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Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources

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Abstract

Land-based nutrient pollution represents a significant human threat to coral reefs globally. We examined this phenomenon in shallow seagrass and coral reef communities between the Content Keys (southern Florida Bay) and Looe Key (south of Big Pine Key) in the Lower Florida Keys by quantifying the role of physical forcing (rainfall, wind, tides) and water management on mainland South Florida to nutrient enrichment and blooms of phytoplankton, macroalgae, and seagrass epiphytes. Initial studies (Phase I) in 1996 involved daily water quality sampling (prior to, during, and following physical forcing events) at three stations (AJ, an inshore area directly impacted by sewage discharges; PR, a nearshore patch reef located inshore of Hawk Channel; and LK, an offshore bank reef at Looe Key) to assess the spatial and temporal patterns in advection of land-based nutrients to the offshore reefs. Concentrations of dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_3^- + NO_2^-$), soluble reactive phosphorus (SRP), and chlorophyll *a* increased at PR and LK following a wind event (~ 15 knots, northeast) in mid-February. The highest DIN (mostly NH_4^+) and SRP concentrations of the entire study occurred at the inshore AJ during an extreme low tide in March. Following the onset of the wet season in May, mean NH_4^+ and chlorophyll *a* concentrations increased significantly to maximum seasonal values at PR and LK during summer; relatively low concentrations of NO_3^- and a low *f*-ratio ($NO_3^-/NH_4^+ + NO_3^-$) at all stations during summer do not support the hypothesis that the seasonal phytoplankton blooms resulted from upwelling of NO_3^-. A bloom of the seagrass epiphyte *Cladosiphon occidentalis* (phaeophyta) followed the onset of the rainy season and increased NH_4^+ concentrations at LK, resulting in very high epiphyte:blade ratios (~ 3:1) on *Thalassia testudinum*. Biomass of macroalgae increased at all three stations from relatively low values (< 50 g dry wt m^{-2})

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in winter and early spring to higher values (~ 100–300 g dry wt m^{-2}) typical of eutrophic seagrass meadows and coral reefs following the onset of the rainy season. The mean δ^{15}N value of *Laurencia intricata* (rhodophyta) during 1996 at AJ (+4.7‰) was within the range reported for macroalgae growing on sewage nitrogen; lower values at the more offshore PR (+3.1‰) and LK (+2.9‰) were at the low end of the sewage range, indicating an offshore dilution of the sewage signal during the 1996 study. However, transient increases in δ^{15}N of *Cladophora catanata* (chlorophtyta) from ~+2% to +5% at LK concurrent with elevated NH_4^+ concentrations following rain and/or wind events in May and July suggest episodic advection of sewage nitrogen to the offshore LK station. The Phase II study involved sampling of macroalgae for δ^{15}N along a gradient from the Content Keys through Big Pine Key and offshore to LK in the summer wet season of 2000 and again in the drought of spring 2001. During the July 2000 sampling, macroalgae in nearshore waters around Big Pine Key had elevated δ^{15}N values (~+4‰) characteristic of sewage enrichment; lower values (~+2‰) at LK were similar to values reported for macroalgae in upstream waters of western Florida Bay influenced by nitrogen-rich Everglades runoff. That pattern contrasted with the drought sampling in March 2001, when δ^{15}N values of macroalgae were elevated (+6‰) to levels characteristic of sewage enrichment over a broad spatial scale from the Content Keys to LK. These results suggest that regional-scale agricultural runoff from the mainland Everglades watersheds as well as local sewage discharges from the Florida Keys are both significant nitrogen sources supporting eutrophication and algal blooms in seagrass and coral reef communities in the Lower Florida Keys. Hydrological and physical forcing mechanisms, including rainfall, water management on the South Florida mainland, wind, and tides, regulate the relative importance and variability of these anthropogenic nitrogen inputs over gradients extending to the offshore waters of the Florida Reef Tract.

Keywords: Nitrogen; Phosphorus; Eutrophication; Coral; Seagrass; Phytoplankton; Macroalgae

1. Introduction

Recognition of the critical interrelationships between water quality, the health of seagrass and coral reef communities, and economic well-being of the Florida Keys led to its designation as an "Area of Critical State Concern" by the Florida Legislature in 1974. Much of the concern centered around the potential impacts of excessive human development on coastal pollution and degradation of water quality. Three decades ago planners recognized that "the high quality of water in and around the Florida Keys is an essential element of the total system. One of the strongest attractions for residents and visitors to the area is the clear, blue waters of the Keys—a resource that rarely exists off the heavily populated, industrialized cities of the mainland" (State of Florida, 1974).

Water quality degradation was also a primary issue leading to designation of the Florida Keys National Marine Sanctuary (FKNMS) in 1990 that specifically included a multi-agency (National Oceanic and Atmospheric Administration, NOAA; United States Environmental Protection Agency, USEPA; Florida Department of Environmental Protection, FDEP) Water Quality Protection Program (WQPP) for the FKNMS (NOAA, 1988; USEPA, 1996). Of particular concern was the impact of local land-based sewage discharges, especially ecological damage from nutrient enrichment and eutrophication (Lapointe and Clark, 1992; Kruczynski and McManus, 2002). Currently, there are ~ 30,000 on-site sewage disposal systems (OSDSs; septic tanks, cesspits, and Class V injection wells cased to 28 m) in subdivisions scattered throughout the Florida Keys (Kruczynski and McManus, 2002). Most of these OSDSs are located adjacent to constructed canal systems that were designed to provide boating access to coastal waters that contain nutrient-sensitive seagrass and coral reef communities (NRC, 2000; Howarth et al., 2000). In addition to OSDSs, the City of Key Colony Beach (1.3 million l/day) and Key West (37 million l/day) have central sewage collection and treatment to secondary and tertiary levels, respectively, prior to injecting into Class V wells (Key Colony Beach) and Class 1 wells (cased to ~ 950 m, Key West).

A growing body of scientific evidence suggests an increasing impact of sewage pollution on degradation of water quality in the FKNMS. Because of high groundwater tables and transmissive limestone substrata, OSDSs in the Florida Keys rapidly contaminate groundwaters with high concentrations of dissolved inorganic nitrogen ($\mathrm{DIN} = \mathrm{NH}_4^+ + \mathrm{NO}_3^- + \mathrm{NO}_2^-$) and to a lesser extent, soluble reactive phosphorus (SRP), which enter coastal waters via submarine groundwater discharge (SGD; Lapointe et al., 1990; Reich et al., 2002). Shinn et al. (1994) examined the fate and pathways of Class V injection well effluent using core drilled monitoring wells and found fecal contamination of groundwaters in three offshore wells in the Lower Florida Keys (Shinn et al., 1994). Lapointe and Matzie (1996) used high-frequency monitoring of dissolved nutrients, salinity, temperature, and dissolved oxygen to track offshore advection of land-based nutrient discharges of NH_4^+ and SRP at the onset of the summer wet season in 1992; this seasonal stormwater phenomenon, identified by decreased salinity and increased NH_4^+ and SRP concentrations, initiated phytoplankton blooms and hypoxia over a broad spatial scale extending from sewage-impacted inshore waters on the west side of Big Pine Key to offshore waters at Looe Key. The problem of sewage pollution is not only one of eutrophication and loss of biotic resources, but also public health because of the known bacterial contamination of shallow groundwaters and contiguous surface waters by OSDSs (Paul et al., 1995a). Paul et al. (1995b), using viral tracers, found that wastewater transport from septic tanks through Key Largo limestone into adjacent surface waters (~ 20 m horizontal distance) occurred in as little as 11 h.

Excessive biomass of macroalgae is a common symptom of nutrient enrichment and eutrophication in seagrass and coral reef ecosystems (NRC, 2000; Howarth et al., 2000). Because macroalgae can rapidly assimilate pulses of NH_4^+ and SRP from the water column (D'Elia and DeBoer, 1978; Hanisak and Harlin, 1978), anthropogenic nutrient pollution can result in expansive blooms in oligotrophic tropical and subtropical waters (Banner, 1974; Johannes, 1975; Lapointe et al., 1994; Bell and Elmetri, 1995; Duarte, 1995; Lapointe, 1997). Macroalgal blooms can physically overgrow seagrasses and adult corals, inhibit recruitment of juvenile corals, lead to hypoxia and/or anoxia, and result in greatly diminished fisheries and biological diversity (NRC, 2000; Howarth et al., 2000). Because of these detrimental effects, excessive biomass levels of macroalgae (above what would be considered "normal" for a particular species in a given environment) are considered Harmful Algal Blooms (HABs; ECOHAB, 1995). Over the past two decades, the Florida Reef Tract has experienced dramatic increases in cover of macroalgae with parallel losses of hard coral cover (Porter and Meier, 1992; Chiappone and Sullivan, 1997; Porter et al., 2002). At Looe Key reef, south of Big Pine Key in the Lower Florida Keys, this "phase-shift" away from hermatypic corals and towards dominance by macroalgae and algal turfs has correlated with increasing annual mean concentrations of DIN and SRP despite the presence of high grazing by abundant schools of Scarid (parrotfishes) and Acanthurid (surgeonfishes) icthyofauna (Lapointe et al., 2002).

Seagrass and coral reef ecosystems impacted by nutrient pollution can require extensive recovery times (Smith et al., 1981; Duarte, 1995). Taxonomic shifts have been used as indicators of nutrient pollution on coral reefs (Littler, 1973; Johannes, 1975; Lapointe, 1997) but because the appearance of "indicator species" often signals an advanced stage of the problem, considerable environmental damage is likely to have already occurred. Measurement of stable nitrogen isotopes ($^{15}N/^{14}N = \delta^{15}N$) in biota has been used widely to discriminate between natural and anthropogenic nitrogen sources (Heaton, 1986; Risk et al., 2001) and could provide an "early warning" tracer of anthropogenic nutrient enrichment such as from sewage or agricultural runoff. For reef corals, tissue is typically enriched in ^{15}N by up to + 5‰ at sewage polluted, eutrophied sites relative to reference sites (Risk et al., 1993; Mendes et al., 1997; Heikoop et al., 2000). Similar levels of enrichment in ^{15}N have been reported for reef macroalgae experiencing sewage pollution. On coral reefs in the Negril Marine Park, Jamaica, macroalgae on shallow, sewage-impacted reefs had enriched $\delta^{15}N$ values ranging from + 4.0‰ to + 6.5‰; macroalgae on deeper reefs less impacted by sewage had lower values of + 0.5‰ to + 2. 5‰ (Lapointe and Thacker, 2002; Table 1). On eutrophic coral reefs off southeast Florida, $\delta^{15}N$ values in *Codium isthmocladum*

Table 1
$\delta^{15}N$ values reported for macroalgae in coastal waters with natural and anthropogenic sources of nitrogen enrichment

Location	Species	$\delta^{15}N$ (‰)	Reference
Boston Harbor, MA	*Ectocarpus* sp. *Ceramium* sp. *Desmarestia* sp. *Entropuria bantrophova* *Chondrus crispus*	+ 6.2	France et al. 1998
Childs River, MA (“high” N loading)	*Cladophora vagabunda*	+ 5.4	McClelland and Valiela (1998)
	Gracilaria tikvahiae	+ 7.6	
	Enteromorpha sp.	+ 8.4	
Sage Lot Pond, MA (“low” N loading)	*Cladophora vagabunda*	+ 3.4	McClelland and Valiela (1998)
	Gracilaria tikvahiae	+ 5.1	
	Enteromorpha sp.	+ 4.9	
Palm Beach County, FL	*Codium isthmocladum*	+ 3.0 – + 11.0	Lapointe (1997)
Western Florida Bay, FL	*Sargassum* sp.	+ 3.4	Zieman and Fourqurean (1985)
	Ulva sp.	+ 0.8	
	Caulerpa sp.	+ 2.6	
	Laurencia intricata	+ 2.0	Lapointe, unpublished data
	“macroalgae”	+ 1 – + 4	Corbett et al. (1999)
Negril, Jamaica	*Chaetomorpha linum*	> + 6.0	Lapointe and Thacker (2002)
	Sargassum polyceratium	+ 4.0 – + 6.0	
Oligotrophic reef, Puerto Rico	21 species of macroalgae	+ 0.3	France (1998)

increased from ~+5‰ to +11‰ following heavy summer rainfall and increased discharges of sewage-contaminated groundwaters enriched in ^{15}N into the Loxahatchee River and coastal waters (Lapointe, 1997; Table 1). Costanzo et al. (2001) recently reported that δ^{15}N values of macroalgae were enriched >+3‰ in areas of Moreton Bay, Brisbane, Australia, that were chronically impacted by sewage nitrogen. In comparison, 21 samples of tropical macroalgae had a mean δ^{15}N of +0.5 ± 1.0‰ in relatively unpolluted coastal waters of southwestern Puerto Rico, a value close to the atmospheric signature of 0‰ and indicative of nitrogen fixation as the source of nitrogen supporting growth (France et al., 1998; Table 1). We hypothesized that if wastewater DIN was impacting seagrass and coral reef communities in coastal waters of the Lower Florida Keys, then macroalgae inhabiting these habitats should have δ^{15}N values >+3‰.

Other regional-scale sources of nitrogen with δ^{15}N signatures different from sewage could also simultaneously contribute to macroalgal HABs and water quality degradation in the FKNMS. Offshore upwelling of cold subsurface water has been hypothesized to be a significant source of NO_3^- and SRP to coastal waters of the Florida Keys (Lapointe and Smith, 1987; Leichter et al., 1996; Szmant and Forrester, 1996; Leichter et al., 2003). However, these deep, offshore cold water intrusions occur primarily in summer months as a result of wind forcing and are highly episodic; when they do occur, they have only minor, if any, effects on NO_3^- concentrations in <30 m water depths in the FKNMS (Lapointe and Smith, 1987; Leichter et al., 1996, 2003). However, previous studies have shown significant long-term gradients in DIN and SRP concentrations from inshore to offshore waters of the FKNMS as a result of land-based nutrient inputs from the Florida Keys and mainland South Florida (Lapointe and Clark, 1992; Lapointe and Matzie, 1996; Lapointe et al., 2002; Brand, 2002). Smith and Pitts (2002), using current meter measurements in the major tidal passes in the Middle and Lower Florida Keys, reported a net long-term Gulf of Mexico to Atlantic flow of seawater. Pitts (1994) showed that these "outwelled" net cumulative flows entering Hawk Channel have an offshore deflection, which combined with elevated DIN concentrations from land-based sources, can result in considerable advection of DIN to the offshore reef tract (Pitts, 2002). Shark River Slough and Taylor Slough in Everglades National Park, watersheds that drain upland agricultural areas, can deliver significant loads of nitrogen (up to 4545 mton N/year for Shark River Slough in 1995) during periods of high flows (Rudnick et al., 1999; Lapointe et al., 2002) and this enrichment has been linked to NH_4^+ enrichment and the generation of phytoplankton blooms and turbidity in both central and western Florida Bay as well as downstream bank reefs at Looe Key (Lapointe et al., 2002; Brand, 2002). The δ^{15}N signatures of macroalgae in western Florida Bay directly influenced by nitrogen loads from Shark River Slough range from +1‰ to +3‰ (Zieman and Fourqurean, 1985; Corbett et al., 1999; see Table 1), values similar to that of fertilizer and peat nitrogen (Heaton, 1986). Annual sewage nitrogen loads in the Florida Keys are also considerable (~400 mtons N/year, Kruczynski and McManus, 2002) but, compared to agricultural sources on mainland South Florida, are relatively constant between wet and dry seasons and years. Because the δ^{15}N method works best when there are multiple sources with different signatures, we hypothesized that we could discriminate among local sewage nitrogen (>+3‰) in the Lower Florida Keys, agricultural nitrogen (+1‰ to +3‰)

associated with Shark River Slough discharges, and natural nitrogen fixation ($< +0.5‰$) in seagrass and coral reef communities of the Lower Keys.

Accordingly, a primary goal of this study was to use $\delta^{15}N$ signatures of macroalgae and seagrass epiphytes to assess the relative importance of anthropogenic DIN generated locally from wastewater in the Lower Keys versus that from regional-scale agricultural runoff to eutrophication in coastal waters of the Lower Florida Keys. Considering that tidal pumping and rainfall events are two important physical mechanisms that result in episodic "pulses" of groundwater-borne wastewater NH_4^+ into surface waters (Lapointe et al., 1990; Shinn et al., 1994; Lapointe and Matzie, 1996), another goal was to assess how episodic (daily) physical forcing associated with rainfall, wind, tides and "wet versus dry" seasonality physically link nutrient discharges from the Florida Keys with the generation of phytoplankton blooms, macroalgal HABs, and seagrass epiphytes in coastal waters of the FKNMS. Such daily and seasonal physical forcing could be important mechanisms not only for the introduction of anthropogenic nitrogen into coastal waters, but also to the resuspension of benthic sediments and recycled pore water nutrients into the water column where they become available to support blooms of phytoplankton and macroalgae.

Our approach involved a two-phased study. During Phase I in 1996 we sampled at three stations (inshore, nearshore, offshore) between Big Pine Key and Looe Key for DIN and SRP concentrations of the water column and phytoplankton biomass (chlorophyll *a*) at daily frequencies prior to, during, and following episodic physical forcing events. Macroalgal biomass and epiphyte loads on turtle grass, *Thalassia testudinum*, were measured monthly at all three stations. We also measured $\delta^{15}N$ at daily and monthly frequencies in macroalgal tissue and monthly in turtle grass epiphyte tissue at the three stations to discriminate the source of DIN enrichment. During Phase II in 2000/2001, we sampled abundant macroalgal populations for $\delta^{15}N$ during the summer 2000 wet season as well as during drought conditions in spring 2001 at five stations over a broad spatial scale (~ 24 km) from the Content Keys (southern Florida Bay) through the Lower Florida Keys (Big Pine Key) and offshore to the bank reef at Looe Key.

2. Materials and methods

2.1. Description of sampling stations

Phase I was conducted between January and October 1996 at three shallow (1–3 m depths) seagrass/coral reef communities between Big Pine Key and the Looe Key Sanctuary Protection Area (Fig. 1). This gradient provides a spatial framework to assess the offshore advection of land-based nutrient enrichment (Lapointe and Matzie, 1996; Pitts, 2002; Fig. 1). The stations consisted of: (1) two offshore sites in the back reef of Looe Key that included "LKBR 1" and "LKBR 2". LKBR 1 is a shallow seagrass meadow immediately shoreward of the reef crest at Looe Key that we have used for monitoring water column nutrients, chlorophyll *a*, and seagrass epiphytes since 1983 (Lapointe and Smith, 1987; Lapointe et al., 1994, 2002). Recognizing the high degree of physical disturbance at this site as a result of wind-driven wave surge and turbulence, we sampled macroalgal biomass at "LKBR 2" located in the rubble zone of the back reef

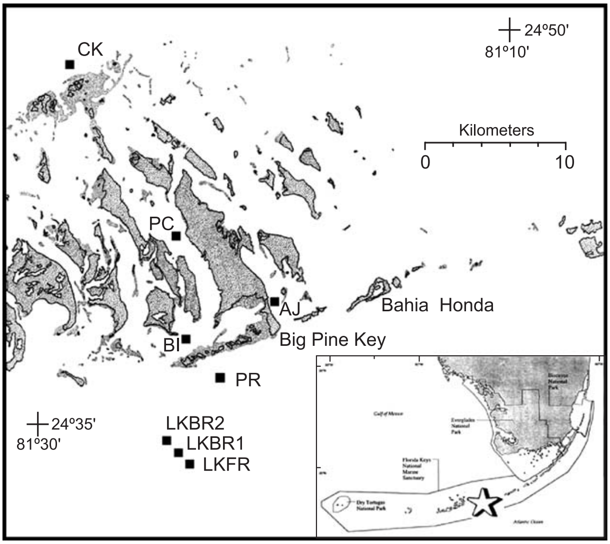

Fig. 1. Map of the Lower Florida Keys showing locations of the three monitoring stations (Avenue J, AJ; Patch Reef, PR; Looe Key Sanctuary Protection Area, LKBR1 and LKBR2) used in the Phase I study in 1996. Also shown are stations (Content Keys, CK; Pine Channel, PC; Bird Island, BI; Looe Key Fore Reef, LKFR) used in the Phase II study in 2000/2001.

where relatively high biomass of macroalgae occurs during summer months (Littler et al., 1986; Fig. 2A); (2) a nearshore station shoreward of Hawk Channel and ∼ 0.5 km southeast of Little Palm Island adjacent to a patch reef ("PR") formed by the lobate form of *Montastrea annularis* (Fig. 2B). During the past two decades these coral formations have experienced overgrowth by the calcareous chlorophyte *Halimeda* spp. as well as fleshy macroalgae and filamentous cyanobacteria; (3) an inshore station located ∼ 0.25 km off the east side of Big Pine Key in Spanish Harbor Channel adjacent to Avenue J ("AJ"). This site was downgradient of hundreds of septic tanks/cesspits (Fig. 2C) and contained seagrasses (*T. testudinum*, *Syringodium filiforme*, *Halodule wrightii*) with abundant macroalgae. At all sites, a 25×5 m study area was established by installing aluminum pins into the sediment and marking with subsurface buoys.

2.2. Collection and analysis of water samples for nutrients and chlorophyll *a*

Water samples were collected between January and September 1996 to determine seasonal variability in DIN, SRP, and chlorophyll *a* concentrations at the three stations. We collected water samples at high (daily) frequencies prior to, during, and following selected physical forcing events throughout the study to elucidate their role in nutrient enrichment of coastal waters. For example, samples were collected daily between 14 and 19 February as daily average wind speed increased from 5 to 15 knots and then subsided during a winter northeaster; again, in mid-July, we sampled as northeast winds built to ∼ 15 knots (July 14) and then subsided. Samples were collected between 15 and 20 March as a

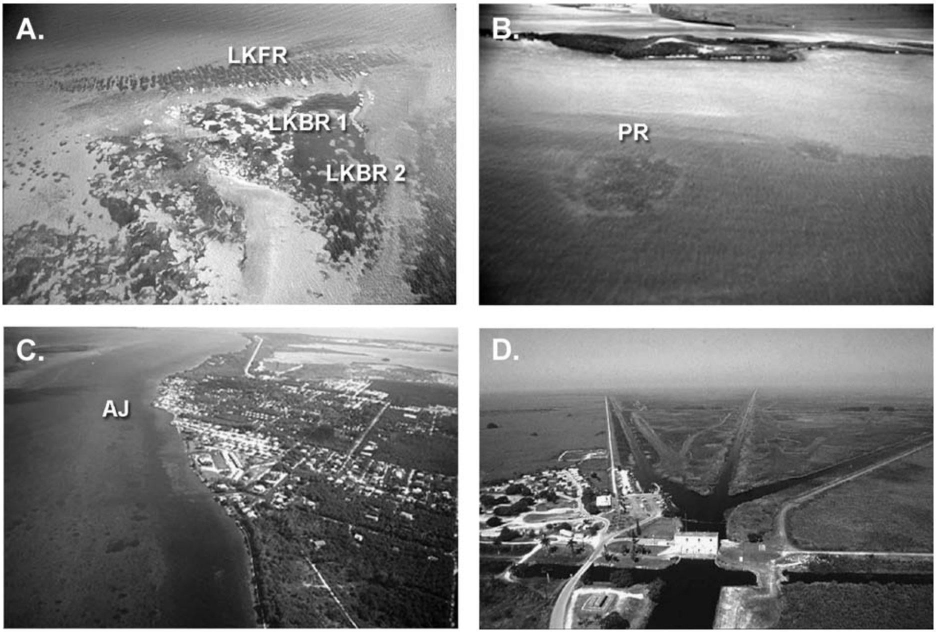

Fig. 2. Aerial photographs showing location of the Phase I sampling sites at Looe Key Sanctuary Protection Area (A), Patch Reef (PR, B), Avenue J (AJ, C) on Big Pine Key and the S-9 pumping structure on mainland South Florida (D, photo courtesy of the South Florida Water Management District) used to convey water southwards to Everglades National Park.

function of tidal stage ranging from a high tide on 15 March (+30 cm) to low tides on 18 March (−10 cm), 19 March (−13 cm), and 20 March (−14.1 cm). Samples were collected through four different rain events on 11 March (~25 mm), 15–25 May (~10 cm), 1–3 July (18.9 cm), and 23–25 September (9.5 cm). Rainfall was continuously monitored (Fig. 3) with a rainfall gauge at the Harbor Branch Oceanographic Institution (HBOI) field station on Big Pine Key during 1996 and by the National Key Deer Refuge on Big Pine Key in 2000/2001. Weather and tide data were obtained from the Key West National Weather Service Station as recorded in the National Climatic Database.

At each sampling for dissolved nutrients, near-bottom (0.15 m above bottom) water samples were collected in triplicate into clean 250 ml Nalgene bottles and held on ice in the dark until return to the HBOI field station on Big Pine Key where they were filtered through 0.45 μm GF/F filters and frozen until analysis. Within 28 days of collection, the samples were analyzed for NH_4^+–N, NO_3^-–N plus NO_2^-–N, NO_2^-–N, and PO_4^{3-}–P (SRP) on a Bran and Luebbe TRAACS Analytical Console at the HBOI Environmental Laboratory in Ft. Pierce, FL. The analytical detection limits were 0.08 μM for NH_4^+, 0.05 μM for NO_3^- plus NO_2^-, 0.003 for NO_2^- and 0.009 μM for SRP. The methods for collection, handling, and processing of the water samples for low-level nutrient analysis followed a quality assurance/quality control protocol designed to prevent problems associated with sample contamination and excessive holding times and to provide accurate and reliable data (Gunsalus, 1997). We used the *f*-ratio ($NO_3^-/NH_4^+ + NO_3^-$) to gauge the

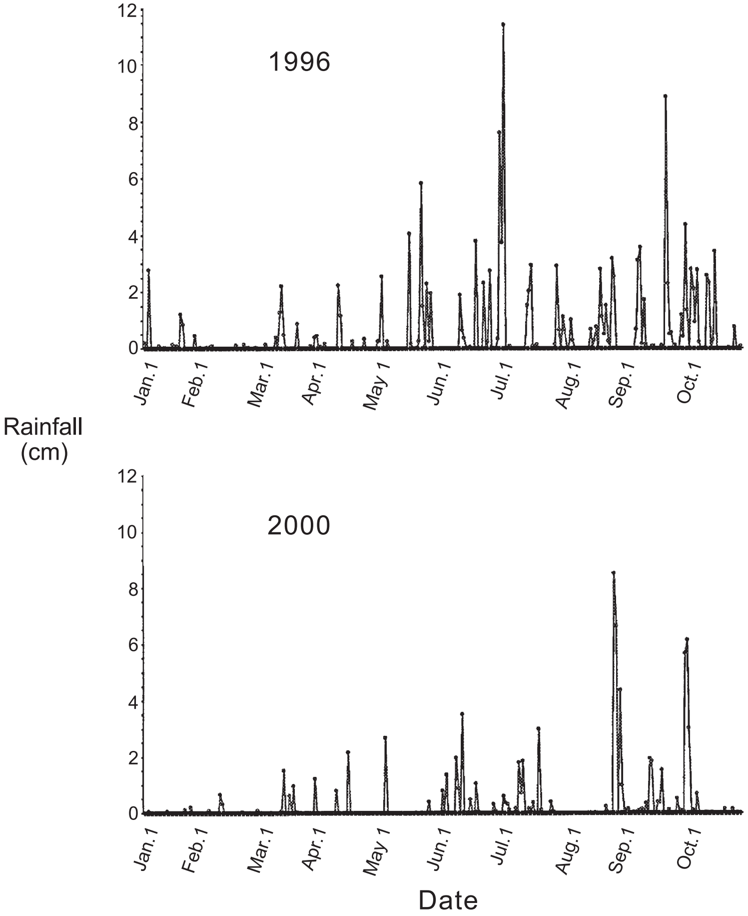

Fig. 3. Rainfall during 1996 (Phase I) and 2000 (Phase II).

potential importance of vertical mixing and offshore upwelling of NO_3^- versus NH_4^+ as a DIN source to algal growth at the three stations; values >0.8 indicate the presence of upwelling (Eppley and Peterson, 1979; Harrison et al., 1987).

For chlorophyll *a* analysis we collected 100 ml water samples in triplicate of near-bottom water that were filtered (after adding 1 mg of $MgCO_3$) through GF/F glass fiber filters and the filters frozen until extraction within 28 days in the laboratory of Dr. Larry Brand, Rosenstiel School of Marine and Atmospheric Science, University of Miami, FL. The filters were extracted for 30 min with 10 ml of dimethyl sulfoxide and then with an added 15 ml of 90% acetone at 5 °C overnight and measured fluorometrically before and after acidification for the measurement of chlorophyll *a* and phaeopigment concentrations (Burnison, 1980; Parsons et al., 1984). Fluorescence measurements were made using a Turner Designs 10-000R fluorometer equipped with a infrared-sensitive photomultiplier and calibrated using pure chlorophyll *a*.

2.3. Sampling biomass and δ^{15}N in macroalgae and seagrass epiphytes

Fleshy (non-calcareous) macroalgae and *Thalassia testudinum* epiphytes were sampled monthly for biomass and tissue δ^{15}N during Phase I. Biomass of macroalgae at the three stations was estimated by haphazard sampling ($n = 10$) with a 0.06 m^2 quadrat within each 125 m^2 study area (Lapointe et al., 1994). The macroalgae were returned to the HBOI field station on Big Pine Key and sorted, cleaned of sediment, epiphytes, identified, and wet weighed. Epiphytes attached to *T. testudinum* were also sampled and included macroalgae (e.g. the phaeophyte *Cladosiphon occidentalis*) and unidentified taxa of microfilamentous algae that were collectively removed by scraping the seagrass blade with a razor blade. Because calcareous epiphytes were only a minor component of the epiphytic community on *T. testudinum* at the three stations during this study, we quantified only the non-calcareous epiphyte loads. Samples of macroalgae, seagrass blade tissue, and epiphytes were processed as described in Lapointe et al. (1994) and dried in a Fisher Isotemp 200 Series laboratory oven (60 °C) to constant weight. Biomass of macroalgae is reported as g dry weight m^{-2} while attached epiphytes (microfilamentous epiphytes and attached macroalgae) are expressed as percent of blade weight to facilitate comparisons with previous studies.

Samples of macroalgae and epiphytes on *T. testudinum* were sampled monthly for δ^{15}N values to discriminate among the various local, regional, and natural sources of DIN potentially supporting their growth. This involved sampling the rhodophyte *Laurencia intricata* that was common to all three stations to allow intraspecific comparisons among the three stations. Samples of macroalgae and microfilamentous epiphytes on *T. testudinum* were obtained by scraping with a razor blade as described above. In addition to the monthly samples, the chlorophyte *Cladophora catenata* was also sampled at higher frequencies periodically throughout the Phase I study at LK to discriminate the nitrogen source of the NH_4^+ spikes that have previously been documented to follow seasonal rain events and decreased salinity at LK (Lapointe and Matzie, 1996). During each sampling for δ^{15}N values, at least five different thalli of *L. intricata*, *C. catenata* and blades of *T. testudinum* with attached epiphytes were collected from each study area and pooled into two replicate ($n = 2$) composite samples. The algal samples were rinsed briefly with deionized water (3–5 s rinse) to remove salt and debris and dried in a laboratory oven (60 °C) to constant weight. After drying, the samples were powdered using a mortar and pestle and analyzed for total N and ^{15}N atom % by Isotope Analytical Services, Los Alamos, New Mexico. The samples were processed through a Carlo-Erba N/A 1500 elemental analyzer using Dumas combustion and the purified nitrogen gas was then measured by a VG Isomass mass spectrometer. The standard used for stable nitrogen isotope analysis was N_2 in air. δ^{15}N values, expressed as ‰, were calculated as $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, with R equal to $^{15}N/^{14}N$ (Shearer and Kohl, 1993).

Phase II of our study was conducted between August 2000 and March 2001 and involved only the collection of macroalgae for δ^{15}N analysis. Sampling stations were located along a ~ 24 km transect extending from a patch reef (the “Rock Pile”) near the Content Keys and southwards through the Lower Florida Keys around Big Pine Key and offshore to LK (Fig. 1). The sampling stations included the Content Keys area (~ 3–6 m), South Pine Channel (2–3 m) in and around the Port Pine Heights subdivision on the northwest side of Big Pine

Key, a guano-rich Bird Island in Newfound Harbor on the southwest side of Big Pine Key (1–2 m), and two shallow stations at Looe Key, one in the back reef (LKBR 2, 3 m) and one on the shallow fore reef (LKFR, 7 m). The August 2000 sampling occurred during the summer wet season when significant Everglades runoff occurred (Abtew et al., 2002). However, Central and South Florida experienced a severe drought and water shortage in the spring of 2001 when the March sampling occurred. Consequently, significantly decreased water deliveries to Shark River Slough occurred prior to and during the March dry season sampling (Fig. 2D; Abtew et al., 2002). Hypothetically, these two different samplings should reflect variable anthropogenic nitrogen inputs from regional-scale agricultural runoff via Shark River Slough (Lapointe et al., 2002).

2.4. Statistical analysis

Statistical significance of variation in water quality (DIN, SRP, chlorophyll *a*, *f*-ratio), biomass of macroalgae and seagrass epiphytes, and δ^{15}N of macroalgae and seagrass epiphytes from the three stations during Phase I was analyzed with two-way ANOVA (location × time) using StatView™ and Statistica™ software. The seasonal data for these variables were pooled into dry season (January to mid-May) and wet season (late-May to October) groups for each station. A non-parametric Mann–Whitney *U*-test was also used to assess seasonal variability in the pooled nutrient data for the three stations. Values for δ^{15}N among the sites and over time during Phase I and Phase II were analyzed using two-way ANOVA (location × time), after homogeneity of treatment means was tested using an *F*-test. A Fisher's PLSD (planned least squared difference) multiple comparisons test was used to compare groups (e.g. sites) within main treatment effects of ANOVA. Additional comparisons of time series data were made using pairwise *t*-tests.

3. Results

3.1. Dissolved inorganic nutrients and chlorophyll a in the water column

Two-way ANOVA revealed that DIN concentrations during Phase I varied significantly with location ($F=40.23$, $P<0.0001$) and the location × season interaction ($F=6.25$, $P=0.0020$, Table 2). Overall, DIN concentrations decreased with increasing distance from shore among the three stations (Table 3). DIN in the dry season averaged 2.35 ± 1.61 μM ($n=64$) at AJ, 0.93 ± 0.46 μM ($n=25$) at PR and 0.83 ± 0.49 μM ($n=22$) at LK. In the wet season, DIN averaged 2.16 ± 1.48 μM ($n=78$) at AJ, 1.43 ± 0.96 μM ($n=63$) at PR and 1.04 ± 0.77 μM ($n=52$) at LK. DIN increased significantly from the dry season to the wet season at PR (Mann–Whitney, $P=0.002$) and LK (Mann–Whitney, $P=0.01$) but not at AJ. Maximum DIN concentrations were 8.50 μM at AJ, 5.77 μM at PR, and 5.59 μM at LK.

Like DIN, NH_4^+ concentrations during Phase I varied significantly with location ($F=40.23$, $P<0.00001$) but with marginally significant effects of the season × location interaction ($F=2.75$, $P=0.065$, Table 2). NH_4^+ was the predominant DIN species (~ 78% of DIN) at the wastewater-impacted AJ site where it did not vary significantly between the

Table 2
Two-way ANOVA for the effects of season (dry vs. wet) and location (inshore, nearshore, offshore) on ammonium, DIN, nitrate, SRP, *f*-ratio, chlorophyll *a*, δ^{15}N of *Laurencia intricata*, δ^{15}N of seagrass epiphytes, macroalgal biomass and epiphyte biomass in Phase I (1996) as well as δ^{15}N of all macroalgae in Phase II (2000/2001)

Effect	Sums of squares	*df*	Mean square	*F*	*P*
Ammonium					
Season	0.99	1	0.99	0.94	0.333
Location	113.1	2	56.54	53.46	**< 0.00001**
Season × location	5.82	2	2.91	2.75	0.065
Within	370.2	350	1.05		
DIN					
Season	1.25	1	1.25	0.90	0.3420
Location	112	2	55.83	40.23	**< 0.0001**
Season × location	17.34	2	8.67	6.25	**0.0020**
Within	491.3	354	1.387		
Nitrate					
Season	0.14	1	0.14	0.97	0.3232
Location	1.40	2	0.70	4.86	**0.0008**
Season × location	3.28	2	1.64	11.39	**< 0.00001**
Within	50.98	353	0.14		
SRP					
Season	0.004	1	0.00429	2.67	0.1026
Location	0.062	2	0.03106	19.35	**< 0.00001**
Season × location	0.007	2	0.00374	2.33	0.09860
Within	0.568	354	0.00160		
f-ratio					
Season	0.41	1	0.41	11.23	**0.00091**
Location	8.068	2	4.034	110.53	**< 0.00001**
Season × location	0.036	2	0.0182	0.49	0.6079
Within	10.87	298	0.0365		
Chlorophyll a					
Season	44.14	1	44.14	29.74	**< 0.00001**
Location	61.64	2	30.82	20.76	**< 0.00001**
Season × location	40.80	2	20.4	13.74	**< 0.00001**
Within	369.6	249	1.48		
δ^{15}N Laurencia					
Season	0.277	1	0.277	0.35	0.55
Location	31.67	2	15.84	20.0	**< 0.0001**
Season × location	8.51	2	4.25	5.37	**0.0008**
Within	34.85	44			
δ^{15}N epiphytes					
Season	0.033	1	0.033	0.045	0.834
Location	6.478	2	3.239	4.413	**0.017**
Season × location	2.649	2	1.324	1.804	0.176
Within	35.24	48	0.734		

Table 2 (*continued*)

Effect	Sums of squares	*df*	Mean square	*F*	*P*
Macroalgal Biomass					
Season	1794	1	1794	92.86	**< 0.00001**
Location	545.4	2	272.7	14.11	**< 0.00001**
Season × location	675.6	2	337.8	17.48	**< 0.00001**
Within	5101	264	19.32		
Epiphyte Biomass					
Season	0.0045	1	0.0045	0.013	0.91
Location	5.44	2	2.72	7.94	**0.00224**
Season × location	0.134	2	0.067	0.196	0.823
Within	8.213	24	0.342		
$\delta^{15}N$ All Macroalgae					
Location	24.92	4	6.23	5.073	**0.0016**
Season	137.502	1	137.502	111.966	**< 0.0001**
Site × Season	20.151	4	5.038	4.102	**0.0057**
Within	65.088	53	1.228		

Significant effects ($P < 0.05$) in bold.

dry and wet seasons—averaging 1.64 ± 1.41 μM ($n = 64$) in the dry season and 1.68 ± 1.38 μM ($n = 78$) in the wet season (Table 3). In contrast, NH_4^+ concentrations at PR increased significantly (Mann–Whitney, $P = 0.01$) from a mean of 0.26 ± 0.13 μM ($n = 25$) in the dry season to 0.62 ± 0.52 μM ($n = 63$) in the wet season, which was accompanied by a parallel decrease in the *f*-ratio from the dry to the wet season (Table 3). Similarly, NH_4^+ increased significantly (Mann–Whitney, $P = 0.04$) at LK from a mean of 0.26 ± 0.14 μM ($n = 22$) in the dry season to 0.44 ± 0.31 μM ($n = 52$) in the wet season, also with a parallel decrease in the *f*-ratio in the summer wet season (Table 3). Maximum NH_4^+ concentrations during the study were 7.95 μM at AJ, 3.96 μM at PR, and 4.42 μM at LK.

Table 3

Mean concentrations (μM) ± 1 SD and sample size (*n*) for ammonium, nitrate, DIN, SRP, DIN:SRP ratio, *f*-ratio, and chlorophyll *a* at the inshore (AJ), nearshore (PR) and offshore (LK) stations in the dry (Januray–April) and wet (May–September) seasons of 1996 in the Lower Florida Keys.

Season	Location	Ammonium	Nitrate	DIN	SRP	DIN/ SRP	*f*-ratio	Chlorophyll *a*
Dry	AJ	1.64 ± 1.41 (64)	0.64 ± 0.50 (64)	2.35 ± 1.61 (64)	0.058 ± 0.049 (64)	40.5	0.32 ± 0.19 (64)	0.62 ± 0.78 (33)
	PR	0.26 ± 0.13 (25)	0.65 ± 0.38 (25)	0.93 ± 0.46 (25)	0.042 ± 0.035 (25)	22.1	0.68 ± 0.18 (25)	0.42 ± 0.30 (33)
	LK	0.26 ± 0.14 (22)	0.56 ± 0.45 (22)	0.83 ± 0.49 (22)	0.051 ± 0.034 (22)	16.3	0.60 ± 0.23 (22)	0.40 ± 0.22 (33)
Wet	AJ	1.68 ± 1.38 (78)	0.34 ± 0.19 (78)	2.16 ± 1.48 (78)	0.069 ± 0.049 (78)	31.3	0.21 ± 0.12 (78)	2.62 ± 2.3 (54)
	PR	0.62 ± 0.52 (63)	0.70 ± 0.41 (63)	1.43 ± 0.96 (63)	0.033 ± 0.027 (63)	43.3	0.59 ± 0.21 (63)	0.64 ± 0.55 (53)
	LK	0.44 ± 0.31 (52)	0.54 ± 0.37 (52)	1.04 ± 0.77 (52)	0.027 ± 0.027 (52)	38.5	0.55 ± 0.24 (52)	0.73 ± 0.53 (50)

NO_3^- concentrations during the Phase I study were generally lower than those of NH_4^+ and varied significantly with location ($F=4.86$, $P=0.0008$) and the season × location interaction ($F=11.39$, $P<0.00001$, Table 2). NO_3^- concentrations in the dry season averaged 0.64 ± 0.50 μM ($n=64$) at AJ, 0.65 ± 0.46 μM ($n=25$) at PR and 0.56 ± 0.45 μM ($n=22$) at LK (Table 3). In the wet season, NO_3^- concentrations averaged 0.34 ± 0.19 μM ($n=78$) at AJ, 0.70 ± 0.41 μM ($n=63$) at PR and 0.54 ± 0.37 μM ($n=52$) at LK. Unlike NH_4^+, there were no significant increases in NO_3^- concentrations from the dry to wet season at any of the three stations. Maximum NO_3^- concentrations were 2.19 μM at AJ, 1.63 μM at PR, and 1.58 μM at LK. Compared to NH_4^+, NO_3^- was a minor component of the DIN pool at AJ (*f*-ratio < 0.32) but relatively more important at the more offshore PR (*f*-ratio of 0.68 and 0.59) and LK (*f*-ratio of 0.60 and 0.55) stations. However, the maximum NO_3^- concentration during the summer wet season at LK was 1.41 μM, nearly three-fold less than that of NH_4^+ (4.42 μM).

Elevated DIN concentrations followed high winds, rain, and low tides throughout the study. For example, DIN (primarily NH_4^+) increased at AJ from ~ 1.0 to 4.0 μM as strong northeasterly winds developed between February 15 and 19, 1996 (Fig. 4). The highest DIN concentration measured during the entire study, 8.50 μM (7.95 μM NH_4^+) occurred at AJ during a low tide sampling on March 19 (Fig. 4). Increased DIN followed rain events between May 15 to 24, on July 1, and on September 23, 1996. Peak DIN concentrations at both PR and LK coincided with high northeasterly winds on July 14 (Fig. 4). Increases in DIN also occurred at PR and LK following rain events on May 24, July 1, and September 23, 1996. In contrast to the inshore station AJ, higher DIN concentrations (mostly NH_4^+) occurred at the more offshore PR and LK in the summer following the onset of the rainy season in mid-May (Fig. 4, Table 3).

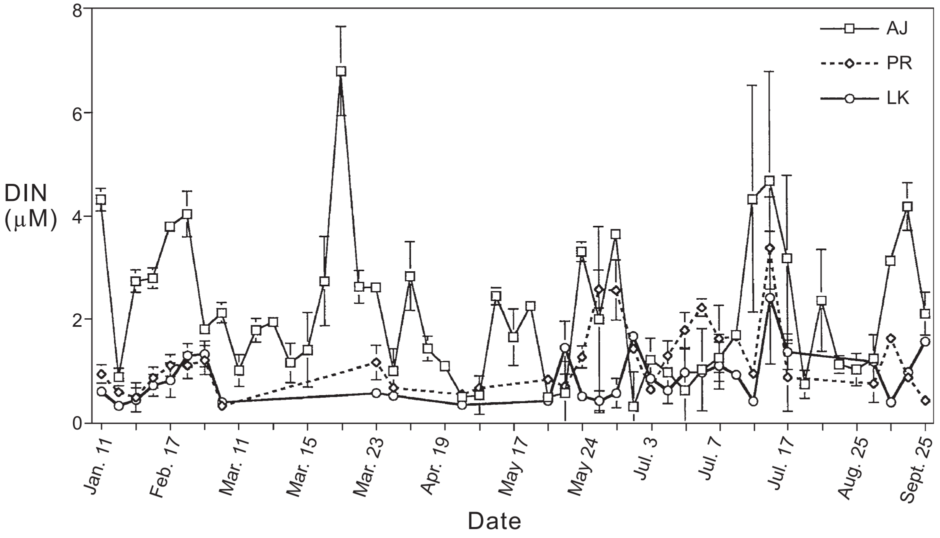

Fig. 4. Dissolved inorganic nitrogen concentrations ($DIN=NH_4^++NO_3^-+NO_2^-$, in μM) at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) stations during the Phase I study in 1996. Values represent mean ± SE ($n=3$).

SRP concentrations during the Phase I study varied significantly with location ($F = 19.35$, $P < 0.00001$, Table 2) and were highest at AJ and decreased at the more offshore PR and LK stations (Table 3, Fig. 5). Concentrations of SRP were relatively low and displayed less variability compared to DIN. In the dry season, SRP concentrations averaged 0.058 ± 0.049 μM ($n = 64$) at AJ, 0.042 ± 0.035 μM ($n = 25$) at PR, and 0.051 ± 0.034 μM ($n = 22$) at LK; in the wet season, SRP concentrations averaged 0.069 ± 0.049 μM ($n = 78$) at AJ, 0.033 ± 0.027 μM ($n = 63$) at PR, and 0.027 ± 0.027 μM ($n = 52$) at LK (Table 3). The only significant seasonal change was a decrease in SRP from the dry to the wet season at LK (Mann–Whitney, $P = 0.02$). The highest SRP concentrations at LK were associated with the northeast wind event between February 15 and 19 (Fig. 5).

Because of significant seasonal changes in NH_4^+ and/or SRP of the water column, the DIN/SRP ratio also varied seasonally at the three stations. During the dry season, the DIN:SRP ratio decreased from a maximum of 40.5 at AJ to 22.1 at PR and 16.3 at LK (Table 3). In the wet season, a different spatial pattern occurred where the DIN:SRP ratio ranged from higher values of 43.3 at PR and 38.5 at LK to a lower value of 31.3 at AJ (Table 3).

Chlorophyll *a* concentrations during the Phase I study varied significantly with season ($F = 29.74$, $P < 0.00001$), location ($F = 20.76$, $P = 0.00001$) and the season × location interaction ($F = 13.74$, $P < 0.00001$, Table 2). Chlorophyll *a* generally decreased with increasing distance from shore and was higher during the summer wet season compared to the dry season at all stations (Table 3, Fig. 6). This seasonal effect was most pronounced at the inshore station AJ where the mean chlorophyll *a* of 0.62 μg/l ($n = 33$) in the dry season increased ~ four-fold to 2.62 μg/l ($n = 54$) in the wet season. At PR, chlorophyll *a* increased from a mean of 0.41 μg/l ($n = 33$) in the dry season to 0.64 μg/l ($n = 53$) in the wet season. At LK, chlorophyll *a* increased from a mean of 0.40 μg/l ($n = 33$) in the dry season to 0.73 μg/l ($n = 50$) in the wet season (Table 3). At AJ, chlorophyll *a* increased to

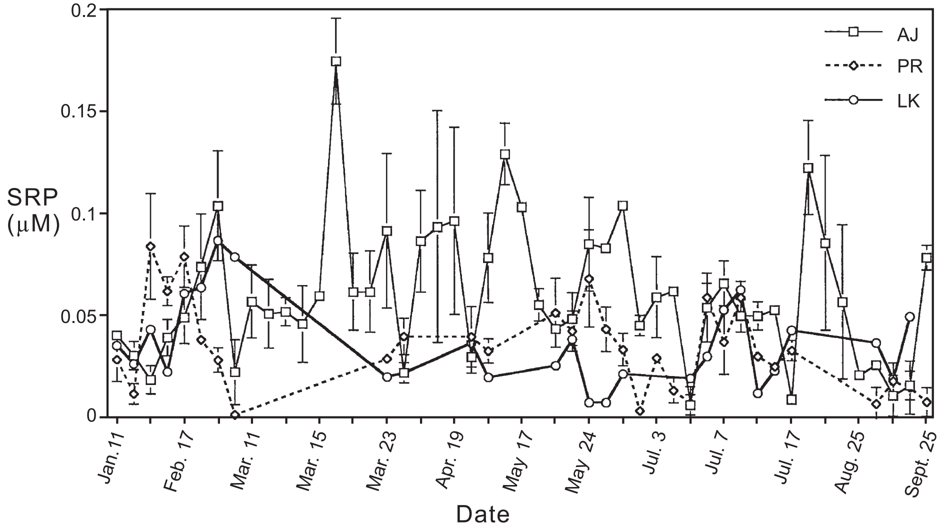

Fig. 5. Soluble reactive phosphorus concentrations (SRP, in μM) at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) stations during the Phase I study in 1996. Values represent mean ± SE ($n = 3$).

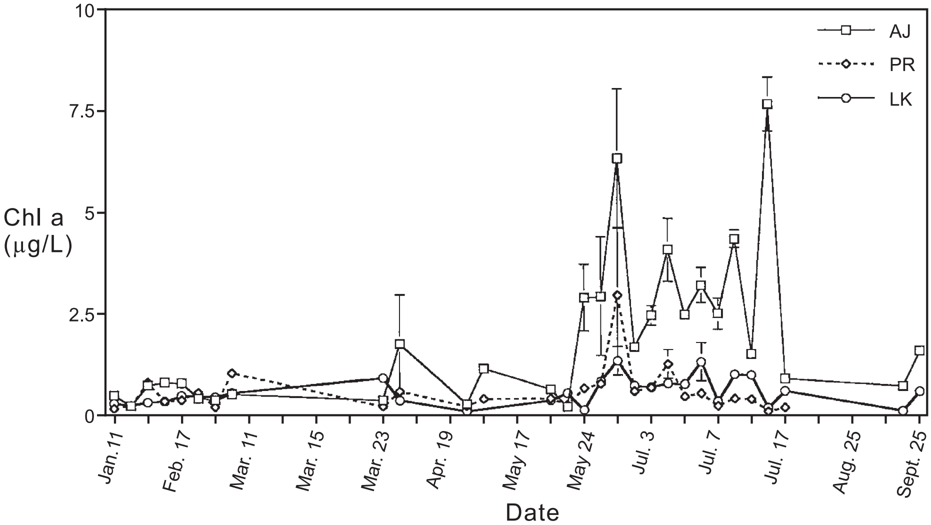

Fig. 6. Chlorophyll *a* concentrations (chl *a*, in µg/l) at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) stations during the Phase I study in 1996. Values represent mean ± SE ($n = 3$).

high values (>5.0 µg/l) on 24 May following several days of intense rainfall (>17.5 cm rain between 15 and 25 May, Fig. 6). A similar pattern was observed at PR where the highest chlorophyll *a* concentrations occurred in late May following heavy rain (Fig. 6).

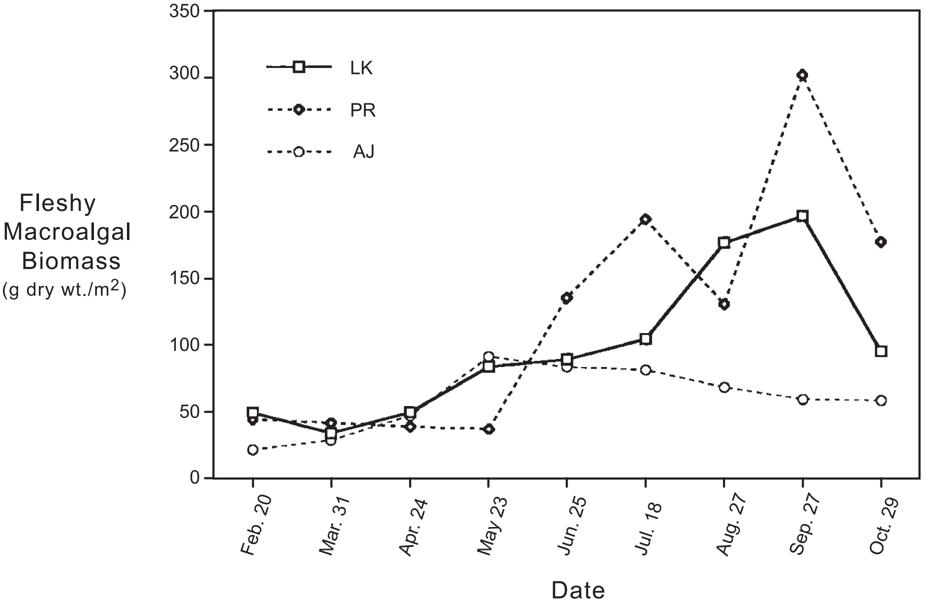

Fig. 7. Monthly mean ($n = 10$) biomass of fleshy macroalgae at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) stations during the Phase I study in 1996.

The seasonal pattern in chlorophyll *a* at LK was similar to that at AJ, increasing in late May following the onset of the rainy season (Fig. 6).

3.2. Biomass of macroalgae and seagrass epiphyte loads

Two-way ANOVA revealed that biomass of macroalgae varied significantly with season ($F=92.86$, $P<0.00001$), location ($F=14.11$, $P=0.00001$), and the season × location interaction ($F=17.48$, $P<0.00001$, Table 2). Macroalgal biomass increased from the dry, winter season into the summer wet season at all three stations (Fig. 7). This "spring–summer bloom" of macroalgae occurred first at AJ when seasonal low values of 25 ± 5 g dry wt m^{-2} in February/March increased to peak values of 87 ± 5 g dry wt m^{-2} in May/June following the onset of the rainy season (Fig. 7). During February and March, the rhodophytes *Laurencia intricata* and *Heterosiphonia wurdmanni* were abundant at AJ and were followed by blooms of the chlorophytes *Cladophora crispula* and *Chaetomorpha gracilis* in late April and May. The rhizomatous chlorophyte *Caulerpa sertulariodes* formed extensive patches in June following heavy rainfall and was followed by blooms of the chlorophyte *Cladophora montagneana* and the cyanobacterium *Lyngbya majuscula* in August and September, respectively.

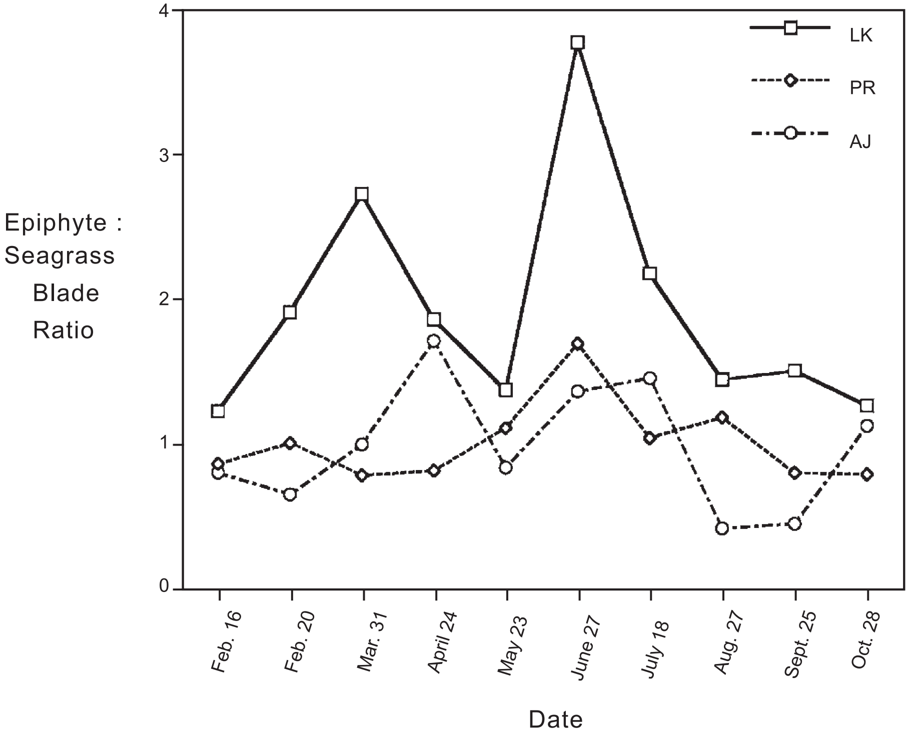

Fig. 8. Monthly mean ($n=10$) epiphyte:seagrass blade dry weight ratio for *Thalassia testudinum* at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) stations during the Phase I study in 1996.

Biomass of macroalgae at PR averaged 40 ± 3 g dry wt m^{-2} between February and May and increased to a mean value of 201 ± 72 g dry wt m^{-2} in July through September following the onset of rain in late May (Fig. 7). Between February and May, the rhodophytes *L. intricata* and *H. wurdmanni* were abundant and blooms of *Ceramium nitens* appeared as an epiphyte on soft corals in late April. Widespread blooms of the cyanobacterium *Lyngbya gracilis* (with *Lyngbya rivularianum* as an epiphyte) developed in June on seagrasses, hard corals, soft corals, and macroalgae.

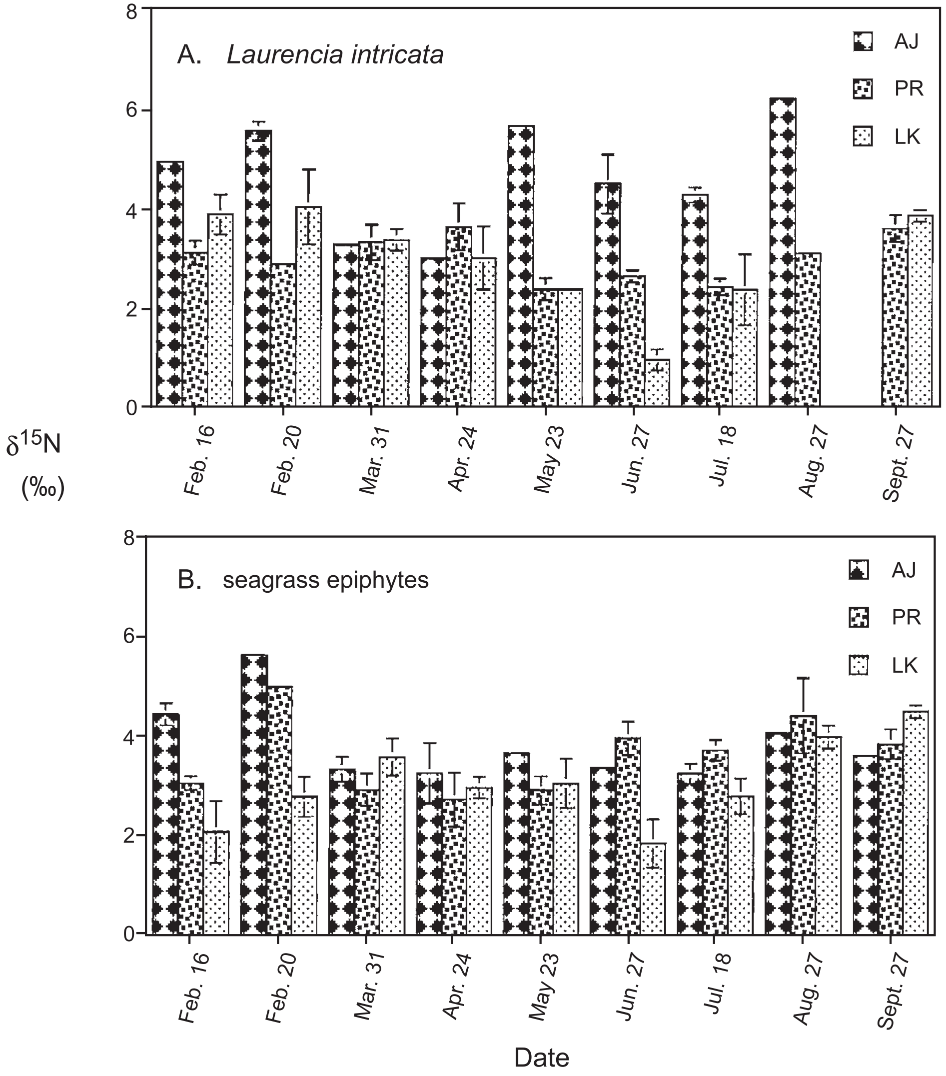

Fig. 9. $\delta^{15}N$ values of *Laurencia intricata* (A) and epiphytes on *Thalassia testudinum* (B) at Avenue J (AJ), Patch Reef (PR), and Looe Key (LK) during the Phase I study in 1996. Values represent mean ± 1 SD ($n = 2$).

Biomass of macroalgae at LK averaged 44 ± 9 g dry wt m^{-2} between February and April and began increasing in May to a mean of 187 ± 14 g dry wt m^{-2} in August/September (Fig. 7). Between February and May, the rhodophyte *L. intricata* and the chlorophyte *Cladophora catenata* were abundant; blooms of the chlorophyte *Cladophora crispata* developed as an epiphyte on sponges, seagrasses, and soft corals between late March and July. Blooms of the cyanobacterium *Lyngbya gracilis* covered much of the benthic biota in June, which was accompanied by blooms of *Lyngbya meneghinana* and *Lyngbya semiplena* in August.

Epiphyte biomass on *Thalassia testudinum* varied significantly with location ($F=7.94$, $P=0.00224$, Table 2) and was generally high (epiphyte:blade ratio>0.50) at all three stations—especially LK. Over the entire Phase I study, the epiphyte/seagrass blade ratio averaged 0.92 ± 0.43 at AJ, 0.94 ± 0.28 at PR, and 1.85 ± 0.81 at LK. The highest epiphyte loads at AJ occurred in April while those at PR and LK occurred in June (Fig. 8). The epiphyte loads at LK were significantly higher than those at PR ($t=-4.309$, $P=0.001$) and AJ ($t=-3.937$, $P=0.003$). The epiphytic algal community at LK between April and June included the phaeophytes *Cladosiphon occidentalis* and *Lophosiphonia saccorhiza*, the rhodophytes *Chondria* sp., *Ceramium fastigatum*, and *Griffithsia* sp., and the cyanobacterium *Lyngbya gracilis*. Following a dense bloom of the chlorophyte *C. montagneana* in September and October at AJ, we observed an extensive die-off of *T. testudinum* manatee grass, *Syringodium filiforme*.

3.3. $\delta^{15}N$ of macroalgae and seagrass epiphytes

The $\delta^{15}N$ values of *Laurencia intricata* during the Phase I study varied significantly with location ($F=20.0$, $P<0.0001$) and the season × location interaction ($F=5.37$,

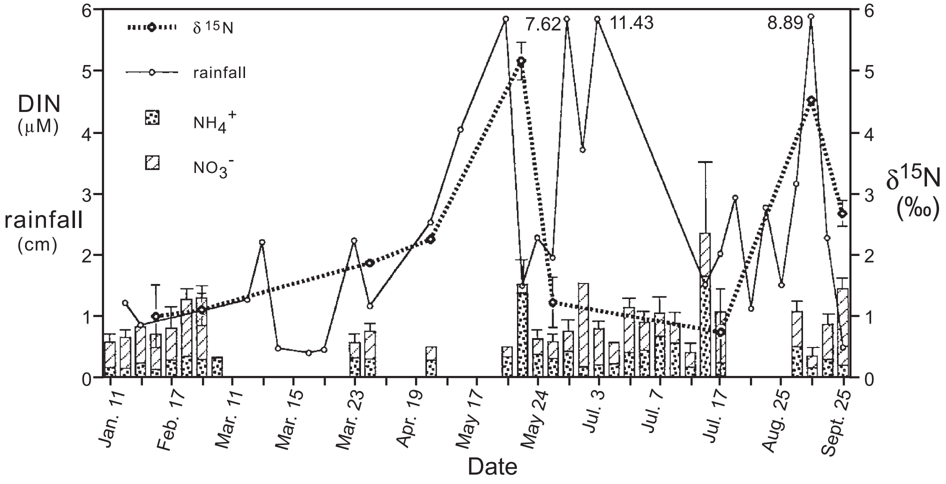

Fig. 10. Dissolved inorganic nitrogen concentrations ($DIN=NH_4^+ + NO_3^- + NO_2^-$, in μM) and $\delta^{15}N$ of *Cladophora catenata* (black dotted line) at Looe Key during the Phase I (1996) study. For DIN, values represent mean ± 1 SD ($n=3$); for $\delta^{15}N$, values represent mean ± 1 SD ($n=2$). Rainfall (cm) recorded on Big Pine Key during the period of record is also shown.

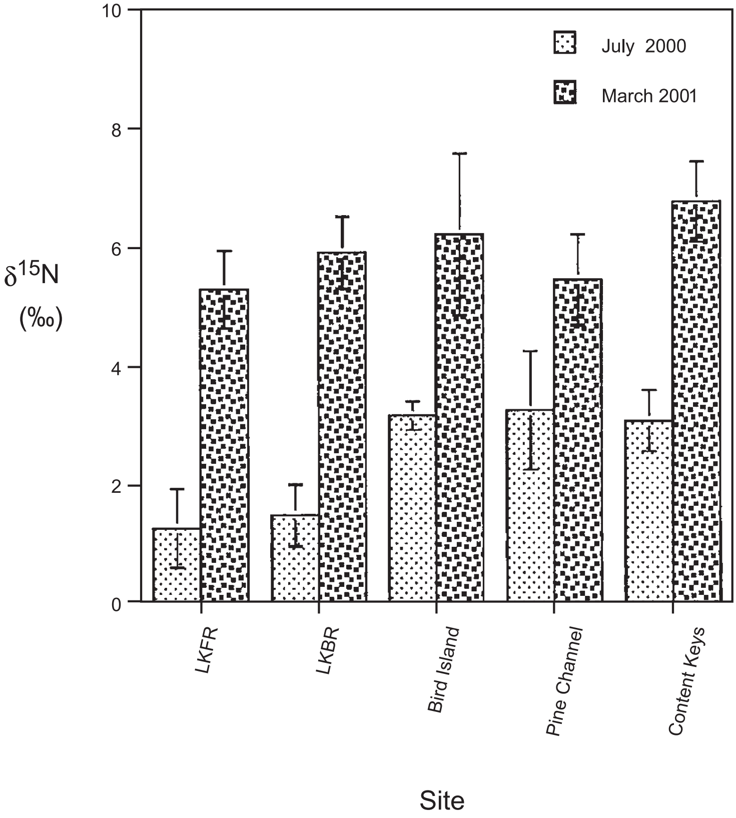

Fig. 11. Pooled δ^{15}N values of macroalgae in the wet (July 2000) and dry (March 2001) seasons of the Phase II study. Values represent mean ± 1 SD ($6 < n < 18$).

$P = 0.0008$, Table 2, Fig. 9A). Overall, the mean δ^{15}N of *L. intricata* was significantly (Fisher's PLSD, $P < 0.00001$) higher at AJ (+4.69 ± 1.14‰) compared to lower and statistically similar (Fisher's PLSD, $P = 0.746$) values at PR (+3.03 ± 0.46‰) and LK (+3.00 ± 1.04‰). At LK, the δ^{15}N values of the chlorophyte *Cladophora catenata* increased following rainfall and DIN (primarily NH_4^+) spikes in May and following a wind event and rainfall in mid-July. Between April 24 and May 23, the δ^{15}N values of *C. catenata* increased from < +3.0‰ to > +5.00‰ following the onset of heavy rain and increases in DIN at LK; the δ^{15}N values increased again on July 14 from values < +1.00‰ to > +5.00‰ following high northeast winds and DIN enrichment (Fig. 10).

The δ^{15}N values of blade epiphytes on *T. testudinum* during the Phase I study varied significantly with location ($F = 4.41$, $P = 0.017$, Table 2, Fig. 9B). Overall, the blade epiphyte δ^{15}N values were highest at AJ (averaged +4.20 ± 1.10‰ in the dry season and

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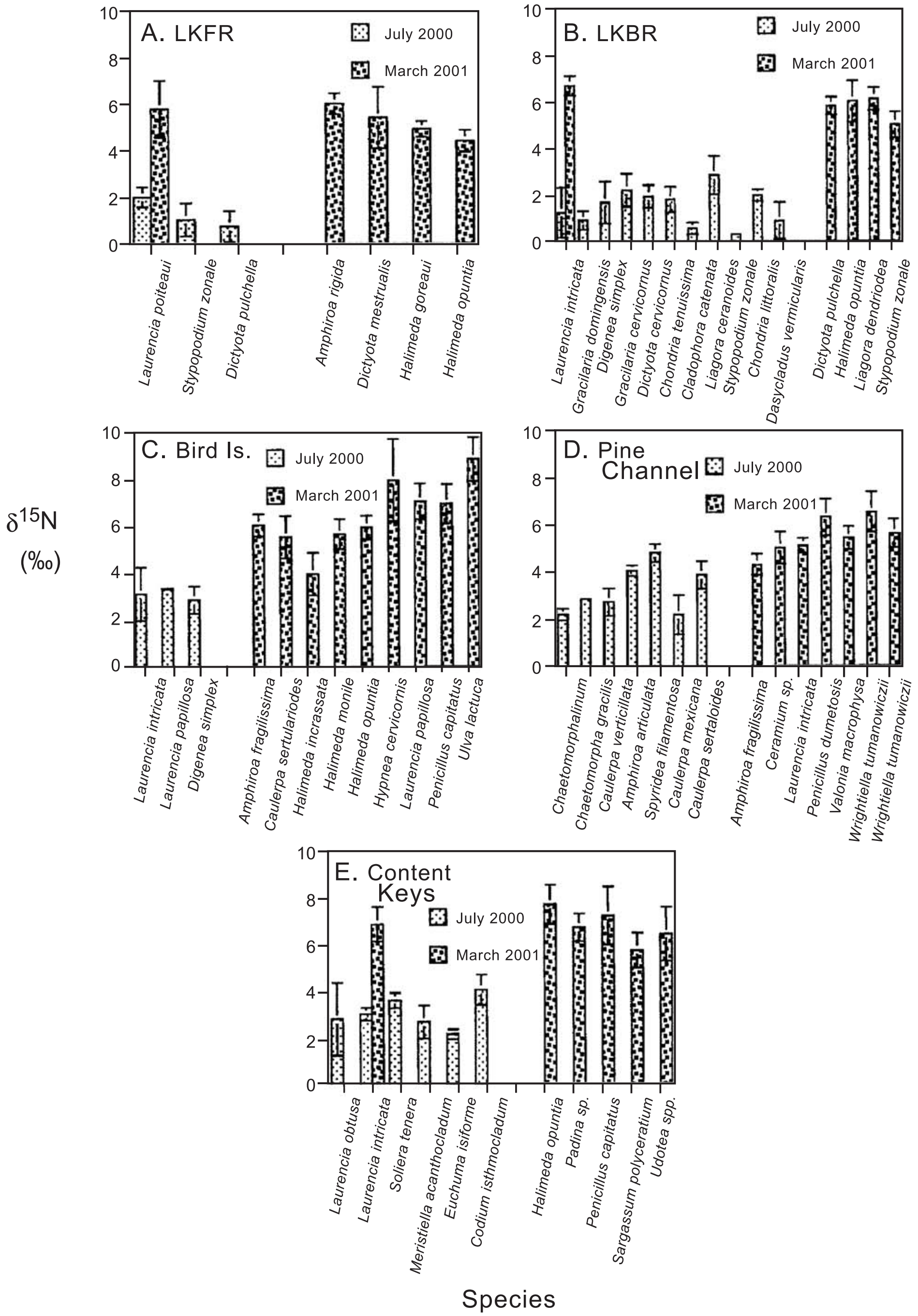
Fig. 12. Species-specific δ^{15}N values of macroalgae at Looe Key Fore Reef (LKFR), Looe Key Back Reef (LKBR), Bird Island, Pine Channel, and the Content Keys in the wet (July 2000) and dry (March 2001) seasons in the Phase II study. Values represent mean ± 1 SD ($n = 2$).


A. LKFR
July 2000
March 2001
10
8
6
4
2
0
Laurencia poiteaui
Stypopodium zonale
Dictyota pulchella
Amphiroa rigida
Dictyota mestrualis
Halimeda goreaui
Halimeda opuntia
B. LKBR
July 2000
March 2001
Laurencia intricata
Gracilaria domingensis
Digenea simplex
Gracilaria cervicornus
Dictyota cervicornus
Chondria tenuissima
Cladophora catenata
Liagora ceranoides
Stypopodium zonale
Chondria littoralis
Dasycladus vermicularis
Dictyota pulchella
Halimeda opuntia
Liagora dendriodea
Stypopodium zonale
C. Bird Is.
July 2000
March 2001
Laurencia intricata
Laurencia papillosa
Digenea simplex
Amphiroa fragilissima
Caulerpa sertulariodes
Halimeda incrassata
Halimeda monile
Halimeda opuntia
Hypnea cervicornis
Laurencia papillosa
Penicillus capitatus
Ulva lactuca
D. Pine Channel
July 2000
March 2001
Chaetomorphalinum
Chaetomorpha gracilis
Caulerpa verticillata
Amphiroa articulata
Spyridea filamentosa
Caulerpa mexicana
Caulerpa sertaloides
Amphiroa fragilissima
Ceramium sp.
Laurencia intricata
Penicillus dumetosis
Valonia macrophysa
Wrightiella tumanowiczii
Wrightiella tumanowiczii
E. Content Keys
July 2000
March 2001
Laurencia obtusa
Laurencia intricata
Soliera tenera
Meristiella acanthocladum
Euchuma isiforme
Codium isthmocladum
Halimeda opuntia
Padina sp.
Penicillus capitatus
Sargassum polyceratium
Udotea spp.
δ15N (‰)
Species

+3.62 ± 0.32‰ in the wet season), intermediate at PR (averaged +3.45 ± 1.04‰ in the dry season and +3.80 ± 0.69‰ in the wet season), and lowest at LK (averaged +2.88 ± 0.74‰ in the dry season and +3.26‰ the wet season). The epiphyte δ^{15}N values at AJ were statistically similar to PR (Fisher's PLSD, $P = 0.42$) but significantly higher than values at LK ($P = 0.008$). In addition, δ^{15}N values at PR were marginally higher (Fisher' s PLSD, $P = 0.058$) than those at LK.

In the Phase II study (2000/2001), the δ^{15}N values of macroalgal communities sampled between the Content Keys (Gulf of Mexico) and LK varied significantly with season, location, and the season × location interaction (Table 2). In the July 2000 wet season sampling, the mean δ^{15}N values at the three inshore sites (Bird Island, Pine Channel, and the Content Keys) were within the range of sewage nitrogen (> +3‰) compared to lower values (< +2‰) at the back reef and fore reef stations at LK; macroalgae along the entire transect from the Content Keys to LK had an overall mean δ^{15}N of +2.46 ± 0.97‰ (Fig. 11). In the March 2001 dry season sampling, the mean δ^{15}N values were > +5‰ at all stations sampled along the transect with an overall mean of +5.93 ± 0.59‰ (Fig. 11). Species-specific differences in the δ^{15}N values of macroalgae sampled during the Phase II study showed little variability compared to the effects of "wet" versus "dry" seasonality (Fig. 12).

4. Discussion

4.1. Land-based nutrient enrichment extends to offshore bank reefs

Our results are consistent with previous studies that used time-series measurements of salinity, temperature, and nutrients to link land-based runoff with phytoplankton blooms and hypoxia at Looe Key following the transition from the dry to wet season in 1992 (Lapointe and Matzie, 1996). The impact of land-based nutrient pollution on the offshore bank reefs of the FKNMS was not recognized by Szmant and Forrester (1996) who concluded that anthropogenic nutrients entering coastal waters of the Florida Keys do not reach either nearshore patch reefs or the offshore bank reefs. Szmant and Forrester (1996) stated for the Lower Keys ("Looe Key area") that "these areas had elevated inshore concentrations of inorganic and organic nutrients and chlorophyll, especially in areas with marinas and developed canals, but concentrations dropped to oligotrophic concentrations within a km of shore (inshore of Hawk Channel)". They also reported that "the steepness of the (nutrient) gradient suggests that these inshore sources are not being transported offshore in measurable quantity" and that nutrients entering coastal waters of the Keys from land-based sources "appear to be taken up by nearshore algal and seagrass communities before they reach reef areas".

To the contrary, our data from the Phase I study showed that mean DIN and SRP concentrations throughout the Lower Keys study area were quite high and exceeded values reported for unimpacted and biologically diverse coral reef communities. All three stations had overall mean DIN concentrations (2.10 μM at AJ; 1.18 μM at PR; 0.87 μM at LK) that were above or similar to threshold concentrations noted for the demise of coral reefs from eutrophication (~ 1 μM, Bell, 1992) and macroalgal blooms (Lapointe, 1997; Lapointe, 1999). The DIN pool at PR and LK was enriched by NH_4^+ rather than NO_3^- in the summer wet season, a phenomenon previously documented to result from stormwater runoff that enhances the transport of NH_4^+-rich sewage effluent into nearshore (Lapointe et al., 1990) and offshore (Lapointe and Matzie, 1996) waters. In contrast to the human-impacted coastal waters of the Florida Keys, DIN concentrations in the naturally enriched, localized waters of a mangrove channel on uninhabited Norman's Pond Cay, Exumas, Bahamas, averaged ~ 1.0 μM but decreased to concentrations < 0.5 μM within 100 m of shore, reaching relatively oligotrophic concentrations of < 0.3 μM at Rainbow Reef and Lang's Reef (Lapointe et al., 2004). For comparison, waters on the outer Great Barrier Reef of Australia have median DIN concentrations of ~ 0.1 μM (Furnas et al., 1997), oligotrophic conditions that support a diverse assemblage of hermatypic (reef-forming) corals. The pattern of decreasing NH_4^+ concentrations in the water column with increasing distance from shore and elevated mean and maximal NH_4^+ concentrations at LK during the summer wet season supports the hypothesis that land-based nutrient enrichment from sewage is contributing to eutrophication not only in the inshore and nearshore waters, but offshore waters as well (Lapointe and Clark, 1992; Lapointe and Matzie, 1996). While the EPA WQPP recognized that inshore and nearshore waters of the Keys are impacted by human-derived nutrients from sewage, they also reported that "no definitive studies on the geographic extent of the impact of anthropogenic nutrients have been conducted" (Kruczynski and McManus, 2002).

Previous studies have suggested that natural upwelling of NO_3^- has supported the recent phytoplankton blooms and proliferation of benthic macroalgae in nearshore as well as offshore waters of the Florida Reef Tract. Szmant and Forrester (1996) stated that offshore upwelling is "a major and persistent natural nutrient source" to coastal waters of the Florida Keys but provided no data demonstrating persistently elevated NO_3^- concentrations or reduced temperatures in either nearshore or offshore waters. It is unlikely that major, persistent upwelling would support the development of the biologically rich and diverse hermatypic coral assemblages that historically occurred at LK. High nutrient loadings associated with upwelling typically favor algal rather than coral reefs, such as the *Halimeda* bioherms that occur where the Caribbean current persistently upwells onto carbonate platforms of the Nicaraguan Rise (Roberts et al., 1992). However, weak and highly episodic upwelling events can occur in deep, offshore waters of the Florida Reef Tract when wind forcing is favorable for offshore transport of surface waters. For example, Lapointe and Smith (1987) first documented such an anomalous upwelling event following several days of ~15–20 knot westerly winds at LK in July 1985. Although this event reduced temperatures from 30 to 21 °C at 30 m and from ~30 to 27 °C at 10 m seaward of the "spur-and groove" zone at LK, only minor enrichment with SRP (< 0.14 μM), and little, if any, NO_3^- enrichment (< 0.31 μM) occurred at either 10 m or 30 m depths during this significant cold water event. More recently, a 5-year study by Leichter et al. (2003) on shallow bank reefs (10–30 m depths) from Key Largo to Key West found no persistent low temperatures (< 20 °C) or high NO_3^- concentrations (> 4 μM) that should occur if upwelling was a major NO_3^- source to the Florida Reef Tract as reported for outer shelf waters in the South Atlantic Bight and the east coast of Florida (Green, 1944; Smith, 1982; Atkinson et al., 1984). The high variability in the temperature/NO_3^- data reported by Leichter et al. (2003) suggest that mechanisms other than upwelling, such as offshore advection of enriched nearshore waters and/or SGD of DIN (Simmons, 1992) may be more important mechanisms contributing to the elevation of NO_3^- and NH_4^+ concentrations on these shallow bank reef communities. In addition, high rates of nitrification by cyanobacterial symbionts are a common feature of coral reef sponges and can result in localized NO_3^- enrichment (Corredor et al., 1988).

The relatively low *f*-ratio values we measured at all three stations in Phase I, especially during the summer, also do not support the hypothesis that upwelling is a major and persistent source of nutrients to our study area. The potential importance of upwelled NO_3^- as a nitrogen source supporting "new production" in oceanic and coastal waters has been gauged through use of the *f*-ratio, which quantitatively compares the relative importance of NH_4^+ versus NO_3^- to phytoplankton growth (Eppley and Peterson, 1979; Harrison et al., 1987). The *f*-ratio has special application in the Florida Keys where it can be used to quantify the relative importance of NH_4^+ derived from sewage pollution and/or Florida Bay outflows (Lapointe and Smith, 1987, Lapointe and Clark, 1992, Lapointe and Matzie, 1996) versus NO_3^- from deep, offshore upwelling to algal growth demands. The generally low *f*-ratio values (overall mean *f*-ratio = 0.49 ± 0.18) at our stations, including the offshore LK station, are typical of oceanic surface waters where NH_4^+ rather than upwelled or vertically mixed NO_3^- supports algal growth (Eppley and Peterson, 1979). Predictably, we observed the highest NH_4^+ concentrations and the lowest *f*-ratios at the inshore AJ station that is most directly influenced by wastewater NH_4^+ enrichment from OSDS discharges (Lapointe et al., 1990). Within our study area, the nutrient concentration gradients consistently decreased from inshore to offshore, a pattern indicating enrichment of coastal waters from land-based nutrient sources rather than offshore upwelling (Lapointe and Clark, 1992). The concentrations of NH_4^+ at PR and LK increased significantly from the dry season to the wet season and averaged ~ 3-fold higher than the maximum concentrations of NO_3^- during the summer period. This pattern resulted in a seasonal decrease in the *f*-ratio, indicating a reduced importance of NO_3^- during summer when the potential for upwelling is maximum (Green, 1944; Lapointe and Smith, 1987; Leichter et al., 2003). Our data support the widely reported pattern of long-term seawater flow in an offshore—not onshore—trajectory in the Lower Keys (Pitts, 1994; Smith, 1994; Smith and Pitts, 2002). A long-term across-shelf flow of ~ 2.0 cm/s during a 13-month study in the Lower Keys in 1992–1993 yielded an average DIN net transport rate of 55 $\mu M\ m^{-2}\ s^{-1}$ toward the outer reef tract (Pitts, 2002).

The broad extent of land-based enrichment in the Lower Keys during our Phase I study was supported by the overall high values and spatial pattern of chlorophyll *a* concentrations among the stations. Chlorophyll *a* is a good index of eutrophication in coral reef waters because it integrates the more variable "pulses" of DIN and SRP over time (Laws and Redalje, 1979). Chlorophyll *a* concentrations on oligotrophic coral reefs are typically < 0.1 μg/l and mean levels of 0.2–0.3 μg/l are considered eutrophic on coral reefs (Bell and Elmetri, 1995; Yentsch et al., 2002). Mean chlorophyll *a* concentrations were highest—1.86 μg/l—at the inshore AJ site and decreased to mean values of 0.55 μg/l at the nearshore PR and to 0.59 μg/l at the offshore LK site—indicating a high degree of enrichment on these offshore bank reefs. The nearly three-fold higher chlorophyll *a* at AJ during summer indicates hypereutrophication at this inshore station, a result of the direct influence of wastewater nutrients from the adjacent watershed and the limited circulation and flushing at this station, which favor development of phytoplankton blooms (Valiela et al., 1997). The chlorophyll *a* concentrations at PR and LK were several-fold higher than typically found on oligotrophic, biologically diverse coral reefs (Yentsch et al., 2002) and are similar to values reported for eutrophic coral reefs. For example, sewage pollution of Kaneohe Bay, Hawaii, resulted in phytoplankton chlorophyll *a* concentrations that ranged from 0.68 to 4.67 μg/l throughout the bay, with the highest values occurring near the sewage outfall (Laws and Redalje, 1979; Smith et al., 1981). This range of chlorophyll *a* for sewage-impacted Kaneohe Bay is similar to the range of values at our stations, including LK. Unlike Kaneohe Bay, however, LK is an offshore bank reef and not a confined inshore bay with restricted flushing. The fact that chlorophyll *a* concentrations were equally high at the nearshore PR and the offshore LK stations during 1996, which are some 6.4 km apart, supports the hypothesis of significant upstream, regional nutrient enrichment from Everglades runoff and Florida Bay outflows adding to the localized sewage pollution in the Florida Keys. These data support previous conclusions of a regional-scale eutrophication event in the Florida Bay/Florida Keys region in 1995–1996 when historically maximum nitrogen loading from Everglades runoff occurred (Lapointe et al., 2002).

4.2. Land-based nutrient enrichment generates blooms of macroalgae and seagrass epiphytes

Macroalgal blooms are symptomatic of eutrophication in the Florida Keys (Lapointe et al., 1994) as in other shallow, tropical coastal waters that receive excessive land-based nutrient loading (NRC, 2000; Howarth et al., 2000). The increasing NH_4^+ concentrations and macroalgal biomass from the dry season into the wet season at all three stations during our study suggests a linkage between seasonal nitrogen enrichment and macroalgal HABs. Such a linkage is supported by experimental in situ enrichment with NH_4^+ and SRP that significantly increased photosynthesis and growth of the rhodophyte *Gracilaria tikvahiae* in winter whereas SRP enrichment alone increased growth during summer (Lapointe, 1987). Those results, combined with the present data demonstrating increased NH_4^+ concentrations and DIN:SRP ratios at PR and LK in the wet season, support the hypothesis that the seasonal increases in macroalgal biomass are linked directly to NH_4^+ enrichment and increased SRP-limitation of macroalgae during late spring and summer. Many species of macroalgae assimilate NH_4^+ much more rapidly than NO_3^- (D'Elia and DeBoer, 1978; Hanisak and Harlin, 1978; Fujita et al., 1989) and the presence of NH_4^+ can inhibit uptake of NO_3^- (Hanisak and Harlin, 1978). Accordingly, the significant increase in mean NH_4^+ concentration to ~ 0.5 μM at PR and LK during the spring–summer wet season, coupled with pulses up to 4.42 μM, could relieve nitrogen limitation and explain the exponential growth rate needed to generate the summertime macroalgal blooms (Lapointe, 1999). Hudson (1985) noted seasonally increased growth of the calcareous chlorophyte *Halimeda opuntia* during summer months in the Marquesas, a pattern that could also result from seasonal increases in NH_4^+ concentrations during summer. Similarly, Lirman and Biber (2000) reported significant increases in macroalgal biomass from winter to summer in reef communities of the northern Florida Reef Tract, a phenomenon that was not correlated with abundance of herbivorous reef fishes that might otherwise result in biomass increases.

The similarity in levels of macroalgal biomass between the relatively low grazing seagrass communities in the present study with those of the highly grazed bank reef communities of the northern Florida Reef Tract (Lirman and Biber, 2000) supports the hypothesis that the expanding macroalgal blooms in the FKNMS are controlled primarily by bottom-up forces, i.e. elevated nutrients (Lapointe, 1997). The maximum seasonal biomass of macroalgae at all three stations in the present study occurred in the spring and summer when mean DIN concentrations in nearshore (PR) and offshore (LK) waters increased to ~ 1 μM, primarily a result of increased NH_4^+ concentrations. The upper range of macroalgal biomass at all three sites ($\sim 100–300$ g dry wt m^{-2}) is similar to that of a localized, naturally DIN-enriched back reef community at Norman's Pond Cay, Exumas, Bahamas (Lapointe et al., 2004), sewage polluted seagrass communities in the Middle and Lower Keys (Lapointe et al., 1994), offshore bank reefs of the northern Florida Reef Tract (Lirman and Biber, 2000), and sewage polluted fringing reefs in the Negril Marine Park, Jamaica (Lapointe and Thacker, 2002). All of these sites have macroalgal blooms and average DIN concentrations of ~ 1 μM, a concentration that can saturate the growth demands of macroalgae (Lapointe, 1997, 1999).

Miller et al. (1999) suggested that blooms of *Dictyota* spp. on the offshore bank reefs of the FKNMS resulted primarily from reduced grazing pressure from herbivorous fishes (especially Scarids, parrotfishes) rather than land-based nutrient pollution. However, Miller et al. (1999) provided no data on the abundance of parrotfish at their experimental site. In the FKNMS, parrotfishes and other herbivorous fishes such as surgeonfishes are not targeted by fishermen and are Federally protected within "no-take zones"—such as the LK Sanctuary Protection Area where abundant, mobile schools of adult and juvenile parrotfishes and surgeonfishes intensively graze (Littler et al., 1986). Furthermore, these mobile schools of herbivorous fishes graze primarily on algal turf rather than frondose macroalgae, and many of the latter are well known to be chemically defended (Hay, 1991). Lirman and Biber (2000) noted that present grazing intensity by abundant reef fishes is insufficient to offset the rapid macroalgal growth and overgrowth of adjacent hermatypic reef corals on the northern Florida Reef Tract. Further, the high DIN (especially NH_4^+) concentrations we measured at LK are adequate to support maximum growth rates of macroalgae where their high rates of production, especially by non-palatable species, cannot be controlled by resident populations of herbivorous fishes (Lapointe, 1997, 1999). In the capital city of Male in the Maldives, Indian Ocean, Maldivians also do not target reef fishes but feed primarily on skipjack tuna; despite the full complement of grazing fishes on their coral reefs, local sewage pollution has led to the demise of these reefs and replacement of corals by macroalgae and bioeroding sponges (Risk and Sluka, 2000).

Previous studies reported that inshore areas experiencing direct wastewater discharges and advanced stages of eutrophication in the Florida Keys can have reduced macroalgal biomass compared to offshore sites with lower nutrient concentrations (Lapointe et al., 1994). We observed a similar pattern in the present study, where maximum biomass at the inshore AJ station was significantly lower than the maximum values for the more offshore PR and LK stations. The lower biomass at the more nutrient-enriched AJ could result from several factors, especially increased light limitation due to selective absorption and attenuation of downwelling irradiance by the three-fold higher chlorophyll *a* concentrations at AJ compared to the other sites during the spring–summer bloom (Yentsch et al., 2002). In addition to elevated phytoplankton biomass, discolored water and associated turbidity/sedimentation at AJ, though not quantified, appeared chronically high in the summer wet season, an environmental condition that would further increase light attenuation and lead to decreased production and biomass of macroalgae at AJ compared to the more offshore stations at PR and LK. We note the die-off of a 0.75-ha meadow of *T. testudinum* and *S. filiforme* at our AJ site in September 1996 following the appearance of the discolored water and a dense bloom of *C. montagneana* that formed thick mats over the seagrasses.

The high seagrass epiphyte loads at all three stations in this study supports previous findings (Tomasko and Lapointe, 1991; Lapointe et al., 1994) of the importance of water column nutrient enrichment to seagrass epiphytization, fragmentation, and die-off. However, a different spatial pattern in epiphyte abundance occurred in the present study compared to our earlier 1992 study (Lapointe et al., 1994). In the 1992 study, seagrass epiphyte loads were generally highest at the inshore HYPER and EUTRO strata (epiphyte biomass up to 97% of blade weight) with generally lower levels on the offshore OLIGO bank reefs (epiphyte biomass $<18\%$ of blade weight; Lapointe et al., 1994). In the present study, the highest epiphyte loads—up to 300% of blade weight—were not only three-fold higher than the highest value measured in the 1992 study, but also occurred on an offshore bank reef at LK—the same site that previously had low epiphyte loads ($<12\%$ of blade weight) and was classified as "OLIGO" (Lapointe et al., 1994). The high epiphyte loads at LK between April and June 1996 coincided with the onset of the rainy season (and increased NH_4^+ concentrations) and included blooms of the phaeophytes *Cladosiphon occidentalis* and *Lophosiphonia saccorhiza*, the rhodophytes *Chondria* sp., *Ceramium fastigatum*, and *Griffithsia* sp., and the cyanobacterium *Lyngbya gracilis*. The present study followed five years of increased discharges of nitrogen-rich agricultural runoff from the Everglades, a management action intended to restore Florida Bay—but which resulted in significant DIN enrichment and phytoplankton blooms in Florida Bay as well as downstream bank reefs at LKNMS (Lapointe et al., 2002; Brand, 2002, Lapointe and Barile, 2004). These record high seagrass epiphyte loads paralleled the maximum chlorophyll *a* concentrations at LK in 1996 following the peak nitrogen loading from the Everglades into the Florida Bay/Florida Keys region (Lapointe et al., 2002).

4.3. Physical forcing of land-based wastewater discharges into coastal waters

That maximum DIN concentrations at AJ coincided with the lowest tide of the present study suggests the importance of tidal pumping to increased rates of SGD and enrichment of coastal waters from wastewater and stormwater runoff. Lapointe et al. (1990), using a heat-pulsing groundwater flowmeter, found that lateral rates of shallow groundwater flow increased by ~ three-fold during ebbing tides as compared to flooding tides. Shinn et al. (1994) also observed tidal pumping and found hydraulic heads sufficient to raise well water as much as 7 cm above sea level during ebbing tides. Lapointe and Matzie (1996), using Hydrolab data loggers to obtain time series environmental data, found minimal dissolved oxygen and salinity in nearshore waters at low tides when maximal rates of SGD occur. Hence, the contamination of coastal waters by discharge of groundwaters enriched by septic tank effluent would be maximum during extreme low tides such as when the highest DIN and SRP concentrations (8.50 and 0.20 μM, respectively) of the study were measured at AJ. In addition to increased tidal pumping and SGD, the extreme low tides also provide minimal dilution of the increased wastewater nutrient load, further increasing nutrient concentrations in the coastal receiving waters.

Wind forcing was another important physical factor resulting in episodic nutrient enrichment and increased chlorophyll *a*, especially during the high northerly winds in February and July 1996. Bell and Elmetri (1995) found a significant correlation between increased turbidity and phytoplankton biomass with wind speed in the Great Barrier Reef Lagoon. Although high turbidity has been observed during winter northeasters as the result of benthic sediment resuspension in the Florida Keys (Lapointe and Clark, 1992), increased nutrient concentrations associated with such wind events have not been previously reported. The episodic increases in nutrient concentrations during wind events potentially result from several factors. First, wind-driven advection associated with these events could increase cross-island hydrostatic potentials by piling up water, for example, on the windward Florida Bay side of the Lower Florida Keys, thereby increasing rates of SGD and enrichment of coastal waters on the lee side towards Hawk Channel and the offshore reefs (Shinn et al., 1994). Reich et al. (2002) reported that Hurricane Georges (September 25, 1998) raised the sea water level on the Atlantic Ocean side of the Keys and lowered the water level in Florida Bay, resulting in a reversal of groundwater flow direction (towards Florida Bay) and increased flow rates. High winds also result in sediment resuspension and enhanced advection and diffusion of pore water nutrients (including nutrients derived from SGD) into the water column. Recent work by Smith and Pitts (2002) reported that a northwest-to-southeast wind stress component was most effective for driving water through tidal channels at Long Key and the Seven Mile Bridge towards Hawk Channel. We observed this effect in mid-February 1996 when high northerly winds transported very turbid waters from the Gulf of Mexico through the tidal channels and offshore of LK when the highest SRP concentration of the study occurred.

Rainfall was another important mechanism causing not only episodic nutrient enrichment but also seasonally increased concentrations of DIN and chlorophyll *a* at LK during the summer wet season. Increased rainfall in the Florida Keys during late spring, summer, and fall results in increased infiltration of NH_4^+-rich septic tank and cesspit effluent plumes into shallow groundwaters and accelerates lateral groundwater flow rates, increasing NH_4^+ and SRP concentrations in inshore surface waters (Lapointe et al., 1990; Lapointe and Clark, 1992). Although rainfall in the Florida Keys can have significant DIN concentrations (~ 15.0 μM; Lapointe and Matzie, 1996), rainfall alone cannot account for the observed magnitude and spatial pattern of enrichment in this study. The relatively low amount of rainfall associated with the "first flush" in May 1996, combined with the magnitude of increase in NH_4^+ concentrations offshore to LK following the rainfall, suggests the importance of the wet season to offshore NH_4^+ transport. Pitts (2002) found that the across-shelf movement of water in the Lower Keys had a distinct seasonal signal; seaward from late spring to winter/early spring and highly variable during the late winter and early spring. This quasi-steady seaward flow from May to November in Pitts's (2002) study in the Lower Keys during 1992 occurred during the wet season, the same time frame when we observed increased mean and maximum concentrations of NH_4^+ and chlorophyll *a* at LK in 1996. This seasonal pattern is important not only because of increased land-based nutrient discharges into nearshore waters during the wet season, but also because of seasonally maximum advection and transport of nutrients to the offshore reefs during the late-spring and summer period (Pitts, 2002).

4.4. Discrimination of nitrogen sources using $\delta^{15}N$ in macroalgal tissue and seagrass epiphytes

Because of their high affinity for wastewater NH_4^+ and ability to integrate pulsed DIN concentrations in the water column over time, macroalgae are considered ecological indicators of sewage pollution on coral reefs (Banner, 1974; Johannes, 1975; Smith et al., 1981; Bell and Elmetri, 1995). The $\delta^{15}N$ values we measured in a wide variety of macroalgae and seagrass epiphytes during 1996 and 2001 were all elevated above values reported for natural nitrogen fixation (Table 1) and provide evidence that anthropogenic nitrogen from local sewage sources in the Florida Keys and agricultural runoff from South Florida are significant sources contributing to nitrogen enrichment in the FKNMS.

The Phase I study in 1996 followed peak Everglades flows and nitrogen loading from Shark River Slough ($\sim$4545 mtons N/year in 1995, Lapointe et al., 2002) and the relatively low $\delta^{15}N$ values ($\sim + 3$‰) of macroalgae and seagrass epiphytes at PR and LK were at the low end of the range reported for sewage enrichment (Costanzo et al., 2001). However, these relatively low $\delta^{15}N$ values were similar to values reported for macroalgae in northwestern Florida Bay that is directly influenced by Everglades runoff via Shark River Slough (Corbett et al., 1999, Table 1). Agricultural fertilizers and peat nitrogen have $\delta^{15}N$ values in the range of + 1‰ to + 3‰ (Heaton, 1986) and their influence, along with DON from the oxidation of Everglades peat, during periods of high Everglades flows and nitrogen loads would reduce $\delta^{15}N$ values normally associated with sewage (> + 3%) to the lower values reported for northwestern Florida Bay and downstream waters at our sites in 1996. The significant increases in $\delta^{15}N$ values to $\sim + 5$‰ in *C. catenata* at LK in 1996 coincident with increased rainfall and pulsed increases in NH_4^+ concentrations provides evidence that wastewater nitrogen from the Florida Keys is advected offshore and contributes to summertime macroalgal blooms at LK. In contrast to the pulsed delivery of sewage nitrogen to offshore waters at LK, the chronic sewage inputs at AJ resulted in consistently elevated $\delta^{15}N$ values of macroalgae and seagrass epiphytes in the range of + 3.0 to + 5‰—values characteristic of nitrogen derived from septic tanks and/or cesspits (McClelland et al., 1997; Lapointe and Thacker, 2002).

A similar spatial pattern occurred in the Phase II study during the wet season of July 2000 when $\delta^{15}N$ values > + 3‰ reflected sewage enrichment of macroalgae in inshore waters of Pine Channel off northern Big Pine Key, a guano-rich bird island in Newfound Harbor on the southside of Big Pine Key, and the Content Keys—but not at the offshore stations at LK. However, a different spatial pattern developed during the record drought in spring of 2001 when Everglades flows were at record lows (Abtew et al., 2002) and $\delta^{15}N$ values were > + 5‰ at all stations from the Content Keys to LK. We attribute this pattern of elevated $\delta^{15}N$ values to widespread advection of sewage nitrogen in shallow waters throughout our study area, including the offshore reef site at LK to $\sim$ 7 m depth on the fore reef. Leichter et al. (2003) reported $\delta^{15}N$ values of + 4.3‰ to + 5.0‰ in *Codium isthmocladum* at 34 m at Conch Reef, which they concluded was the result of upwelled NO_3^-. However, Leichter et al. (2003) did not consider the possible importance NH_4^+ that was >2 μM on Conch Reef during their study and could have contributed to δ^{15}N enrichment of *C. isthmocladum*. As noted previously, uptake of NH_4^+ by *Codium fragile* is seven-fold faster than uptake of NO_3^- and the presence of NH_4^+ inhibits uptake of NO_3^- (Hanisak and Harlin, 1978). This suggests an alternative hypothesis that the elevated δ^{15}N values in *C. isthmocladum* at Conch Reef could have resulted from advection of NH_4^+-rich nearshore waters to these deep reef environments (Lee et al., 1994; Pitts, 2002) as well as the SGD of NH_4^+- and/or NO_3^- enriched water into the near-bottom waters (Simmons, 1992). The latter pathway would be of special concern because of the common disposal of partially treated, nutrient-rich sewage effluent under pressure into either Class I (~ 950 m depth) or Class V (27 m depth) injection wells in adjacent upland watersheds of the Florida Keys and mainland South Florida.

Similar broad-scale spatial patterns of land-based runoff of nitrogen have been reported for other coral reef ecosystems. Sammarco et al. (1999) measured δ^{15}N values in coral tissue and reported effects of land based nitrogen enrichment for ~ 70 km from shore on the Great Barrier Reef (GBR), Australia. In that study, the pattern of δ^{15}N values formed a spatial pattern referred to as the "Australian Smile" as the land-based δ^{15}N enrichment decreased in the mid-lagoon of the GBR and increased again on the outer reef, presumably due to upwelling of denitrified waters (Sammarco et al., 1999). In the Negril Marine Park, Jamaica, δ^{15}N values of macroalgal communities were higher on shallow reefs ($+3.56 \pm 1.65$‰) compared to deep reefs ($+1.84 \pm 0.72$‰) and the lowest values consistently occurred at the most offshore station least impacted by land-based runoff (Lapointe and Thacker, 2002). Those δ^{15}N results were corroborated by water column nutrient data in which elevated NH_4^+ concentrations on shallow reefs were significantly higher than on deep reefs, suggesting sewage and agricultural runoff as land based sources of nitrogen pollution to the Negril Marine Park (Lapointe and Thacker, 2002). The results of Lapointe and Thacker (2002) mirror these results from the FKNMS where elevated NH_4^+ concentrations in the water column were associated with elevated δ^{15}N values in macroalgae at levels reported for nitrogenous pollution from sewage and/or agricultural runoff.

5. Conclusions

Results of this study support the hypothesis that land-based nutrient enrichment is a primary factor in the seasonal development of phytoplankton, macroalgae, and seagrass epiphyte blooms in the inshore, nearshore, and offshore waters of the FKNMS. Our data suggest that chronic nutrient enrichment of coastal waters of the Florida Keys from local (sewage) and regional (agricultural) land-based sources has led to elevated NH_4^+ (and to a lesser extent, SRP) concentrations in the water column that meets or exceeds the threshold concentrations known to support eutrophication and macroalgal HABs. Our results do not support the hypothesis that the recent expansion of macroalgal blooms on shallow bank reefs (< 30 m) of the FKNMS resulted from NO_3^- enrichment associated with upwelling (Szmant and Forrester, 1996). Wind-driven upwelling does develop along Florida's northeast coast during summer when wind and other factors are favorable (Green, 1944; Smith, 1982; Atkinson et al., 1984) but has relatively minor and infrequent effects on shallow coral reef communities of the Florida Reef Tract (Lapointe and Smith, 1987; Lee et al., 1994; Leichter et al., 2003).

Our conclusion that chronic anthropogenic nutrient enrichment has supported algal blooms, eutrophication, and reef coral die-off in the FKNMS is supported by several recent studies. During the early 1990s water managers increased flows and nitrogen-loading into Florida Bay and downstream waters of the FKNMS from upland agricultural watersheds (Lapointe et al., 2002); this massive increase in regional nitrogen loading was followed by 38% mortality of living coral in the FKNMS between 1996 and 1999 (Porter et al., 2002). Much of the coral die-off was attributed to microbial diseases, which, like algae, respond to increased nutrient loading and the production of "new" organic matter. The white-pox disease that caused widespread mortality of *Acropora palmata* (elkhorn coral) at LK and other bank reefs of the FKNMS was identified as *Serratia marescens*, an opportunistic fecal coliform bacterium (Patterson et al., 2002). *S. marescens* exhibits significant seasonal variability, increasing during the summer wet season (Patterson et al., 2002) in similar periodicity to the blooms of macroalgae, phytoplankton, and seagrass epiphytes we documented to correspond with seasonally higher NH_4^+ concentrations. Additional studies by Ward-Paige and Risk (2002) have reported increased growth of the fecal bioindicator *Cliona delitrix*, which is now causing rapid rates of bioerosion of coral reef frameworks over broad areas of the FKNMS.

Despite wide recognition by resource managers of the importance of water quality to the economic health of the Florida Keys and a well-funded water quality monitoring program for the FKNMS, "no definitive studies have been conducted on the geographic extent of anthropogenic nutrients" in the FKNMS (Kruczynski and McManus, 2002). Part of this failure can be traced to limitations in the design of the water quality monitoring program for the FKNMS. The necessity for high frequency water quality monitoring to resolve on physically mediated, short-term "pulses" of land-based nutrient discharges and their ecological effects in the FKNMS was documented nearly a decade ago (Lapointe and Matzie, 1996). Although the FKNMS water quality monitoring program is spatially intensive (~ 200 stations), it is conducted at only quarterly intervals so, by design, it cannot detect episodic nutrient inputs, seasonal patterns, or even year to year trends. Following 3 years of sampling in the FKNMS, Boyer and Jones (2002) recently concluded that "the monitoring program based on quarterly sample intervals may require up to 10 years before small trends may be detected because of seasonal variability and background noise". Obviously, such a protracted time frame to determine significant changes in water quality is not appropriate for management of the natural resources of the FKNMS where average coral cover declined to ~ 6.0% between 1996 and 1999 (Porter et al., 2002).

Our results support the conclusions of others (Risk et al., 2001) that the use of stable nitrogen isotope ratios provides a rapid and cost effective method for detection of land based nitrogen stress in coral reef communities. These advanced biogeochemical techniques have been used to trace sewage nitrogen in a wide variety of developing countries yet have not been used in a systematic, comprehensive fashion to quantify the spatial and temporal patterns of sewage and/or agricultural pollution in the FKNMS. While it is tempting for coral reef resource managers to attribute recent coral reef die off in the FKNMS simply to "global change", local and regional sources of anthropogenic nutrient pollution have long been known as a primary cause of coral reef destruction (Banner, 1974; Johannes, 1975; Weiss and Goddard, 1977; Smith et al., 1981; Bell, 1992; Risk, 1999; NRC, 2000) and a threat to the sustainability of coastal resources in the Caribbean region (UNEP, 1994). The emerging problem of land based nitrogen enrichment of coastal waters and its ecological consequences, as documented here for the FKNMS, should now be considered a primary local and regional threat to coral reef ecosystems worldwide.

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