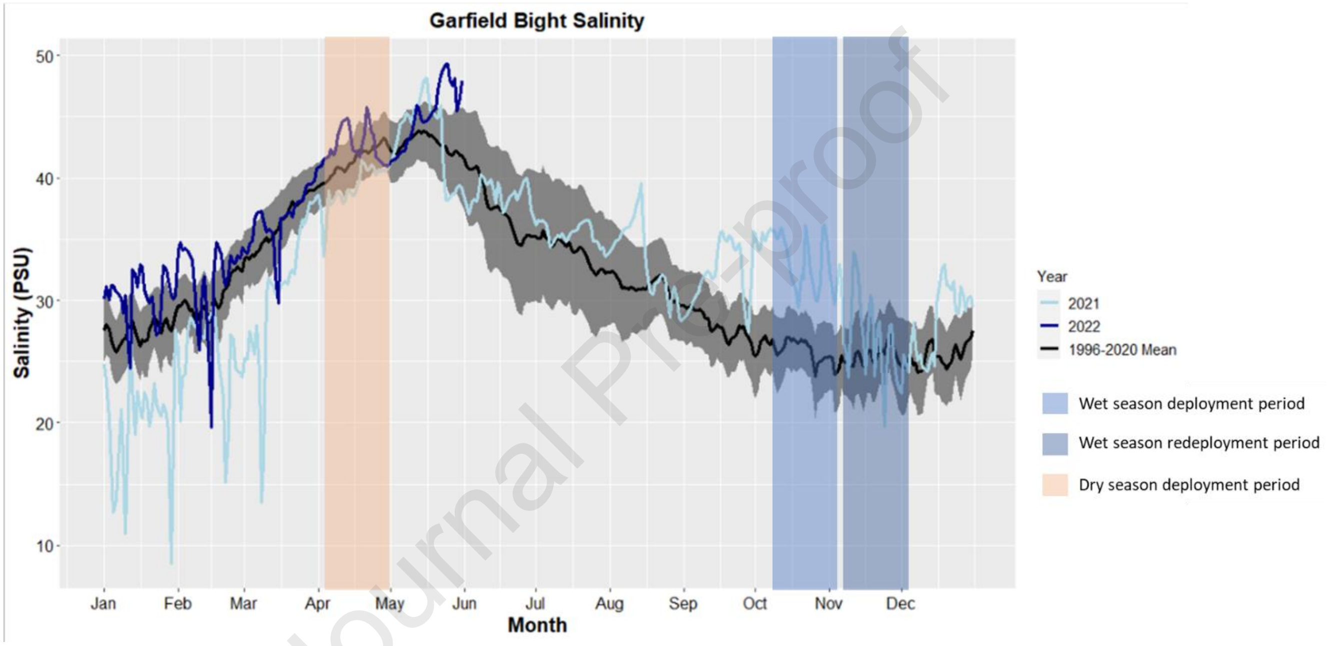
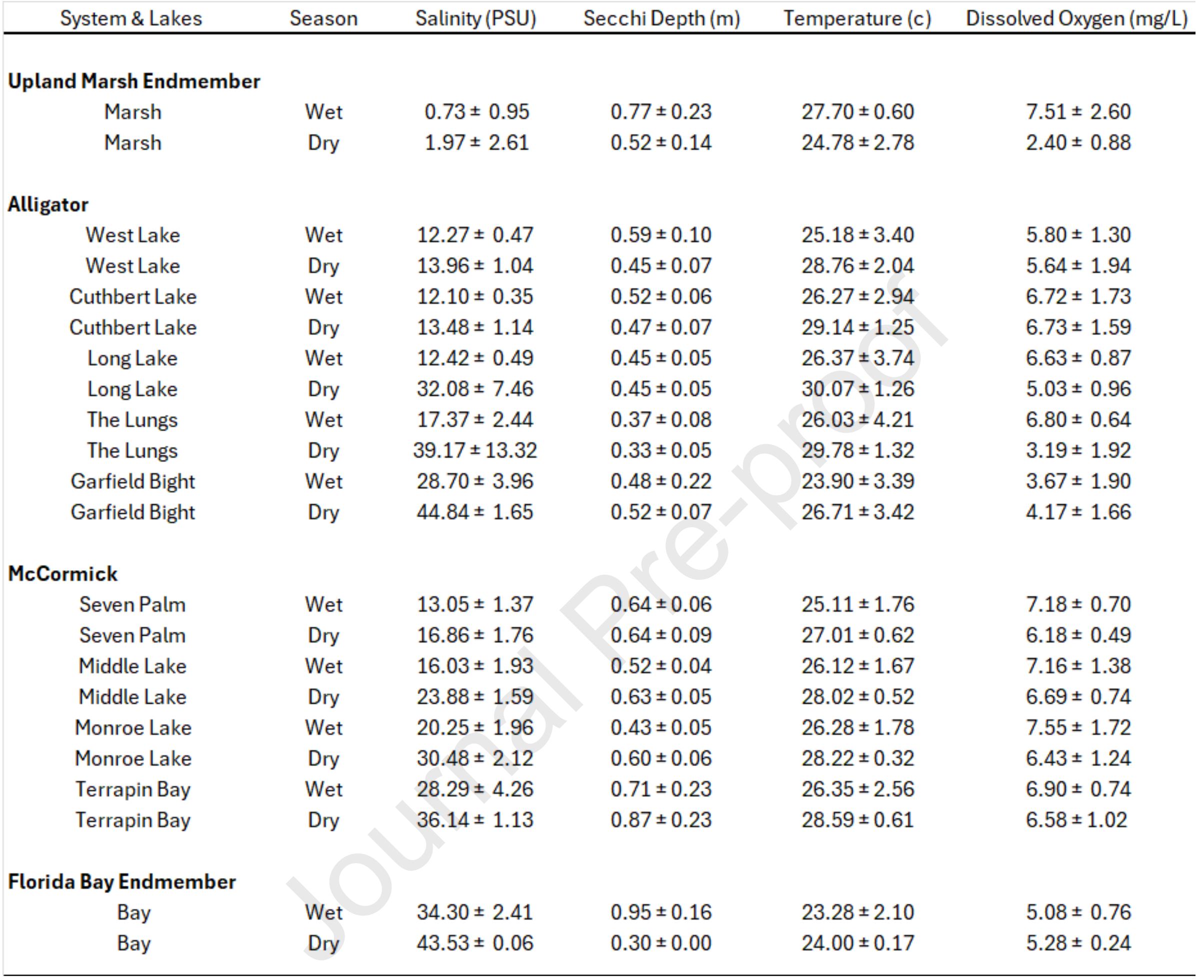


Fig. A.2 Environmental conditions (means ± SD) across all waterbodies at the time of seasonal sampling.



System & Lakes	Season	Salinity (PSU)	Secchi Depth (m)	Temperature (c)	Dissolved Oxygen (mg/L)
Upland Marsh Endmember					
Marsh	Wet	0.73 ± 0.95	0.77 ± 0.23	27.70 ± 0.60	7.51 ± 2.60
Marsh	Dry	1.97 ± 2.61	0.52 ± 0.14	24.78 ± 2.78	2.40 ± 0.88
Alligator					
West Lake	Wet	12.27 ± 0.47	0.59 ± 0.10	25.18 ± 3.40	5.80 ± 1.30
West Lake	Dry	13.96 ± 1.04	0.45 ± 0.07	28.76 ± 2.04	5.64 ± 1.94
Cuthbert Lake	Wet	12.10 ± 0.35	0.52 ± 0.06	26.27 ± 2.94	6.72 ± 1.73
Cuthbert Lake	Dry	13.48 ± 1.14	0.47 ± 0.07	29.14 ± 1.25	6.73 ± 1.59
Long Lake	Wet	12.42 ± 0.49	0.45 ± 0.05	26.37 ± 3.74	6.63 ± 0.87
Long Lake	Dry	32.08 ± 7.46	0.45 ± 0.05	30.07 ± 1.26	5.03 ± 0.96
The Lungs	Wet	17.37 ± 2.44	0.37 ± 0.08	26.03 ± 4.21	6.80 ± 0.64
The Lungs	Dry	39.17 ± 13.32	0.33 ± 0.05	29.78 ± 1.32	3.19 ± 1.92
Garfield Bight	Wet	28.70 ± 3.96	0.48 ± 0.22	23.90 ± 3.39	3.67 ± 1.90
Garfield Bight	Dry	44.84 ± 1.65	0.52 ± 0.07	26.71 ± 3.42	4.17 ± 1.66
McCormick					
Seven Palm	Wet	13.05 ± 1.37	0.64 ± 0.06	25.11 ± 1.76	7.18 ± 0.70
Seven Palm	Dry	16.86 ± 1.76	0.64 ± 0.09	27.01 ± 0.62	6.18 ± 0.49
Middle Lake	Wet	16.03 ± 1.93	0.52 ± 0.04	26.12 ± 1.67	7.16 ± 1.38
Middle Lake	Dry	23.88 ± 1.59	0.63 ± 0.05	28.02 ± 0.52	6.69 ± 0.74
Monroe Lake	Wet	20.25 ± 1.96	0.43 ± 0.05	26.28 ± 1.78	7.55 ± 1.72
Monroe Lake	Dry	30.48 ± 2.12	0.60 ± 0.06	28.22 ± 0.32	6.43 ± 1.24
Terrapin Bay	Wet	28.29 ± 4.26	0.71 ± 0.23	26.35 ± 2.56	6.90 ± 0.74
Terrapin Bay	Dry	36.14 ± 1.13	0.87 ± 0.23	28.59 ± 0.61	6.58 ± 1.02
Florida Bay Endmember					
Bay	Wet	34.30 ± 2.41	0.95 ± 0.16	23.28 ± 2.10	5.08 ± 0.76
Bay	Dry	43.53 ± 0.06	0.30 ± 0.00	24.00 ± 0.17	5.28 ± 0.24

Fig. A.3 Depiction of an algal sampler. A) Each sampler consisted of two 22.86 cm x 22.86 cm clear acrylic plexiglass zip tied and taped to a 1.9 m PVC pipe staked in the sediment. The plexiglass plates were identical and were spaced 10 cm apart. The PVC was cut to size such that it could be staked down through the sediment to the bedrock, at least 0.5 m, and would position the plexiglass to sit exactly 30 cm below the water line. All samplers were constructed anew for each sampling event. B.) Image of sampler staked in sediment.

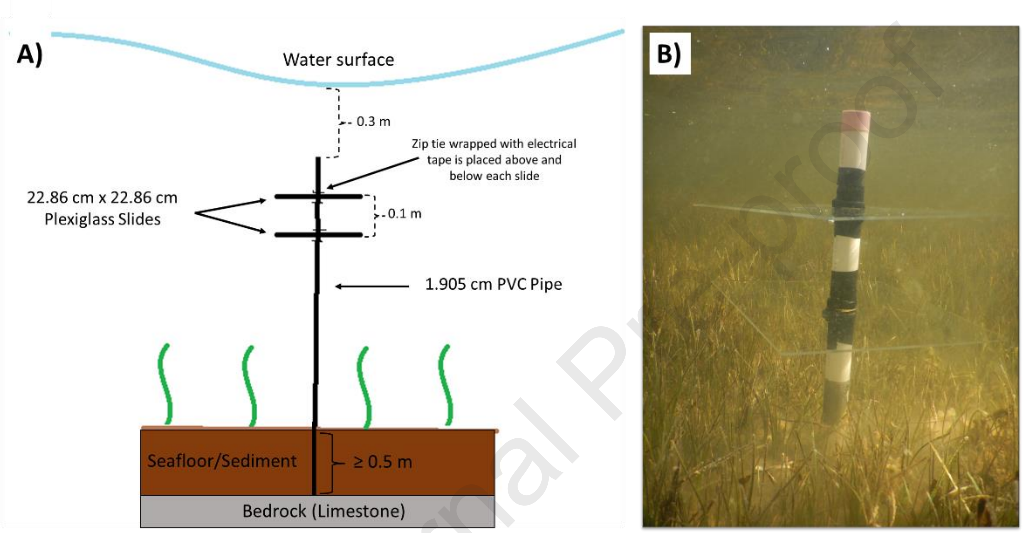


Fig. A.4 Microscopic image of algae samples collected on artificial substrates, illustrating that the community was dominated by diatoms; this was consistent across sites.

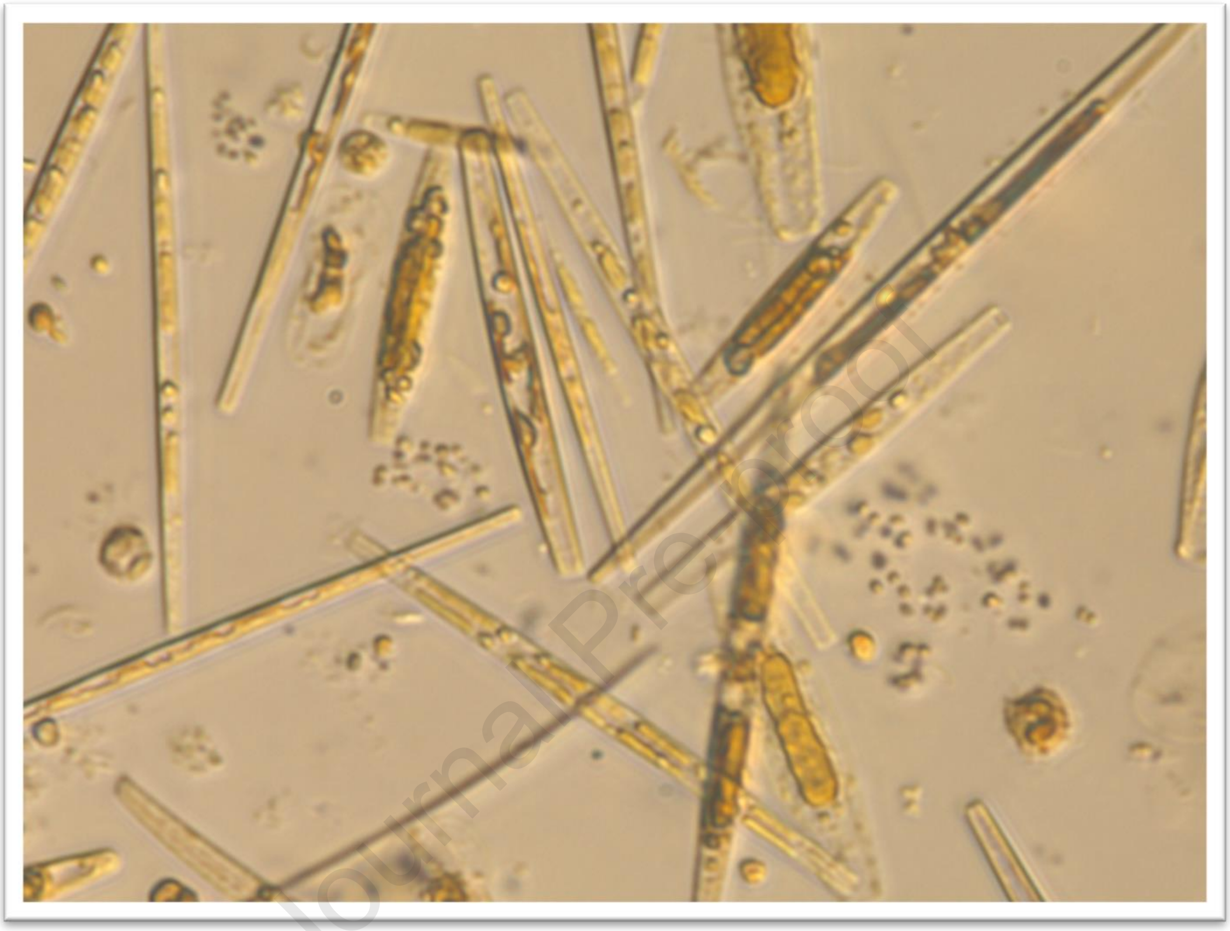


Fig A.5 Boxplot representing the distribution of δ^{15}N, δ^{13}C, and δ^{34}S across all lakes and both endmembers within the study system. The central line within each box indicates the median value, while the lower and upper edges of the box represent the first and third quartiles, respectively. The whiskers extend to 1.5 times the interquartile range (IQR) from the lower and upper quartiles, with outliers depicted as individual points beyond this range.

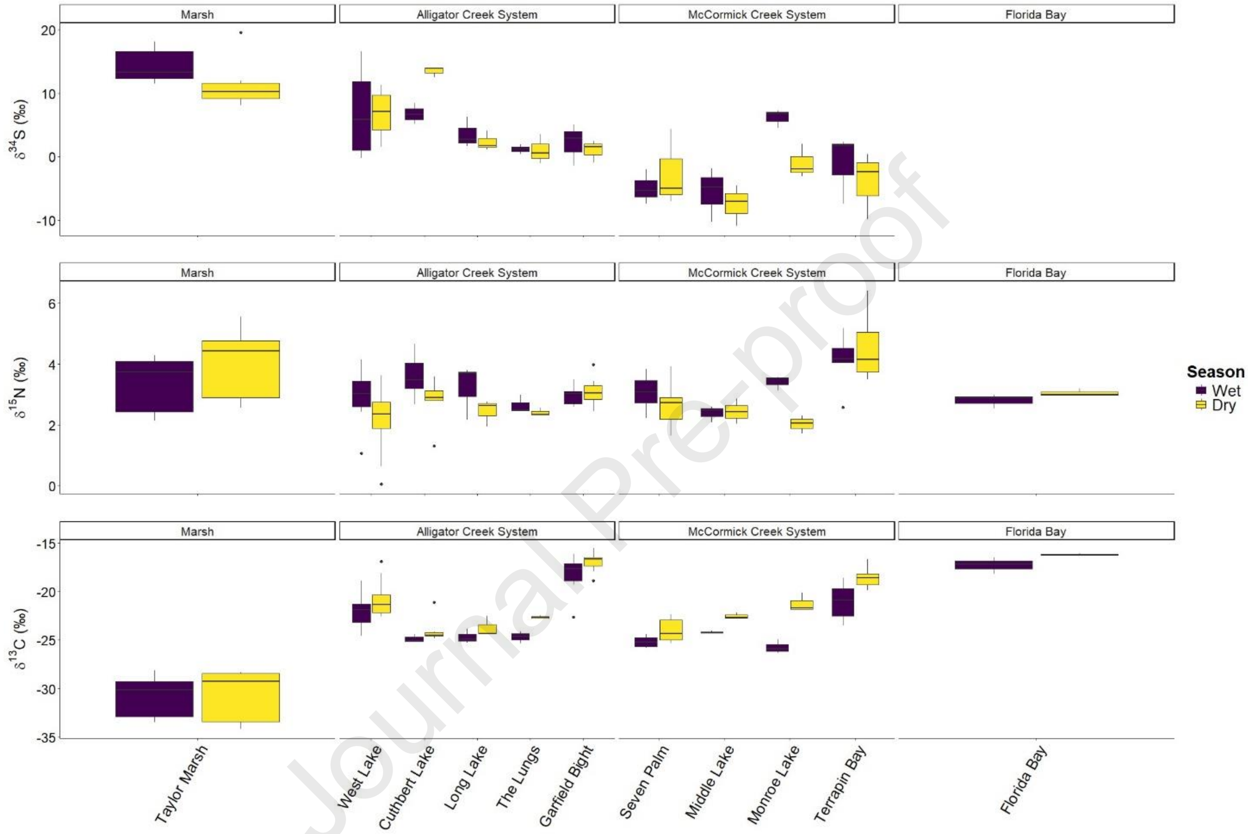


Table 1. δ^{15}N, δ^{13}C, and δ^{34}S values (means ± SD) for all algal samples in both seasons throughout the study system, along with mixing model results using the δ^{15}N and δ^{13}C values to determine the proportion of bay and marsh source nutrient inputs to the ECLS.

	Stable Isotope Results				**Mixing Model Results**	
Season	δ^{15}N	δ^{13}C	δ^{34}S	Lake	Bay	Marsh
				Taylor Slough Endmember		
Wet	3.32 ± 0.89	-30.67 ± 2.09	14.32 ± 2.81	Marsh		
Dry	4.05 ± 1.09	-30.49 ± 2.55	11.50 ± 4.18	Marsh		
				Alligator		
Wet	2.97 ± 0.87	-21.86 ± 1.75	7.00 ± 7.91	West Lake	0.60 ± 0.14	0.40 ± 0.14
Dry	2.16 ± 1.11	-20.78 ± 1.93	6.75 ± 4.34	West Lake	0.73 ± 0.11	0.27 ± 0.11
Wet	3.61 ± 1.65	-24.92 ± 0.31	6.70 ± 1.65	Cuthbert Lake	0.37 ± 0.03	0.63 ± 0.03
Dry	2.88 ± 0.63	-24.12 ± 1.08	13.47 ± 0.84	Cuthbert Lake	0.49 ± 0.09	0.51 ± 0.09
Wet	3.22 ± 0.92	-24.67 ± 0.74	3.53 ± 2.46	Long Lake	0.39 ± 0.06	0.61 ± 0.06
Dry	2.45 ± 0.45	-23.73 ± 1.08	2.27 ± 1.53	Long Lake	0.53 ± 0.09	0.47 ± 0.09
Wet	2.64 ± 0.30	-24.67 ± 0.62	1.17 ± 0.75	The Lungs	0.40 ± 0.04	0.60 ± 0.04
Dry	2.41 ± 0.12	-22.64 ± 0.07	0.97 ± 2.34	The Lungs	0.61 ± 0.01	0.39 ± 0.01
Wet	2.98 ± 0.34	-18.39 ± 2.33	2.17 ± 3.26	Garfield Bight	0.78 ± 0.18	0.22 ± 0.18
Dry	3.10 ± 0.50	-16.94 ± 1.11	0.97 ± 1.76	Garfield Bight	0.87 ± 0.07	0.13 ± 0.07
				Cuthbert Rookery		
Wet	2.69 ± 0.49	-23.34 ± 1.15	NA	Microalgae		
Dry	3.22 ± 0.71	-22.58 ± 1.24	NA	Microalgae		
Wet	7.36 ± 0.53	-25.67 ± 4.37	NA	Bird Excrement		
Dry	6.63 ± 0.73	-28.12 ± 3.34	NA	Bird Excrement		
				McCormick		
Wet	3.07 ± 0.51	-25.19 ± 0.54	-4.97 ± 2.68	Seven Palm	0.36 ± 0.04	0.64 ± 0.04
Dry	2.62 ± 0.65	-24.02 ± 1.17	-2.60 ± 6.07	Seven Palm	0.51 ± 0.10	0.49 ± 0.10
Wet	2.39 ± 0.27	-24.26 ± 0.14	-5.67 ± 4.27	Middle Lake	0.44 ± 0.01	0.56 ± 0.01
Dry	2.44 ± 0.41	-22.54 ± 0.35	-7.53 ± 3.17	Middle Lake	0.62 ± 0.03	0.38 ± 0.03
Wet	3.40 ± 0.25	-25.73 ± 0.02	6.17 ± 1.47	Monroe Lake	0.32 ± 0.04	0.68 ± 0.04
Dry	2.02 ± 0.29	-21.23 ± 0.98	-1.03 ± 2.68	Monroe Lake	0.72 ± 0.06	0.28 ± 0.06
Wet	4.15 ± 0.82	-21.05 ± 1.86	-1.13 ± 5.44	Terrapin Bay	0.66 ± 0.14	0.34 ± 0.14
Dry	4.52 ± 1.07	-18.56 ± 1.09	-3.97 ± 5.33	Terrapin Bay	0.86 ± 0.04	0.14 ± 0.04
				Florida Bay Endmember		
Wet	2.81 ± 0.24	-17.26 ± 0.84	NA	Bay		
Dry	3.05 ± 0.13	-16.16 ± 0.10	NA	Bay		

Manuscript Highlights:

- C, N and S isotopes distinguish marsh-derived and marine-derived nutrient sources
- Isotopic values vary spatially across the marsh-bay transition in Florida Bay
- Bayesian mixing models reveal seasonally shifting nutrient transport pathways

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: