The Sanibel River and the Impaired Waters Rule: How Stable Nitrogen Isotopes and Phytoplankton Community Analyses Can Indicate Nutrient Sources and Evaluate Nutrient Reduction Efforts

Final Project Report

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EXECUTIVE SUMMARY

Background

Due to high nutrient and phytoplankton abundance, water quality in the Sanibel River does not meet State of Florida standards for Class III surface waters, for which designated uses described as "recreation, and propagation and maintenance of a healthy, well-balanced population of fish and wildlife" are expected. The goal of this pilot project was to investigate the utility of stable nitrogen isotope analyses and phytoplankton community description in linking potential sources of nutrients on Sanibel Island to nutrient concentrations and phytoplankton community characteristics in the surface waters of the Sanibel River.

Sampling

To coordinate this pilot project with the City of Sanibel's existing ambient water quality monitoring program, all samples were collected on Monday, July 28, 2009. In addition to the six regularly visited stations along the Sanibel River, samples for stable nitrogen isotope analysis ($\delta^{15}N$) were collected at several additional locations around the island, including two other locations along the river, the Donax Water Reclamation Facility (WRF), and a holding pond used for golf course irrigation. Samples of pellet fertilizer and reclaimed water were also analyzed to bracket stable nitrogen isotope signatures of potential nutrient sources. Finally, the contributions to chlorophyll concentration made by eight phytoplankton taxa were calculated based on samples collected at the same six ambient surface water monitoring stations, plus two additional locations along the river.

Results

Between ammonia and nitrate, ammonia was the more abundant and more important source of inorganic nitrogen to phytoplankton, particularly in the eastern sub-basin. Stable nitrogen isotope signatures of ammonia and nitrate sampled from the eastern sub-basin were generally isotopically heavy, indicating that a greater proportion of the nutrients in these areas originated from organic sources (*e.g.* WRF or septic tanks effluent) than from fertilizer, though we did observe some exceptions. Isotope signatures in the western sub-basin indicated more even proportions of nutrients from these types of sources, *e.g.* both fertilizer and organic sources of ammonia were used by phytoplankton. It is important to note that *concentrations* of nitrogen-nutrients were higher in the eastern sub-basin than the western.

Our phytoplankton community results indicate that dinoflagellates and cyanophytes (also known as blue-green algae or cyanobacteria) become proportionately more abundant at the downstream stations in each sub-basin. This may be a response by phytoplankton to the increased nutrient availability noted above, or to the abundance of phosphorus relative to nitrogen. However, besides nutrient supply, the rate of grazing by zooplankton also influences phytoplankton abundance. As zooplankton are selective grazers, favoring diatoms, chrysophytes, and cryptophytes over dinoflagellates and cyanophytes, grazing by zooplankton will exert less control over phytoplankton abundance in these locations. This observation suggests that phytoplankton abundance may remain high in these downstream locations for some time, even as nutrient inputs from fertilizers and reclaimed water are controlled.

A second important result based on our analysis of distribution of phytoplankton community taxa is that most of the phytoplankton groups at all locations are capable of utilizing a variety of nutrient forms, including dissolved organic nitrogen-containing compounds. Concentration of dissolved organic matter, as indicated by CDOM, was high at all stations. This observation also suggests that even as new inputs of nutrients are controlled and reduced, phytoplankton (as indicated by the concentration of chlorophyll) will remain abundant in the water column for some time.

Therefore, we recommend that nutrient concentrations (both nitrogen and phosphorus) and the proportions of nutrients from inorganic and organic sources be measured in other seasons during the year, *e.g.* June, at the end of the dry season, and October, at the end of the wet season. Combined with the current project's results, this would provide a more complete picture of nutrient cycles, nutrient phytoplankton dynamics, and the influence of nutrient reduction practices such as the fertilizer ordinance

on the concentrations of surface water nutrients and phytoplankton. In addition, future studies of nutrients in the Sanibel River should take two other potential sources into account: dissolved organic matter and atmospheric deposition.

We also recommend that the distribution of dinoflagellate and cyanophyte groups of phytoplankton be monitored to detect shifts in community composition in response to nutrient reduction measures that may not be identified by chlorophyll concentration alone.

Finally, we recommend an investigation be made into the causes of the very low dissolved oxygen levels at all ambient surface water quality stations observed along the Sanibel River, and the relationship between low dissolved oxygen and high dissolved organic matter.

In closing, we note that the Sanibel River is managed for several purposes in addition to the IWR mandate describing “recreation, and propagation and maintenance of a healthy, well-balanced population of fish and wildlife”. For example, water levels in the river are monitored at least once per week, and manipulated to reduce the potential for flooding during the rainy season. Given these complex expectations for natural resource management, we believe future studies of Sanibel River water quality issues should be made in the context of the integrated management of surface water nutrients in conjunction with other water quality variables.

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INTRODUCTION

Project Background

The Sanibel River is currently listed as impaired under the Impaired Waters Rule (IWR, F.A.C. 62-303) for Trophic State Index (TSI; see Florida Department of Environmental Protection 2006), an aggregate measure of water quality based on nutrient (nitrogen and phosphorus) concentration and phytoplankton abundance. The TSI was originally developed to assess the health of freshwater ecosystems, where phosphorus is generally the nutrient that limits algal biomass. In these types of systems, addition of phosphorus generally results in increased abundance of algae (*e.g.* Schindler *et al..*, 2008). In contrast, marine systems are generally thought to be limited by nitrogen – an increase in nitrogen availability, usually as either ammonia or nitrate, generally leads to an increase in phytoplankton production (see review in Smith, 2006). However, algae abundance in brackish or estuarine systems, such as the Sanibel River and San Carlos Bay, may be limited by nitrogen or phosphorus. For this reason, in the context of the IWR, the TSI has been revised, and now includes thresholds for both nitrogen and phosphorus, as well as for chlorophyll.

However, it is difficult to differentiate between the sources of nutrients in surface waters, or to evaluate the effectiveness of specific nutrient reduction practices using conventional water quality data (*e.g.* concentration of ammonia) alone. Furthermore, although chlorophyll abundance is a useful index of ecosystem production, it represents a collection of all groups of phytoplankton in a system, each of which may react to changes in their environment (*e.g.* nutrient availability, salinity) in different ways; ways that are difficult to distinguish using chlorophyll concentration data alone. A clearer picture of the response of the phytoplankton community to environmental changes, whether natural or due to environmental management decisions, would help evaluate the effectiveness of management efforts intended to resolve water quality issues identified by the IWR.

Water Quality Variables

The existing ambient surface water quality sampling program administered by Sanibel-Natural Resources Department (NRD) contributes to the dataset used by the Florida Department of Environmental Protection (DEP) to evaluate surface waters for IWR. For this application, the TSI relates the health of a water body to the concentrations of three water quality variables: total nitrogen, total phosphorus, and chlorophyll. Two of these components are of interest to the present study; total nitrogen and chlorophyll. *Total* nitrogen is the sum of all biologically available nitrogen in a system, and includes a number of different compounds: ammonia, nitrate and nitrite, urea, as well as other dissolved and particulate organic compounds that contain nitrogen. The compounds that bring a “tea stain”, tannic, or colored appearance to river or lake water also contribute nitrogen compounds to *total* nitrogen. High values of total nitrogen in a river or lake may be a natural condition, or they may indicate a number of characteristics of unhealthy ecosystems – high concentrations of inorganic nutrients or dissolved organic matter, or excessive microbial and phytoplankton abundance.

The concentration of chlorophyll is a useful index of phytoplankton abundance – higher concentration of chlorophyll implies greater phytoplankton biomass. As generators of oxygen and food for zooplankton, phytoplankton are very important components of aquatic ecosystems. However, an overabundance of phytoplankton can cause environmental problems, such as hypoxia (by consuming oxygen at night), or by intercepting light required for submerged aquatic vegetation, such as seagrasses. In addition, certain types of phytoplankton can threaten healthy ecosystems as well, either by producing toxins (*e.g.* red tide, a dinoflagellate, or *Lyngbya* sp., a cyanophyte), or by out-competing other species or groups that are more nutritious to zooplankton grazers such as diatoms or chrysophytes. As many of these zooplankton grazers ultimately become food for fish, the importance of the IWR mandate for the “maintenance of a healthy, well balanced population of fishes” becomes apparent.

In response to degraded water quality conditions, the City of Sanibel has implemented a variety of measures to reduce nutrients in the Sanibel River. These include public purchase of conservation

easements, increased development setbacks from wetlands to reduce the impacts of storm water runoff, implementation of an island-wide central sewer system. In addition, the City of Sanibel adopted a fertilizer ordinance in March, 2007 (amended September, 2007), restricting the use of fertilizers to products with no more than 20% nitrogen by weight, at least 50% of which must be "slow release", and less than 2% phosphorus. In addition, a setback from open surface waters (*e.g.* canal) of 25 feet is required, and fertilizers may not be applied between 1 July and 30 September.

Stable Nitrogen Isotopes

An isotope is a form of an element (*e.g.* nitrogen, carbon) that differs slightly in atomic weight, but functions the same in most other respects (Peterson and Fry 1987). There are two stable (non-radioactive) isotopes of nitrogen, and both are incorporated into nitrate, ammonia, organic nitrogen compounds, and the atmospheric nitrogen that makes up 80% of the atmosphere. The only difference is that individual molecules of ammonia (or nitrate, or organic matter, etc) made with the "heavy" isotope of nitrogen weigh slightly more than molecules composed with the "light" isotope. And while biochemical reactions involving the heavy isotope are no different than those involving the light, these small differences in mass are detectable in the laboratory, and, when expressed as the ratio of heavy to light isotope, can be used to investigate certain patterns in nature.

Isotope ratios are represented by the "del" notation, δ, which corresponds to the difference between a sample's stable isotope ratio and that of the "standard" ratio (N_2 in air, for nitrogen). Isotope ratios are reported in unit parts per thousand, ‰, pronounced and often written "permil", and calculated by the following formula:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000,$$

where R, for both sample and standard, is the molecular ratio of heavy isotope to light isotope in a sample:

$$R = \frac{\text{(number of nitrogen molecules with atomic weight of 15)}}{\text{(number of nitrogen molecules with atomic weight of 14)}}$$

For example, if a sample's ratio of heavy to light isotope equals the "standard" ratio, the quantity goes to 1, and $\delta^{15}N$ = 0‰. Samples with more "heavy" nitrogen have positive values (greater than 0‰), while samples with less "heavy" nitrogen have negative values. When comparing two samples, the one with the higher ratio has a relatively greater proportion of the heavier isotope, while the sample with the lighter ratio has relatively less heavy isotope. It's important to remember that a $\delta^{15}N$ value of 0‰ does not mean "zero" or "nothing".

Because the isotopically "lighter" molecules are transported at a slightly quicker rate than isotopically "heavier" molecules, processes such as nutrient assimilation, trophic interactions, and photosynthesis cause fractionation between the ratios of stable isotopes – fractionation describes the differences in $\delta^{15}N$ ratios between a source (*e.g.* nutrients) and a product (*e.g.* phytoplankton). Phytoplankton assimilating ammonia will use the "lighter" ammonia molecules first, resulting in relatively "light" phytoplankton, while the remaining ammonia becomes relatively "heavier". In other words, the process of nutrient assimilation produces biomass (*e.g.* phytoplankton mass) with a $\delta^{15}N$ ratio related to, but lighter than, its nutrient source.

In this way, stable nitrogen isotopes can be used to identify nutrient sources in locations where differences in $\delta^{15}N$ ratios between the likely sources in a given environment exist (review by Robinson, 2001). For example, ammonia from artificial fertilizer (also known as an "inorganic" source of nutrients), carries a characteristic $\delta^{15}N$ ratio which is generally less than 0‰ (reviews in Peterson and Fry 1987, and

Goffman *et al..* 2006). On the other hand, septic tanks and wastewater treatment plants, which are considered "organic" sources of nutrients, produce ammonia, nitrate, and organic nitrogen compounds with relatively heavier ratios, often greater than +10‰ (review in Valiela *et al..* 2000). WRF that employ an alternating aerobic/anaerobic cycle to achieve a higher degree of nutrient reduction generate effluent and residuals with δ15N ratios more enriched than most other naturally occurring signatures, reportedly as high as +26‰ (Savage and Elmgren 2004) and +40‰ (Jordan *et al..* 1997).

Therefore, the source of nutrients used by phytoplankton in a water sample may be estimated by comparing the isotope ratio of phytoplankton with those of the potential nutrient sources for a particular environment. For example, phytoplankton using ammonia originating from "inorganic" fertilizer will acquire a $\delta^{15}N$ somewhat "lighter" than phytoplankton using nutrients originating from WRF effluent. Regional examples of this kind of nutrient source/sink investigation include Lapointe and Bedford (2006), Demers (2008), and Hale and Duffey (in review).

Furthermore, when sources of nutrients such as these make large contributions to the total amount of surface water nutrients, stable isotope techniques become a useful way to identify the proportion of nutrients from each source being used by phytoplankton. Under these circumstances, contributions of particular land uses toward the calculation of total maximum daily loads of pollutants (TMDL, see Florida Department of Environmental Protection 2006) can be estimated.

Phytoplankton, Chlorophyll, and Photopigments

Understanding the composition of phytoplankton community can be a useful tool when investigating the impacts of degraded water quality (*e.g.* Paerl *et al..* 2003, 2007, 2008; Livingston 2007). For example, nitrogen and phosphorus concentrations based on a recent data evaluation by Sanibel-NRD suggest increasing concentration of phosphorus from upstream to downstream locations in the Sanibel River. High phosphorus concentration relative to nitrogen concentration may promote the dominance of phytoplankton communities by cyanophytes (a.k.a. cyanobacteria, blue-green algae), which are capable of "fixing" atmospheric nitrogen to use as a nutrient source, and so take advantage of available phosphorus in a way that other phytoplankton groups cannot. However, water quality data including only the concentration of chlorophyll would not detect the dominance of cyanophytes over other phytoplankton groups.

Besides nutrients, another control on phytoplankton abundance is the rate of grazing by zooplankton. However, zooplankton do not consume all types of phytoplankton evenly; diatoms, chrysophytes, and cryptophytes are generally favored over dinoflagellates and cyanophytes. Therefore, a phytoplankton community dominated by these latter groups may not sustain a healthy a zooplankton community which, in turn, will negatively impact a "healthy and well balanced fish and wildlife population" required by the IWR. Clearly, information on the composition of phytoplankton groups would be useful information when describing the environmental impacts of excess nutrients on surface waters, as well as when estimating the efficacy of environmental restoration.

<u>Analyzing</u> <u>photopigments</u>. To characterize phytoplankton communities in aquatic ecosystems, high performance liquid chromatography (HPLC) of photopigments is part of a system developed to detect a variety of photopigments (*e.g.* chlorophyll *a*, fucoxanthin) in a given sample (Wright *et al..* 1991). Combining HPLC with a customized statistical analysis, separation and quantification of combinations of photopigments can be an effective way of determining the species or group composition of phytoplankton communities based on the photopigments associated with each algal group. Phytoplankton taxa that can be quantified using this method include: dinoflagellates, diatoms, chlorophytes, cryptophytes, haptophytes, chrysophytes, euglenophytes, and cyanophytes.

The following report includes descriptions of two types of analyses, natural abundance of stable isotopes and phytoplankton community composition. We proposed that these datasets may be used in conjunction with existing City of Sanibel-Natural Resources Department (NRD) water quality sampling program to (1) identify and quantify the sources of nutrient inputs into the Sanibel River, and (2) describe the relationship between nutrient sources, chlorophyll concentration, and phytoplankton groups in the

Sanibel River. In turn, these results may be used to identify sources of nutrients in the Sanibel River, to evaluate the effectiveness of nutrient reduction actions, and to guide future water quality data analyses and monitoring programs.

METHODS

Overview

Samples for water quality analysis were collected at eleven locations on July 28, 2008, (Figure 1 and Table 1), early in the "restricted fertilizer use" season, which began July 1. In addition to six stations along the Sanibel River that are monitored monthly for surface water quality variables, collections were made at the following locations for both $\delta^{15}N$ ratios and phytoplankton community characteristics: Sanibel River at Dimmick Drive, Sanibel River at Tarpon Bay Road. Additional samples were collected for $\delta^{15}N$ only: a re-use/irrigation holding pond on Beachview Golf Course, a ground water sample collected from the unsaturated zone on the Donax WWTP property, and two storm water swales (1740 Periwinkle Way, called "Church swale", and across from the corner of Periwinkle Way and Purdy Dr., called "Periwinkle swale"). Finally, two types of samples were collected to bracket the $\delta^{15}N$ of potential nutrient sources: inorganic fertilizer, and water samples from the Donax WRF facility,

Water Quality Variables

Ambient monthly and nutrient concentration sampling. A complete description of this sampling program is on file at Sanibel-NRD. Briefly, surface water (depth < 0.3 m) samples were collected at six regularly sampled locations along the Sanibel River in dark bottles (for chlorophyll) or pre-acidified bottles (nutrients), and physical water quality variables were recorded, including temperature, conductivity, and dissolved oxygen. Bottles were transported on ice and delivered to Benchmark EnviroAnalytical, Inc. and Lee County Environmental Labs within allowable holding times.

RECON. A portable model of the RECON (River, Estuary, and Coastal Observing Network), operated by the Sanibel-Captiva Conservation Foundation – Marine Lab, was deployed at each of the six regular monitoring sites, plus the two additional sites along the river, Dimmick Drive and Tarpon Bay Road. Supplementary water quality variables recorded at a depth of approximately 0.3 m included: CDOM (colored dissolved organic matter (related to apparent color), in QSDE units), turbidity (NTU), temperature (degrees C), conductivity (reported as mmhos/cm), and dissolved oxygen (mg/L).

Stable Nitrogen Isotopes

Stable nitrogen isotopes: nitrate and ammonia. We collected duplicate water samples from 15 locations and samples. Sample preparation for $\delta^{15}N$ analysis of both ammonia and nitrate followed Sigman *et al..* (1997), Holmes *et al..* (1998), and Sebilo *et al..* (2004), and is briefly described here. Filtered water samples (pre-combusted quartz fiber filters < 1.0 µm pore size) were buffered to pH of 9.6 with magnesium oxide (MgO) and osmotically balanced to a salinity of 50 ppt with reagent grade sodium chloride (NaCl) before adding an acidified disc (10 µm H_2SO_4) sandwiched in teflon to capture gaseous ammonia. Sample bottles were tightly capped and incubated at 60° C for 4 wks to promote volatilization of ammonium ion to ammonia which was then captured on the disc. After removing the first acidified disc, samples were evaporated to dryness, rehydrated with 100 ml of Milli-Q (ultra-pure) water, then mixed to re-dissolve salts and allowed to settle before Devarda's alloy was added. Another acidified disk was added to each sample bottle, which was then tightly capped and incubated for another 2 weeks. Devarda's alloy chemically reduces nitrate in the sample to ammonium ion, which is again volatilized and captured on the second acidified disc. Sample support volumes (400 ml initial volume), incubation temperatures, and incubation intervals followed the methods and recommendations in the citations listed above. Based on linear regressions in Holmes *et al..* (1998), corrections were made for two processes affecting ammonia extraction using these methods: sample volume (1 ‰ added to measured δ15N value for ammonia) and incubation period (4 ‰ added to measured δ15N value for ammonia). These and all other δ15N samples were analyzed on a Finnegan-MAT DeltaPlus mass spectrometer at the Center for Isotope Geoscience, Department of Geological Sciences, University of Florida.

Stable nitrogen isotopes: sources of inorganic nitrogen. Two brands of fertilizer were analyzed for δ^{15}N-ammonia. A sample of 15-0-15 fertilizer (probably "Fairway") was acquired from the Beachview Golf Course maintenance facility (provided by the golf course superintendent) on Donax Street. A second off-island sample of fertilizer ("Vigoro All-Purpose 6-6-6 Fertilizer") was also used in this study. Both fertilizers comply with nitrogen requirements of Sanibel's Fertilizer Ordinance (07-012) for both content by weight and percent slow release form (6-6-6 does not comply with respect to phosphorus). To prepare the fertilizers for analysis, a 15-g sample of each was ground by mortar and pestle. Next, a 1 g subsample of the powdered fertilizer was allowed to dissolve in 1 liter of ultra-pure water using a stir bar for 20 minutes. This solution was diluted to yield a concentration of nitrogen in the same support volume comparable with our surface water samples (approximately 0.050 mg/L ammonia-N in 400 mL initial volume). Because ammonia was more abundant in our environmental samples while nitrate concentration has generally been below detection limits (data available from City of Sanibel-NRD), we analyzed both fertilizers for δ^{15}N-ammonia only.

Samples of treated wastewater were collected from the Donax WRF on July 28. Duplicate samples of two types of "after filtration" ("AF") treated wastewater were collected, namely before and after chlorination, identified as AFBC and AFAC, respectively (only AFAC water is used in "re-use" applications, *i.e.* irrigation). Duplicate 400-mL samples of each were prepared and analyzed for δ^{15}N in both ammonia and nitrate as described above. While this is not the only likely source of "organic" nutrients on the Sanibel River, WRF effluent is a widely used representative (*e.g.* Valiela *et al.*, 2000, Savage *et al..* 2004)

The proportions nutrient-nitrogen from different land uses were calculated according to a simple, 2-end member mass balance formula (Phillips 2001; Phillips and Gregg 2003):

$$\delta^{15}N(obs) = f(A)^* \delta 15N(A) + f(B)^* \delta 15N(B),$$

and

$$1 = f(A) + f(B),$$

where δ^{15}N(obs) represents δ^{15}N-ammonia at a location; δ^{15}N(A) and δ^{15}N(B) are δ^{15}N-ammonia associated with two potential sources, A (fertilizer) and B (WRF effluent), based on values observed by this project. Finally, f(A) and f(B) are the fractions of observed ammonia concentration. Given the cautions in Peterson and Fry (1987), Philips (2001), and Philips and Gregg (2003), the contributions of ammonia from fertilizer (*e.g.* f(A)) and organic sources (*e.g.* WRF, f(B)) can be calculated.

Stable nitrogen isotopes: organic matter. Each water sample used for δ^{15}N ammonia and nitrate preparation was passed through a pre-combusted quartz fiber filter of maximum pore size 1.0 µm until it clogged. The filters were dried at 60° C, and the organic material remaining on each filter, which contained phytoplankton as well as other forms of particulate organic matter, was peeled off the top and divided into two duplicate samples.

Organic matter δ^{15}N was also analyzed in the leaves of two stream-side trees, Red mangrove (*Rhizophora mangle*) and Buttonwood (*Conocarpus erectus*), the emergent plant cattail (*Typha* sp.), a sample of filamentous macroalgae (possibly *Lyngbya* sp.), and sediment organic matter collected from the Tarpon Bay Road station. These samples were dried at 60 °C, ground with mortar and pestle, and analyzed for δ^{15}N.

Phytoplankton, Chlorophyll, and Photopigments

Phytoplankton community: pigment analysis of phytoplankton. Whole water samples collected from each of eight stations (Table 1) were filtered though a 0.7 µm GF/F glass fiber on site and immediately frozen for transport to Mote Marine Laboratory. Sample filters were then extracted in approximately 1.5 ml of cold 98:2 methanol:ammonium acetate, sonicated in an ice bath and placed in a freezer for approximately

two hours. The extract was clarified by refrigerated centrifugation and placed in amber vials in a refrigerated auto-injector (SIL-10A, Shimadzu, Inc). The HPLC protocol was conducted according to Wright *et al..* (1991). Chromatographic peaks were detected by a photodiode array uv-vis detector (SPD-M10AVP, Shimadzu, Inc.) and identified by retention time and comparison of absorbance spectra with spectra of pigments from authentic standards and standard microalgae cultures.

The chlorophyll *a* attributed to each phylogenetic group and the percent contribution of each group to total chlorophyll *a* were derived from chemotaxonomic photopigments using ChemTax®, a software package incorporating factor analysis and a steepest descent algorithm (Mackey *et al..* 1996, and Mackey *et al..* 1997). This statistical program optimizes chlorophyll and carotenoid pigment ratios, based on published chlorophyll *a*:diagnostic pigment ratios within the representative phylogenetic groups, in order to determine phytoplankton community composition. For this project, ChemTax routines followed the guidance of Latasa (2007), and initial pigment ratios for freshwater phytoplankton were incorporated from Schlüter *et al..* (2006).

RESULTS

Water Quality Variables

Nutrient and other water quality data are presented in Table 2a. Salinity was low but detectable at all stations, generally ranging between 1.6 and 2.8 g/kg – the highest salinity, 6.4 g/kg, was measured at WQ5 (Tarpon Bay Weir). In addition, all locations along the Sanibel River were characterized by very low levels of dissolved oxygen, and only WQ5 exhibited a value above 2.5 mg/L.

Nitrate and nitrite-nitrate concentrations were below detection limit for every station, as is generally the case in the Sanibel River. In contrast, all stations had detectable amounts of ammonia except for the Tarpon Bay Road station. Therefore, ammonia is likely the more important source of nitrogen for phytoplankton biomass.

The ambient monthly monitoring program recorded very high total nitrogen and total phosphorus concentrations in all stations along the Sanibel River, where significantly higher nutrients were consistently observed in the eastern sub-basin than in the western sub-basin (TN west mean 1.8 mg/L; east mean 2.4 mg/L. t-test: $p < 0.05$, n=4, df=3. TP west mean 0.07 mg/L; east mean 0.15 mg/L. t-test: $p < 0.05$, n=4, df=3).

The ambient monitoring program also recorded very high chlorophyll concentrations at all locations, where observations at five of six stations were between 29.1 and 76.7 µg/L. The highest chlorophyll concentration of 197 µg/L was recorded at WQ8. However, in spite of this very high value, there was no significant difference between chlorophyll concentrations measured in each sub-basin (t-test: $p = 0.28$, n=4, df=3; Dimmick Dr. and Tarpon Bay Rd. as measured by the RECON were included in the analysis). When considering relationships both in each sub-basin and along the length of the river, chlorophyll *a* concentrations seemed to be more closely related to total phosphorus concentrations than to total nitrogen (Figure 2).

Finally, CDOM (colored dissolved organic matter) concentrations, which reflect the presence of organic compounds such as humics and tannins, were also high, and significantly greater in west sub-basin stations than in east sub-basin stations (means 140 and 110 QSDE, respectively. t-test: $p< 0.05$, n=4, df = 3).

Stable Nitrogen Isotopes

The lightest $\delta^{15}N$ ammonia we recorded was -8.89‰ from our 15-0-15 fertilizer sample (Table 2b); the average for all fertilizer samples was -7.7‰. $\delta^{15}N$-ammonia in AFAC (after filtration after chlorination) water was 8.0‰. $\delta^{15}N$-ammonia in AFBC (after filtration before chlorination) water was our “heaviest”

value, at 30.5‰, but this water is not released off site. Therefore, our nutrient source isotope ratio "bounds" were -8.9‰ for "inorganic" sources such as fertilizer, and 8.0‰ for "organic" sources (Fig. 3).

Across all water samples, δ^{15}N-nitrate ranged from -7.5‰ (WQ5) to 5.4‰ (Donax WRF core). Ammonia δ^{15}N in surface water samples ranged from 1.9‰ (Dimmick) to 6.1‰ (WQ7), while nitrate δ^{15}N values in the Sanibel River ranged from -7.5‰ (WQ5) to 4.7‰ (WQ7), although most observations were above 0‰. Organic matter δ^{15}N ranged from -0.04‰ (*Rhizophora mangle* leaves) to 6.8‰ (wastewater treatment AFAC). Suspended particulate organic matter δ^{15}N sampled from the Sanibel River ranged from -0.14‰ (Dimmick Dr.) to 4.5‰ (WQ6). Unfortunately, AFBC and AFAC samples as prepared contained too much nitrogen for accurate δ^{15}N-nitrate analysis.

An analysis of the proportions of nutrients from each source based on δ^{15}N-ammonia revealed a difference between western and eastern sub-basins, but trends from upstream to downstream locations in either sub-basin were not evident (Table 3). Between a quarter and a third of available ammonia in the western sub-basin may have originated from an inorganic source such as fertilizer. This proportion drops to 16% or below in the eastern sub-basin, with the majority of ammonia likely coming from organic sources.

SPOM (suspended particulate organic matter, including phytoplankton), ammonia, and nitrate δ^{15}N ratios in the Beachview re-use irrigation pond reflected the Donax WRF isotope ratio of AFAC effluent which is stored there. Nutrient isotope ratios in the two storm water swales along Periwinkle generally suggested an organic source, although δ^{15}N-ammonia in the Church swale was unusually light, suggesting nearly half of the ammonia in that location may have come from fertilizer. Similarly, isotope ratios of streamside vegetation collected along the river at Tarpon Bay Road, including red mangrove, buttonwood, and cat-tail, may reflect either ammonia or nitrate as a nutrient source.

As noted above, ammonia was the more abundant nitrogen nutrient at nearly all locations, as demonstrated by data collected by the ambient surface water sampling program. Recognizing that phytoplankton biomass will generally exhibit a δ15N-SPOM ratio a few permil lighter (but not heavier) than its nutrient source, δ^{15}N-ammonia and δ^{15}N-nitrate data confirm that phytoplankton, as a component of SPOM, probably assimilate more ammonia than nitrate at most locations, particularly in the eastern sub-basin (Fig. 3).

Phytoplankton, Chlorophyll, and Photopigments

Pigment and ChemTax® analyses were conducted on samples from eight stations along the Sanibel River (Table 4). In both west and east sub-basins, upstream stations were dominated by chrysophytes. And in both sub-basins, there was an upstream to downstream shift in proportional dominance, from chrysophytes to dinoflagellates and/or cyanophytes. This shift is more dramatic in the eastern basin: chrysophytes were replaced by dinoflagellates and cyanophytes by the second station downstream, and to a greater degree. Diatoms were present at only one station sampled during this project, WQ5, in the western sub-basin.

DISCUSSION

Stable nitrogen isotope signatures of both ammonia and nitrate sampled from the eastern sub-basin were generally closer to our AFAC δ^{15}N ammonia observations than those in the western sub-basin (Fig. 3), indicating that a greater proportion of the nutrients in these areas originated from organic sources – WRF effluent, septic tank effluent, or pet waste – than from fertilizer, though we did observe some exceptions. Isotope signatures in the western sub-basin were lighter than those in the eastern sub-basin, suggesting that a slightly higher proportion of nutrients in those areas may be from fertilizer. Overall, though, nutrients from organic sources are more abundant than nutrients from fertilizer sources. However, this result should not be confused with the *concentrations* of nitrogen-nutrients. Both nitrogen and phosphorus concentrations were greater in the eastern sub-basin than the western.

The mass balances calculated for contributions of fertilizer and organic sources of ammonia to total ammonia observed in the Sanibel River suggest that fertilizers currently play a significant but subordinate role to organic sources of ammonia. Furthermore, our results have provided a baseline against which future monitoring projects may be able to compare the efficacy of management actions such as the fertilizer ordinance or timing of irrigation using WRF effluent on the overall reduction of nitrogen nutrients in the Sanibel River. For example, it would be useful to measure $\delta^{15}N$-ammonia during other times of the year, *e.g.* outside of the restricted fertilizer use months, or later in the rainy season. A decrease in the $\delta^{15}N$ ratio (lower number), or a change in proportions supplied by each source (*cf*. Table 3) would suggest fresh nutrients from fertilizer are reaching the Sanibel River.

The contribution of this method to TMDL (total maximum daily load) calculations would require estimates of sub-basin areas as well as stream flow and total volume of water in reaches within the Sanibel River. In addition, the results from the analysis of proportions by mass balance should be considered in light of the fact that two potential sources of nitrogen-nutrients were not quantified in this study, namely atmospheric deposition, and autochthonous processing of dissolved organic matter processed by bacteria. The potential contributions from all likely sources of nutrients into this system should be considered when constructing a mass balance to support TMDL calculation.

$\delta^{15}N$ of *Rhizophora mangle* leaves collected along the river at the Tarpon Bay Road station (furthest upstream in the eastern sub-basin) may reflect either ammonia or nitrate as a nutrient source for these plants. Hale and Duffey (in review) also analyzed mangrove leaves for $\delta^{15}N$ along the Peace River, upstream from Punta Gorda – their results of approximately 5 ‰ suggest higher proportion of organic sources of nitrogen. However, a number of differences exist between the Sanibel River and Peace River (*e.g.* salinity and tidal influence, sediment substrate), and comparisons across these systems should be made with caution. Hale and Duffey (in review) also sampled young leaves from stream-side willow (*Salix* sp.) along Peace River basin creeks and rivers, noting a generally increasing trend downstream in leaf $\delta^{15}N$ in creeks, but slightly decreasing trend downstream in leaf $\delta^{15}N$ along the Peace River. These trends may reflect changes in nitrogen concentration (and degree of nutrient limitation), or hydroperiod other soil sediment characteristics. If future collections of stream-side vegetation are made along the Sanibel River, these considerations should be kept in mind.

More important, though, is the contribution of stream-side vegetation to the overall amount of organic matter in the river. Mangrove, buttonwood, cat-tail, willow (*Salix* sp.) and other species no doubt contribute considerable leaf litter to the river, particulate organic matter which, over time, becomes part of the pool of dissolved organic matter adding color, as well as organic nitrogen phosphorus compounds to the river, compounds which are eventually used by bacteria and phytoplankton (Carlsson and Graneli 1993). Compared to the $\delta^{15}N$ of WRF effluent, these “organic” sources of nitrogen would appear isotopically “lighter” to decomposing bacteria, but “heavier” than ammonia from fertilizer. This internal nutrient cycling of dissolved organic matter likely accounts for some fraction of the ammonia that is consistently observed in the Sanibel River.

As an indicator of the presence of humic substances dissolved in water, CDOM (colored dissolved organic matter) values measured by the RECON unit deployed in the Sanibel River for this project (140 and 110 QSDE in western and eastern sub-basin, respectively) are comparable to continuous measurements made by SCCF-Marine Lab instruments in the Caloosahatchee River at Moore Haven and Ft Myers. Most of the phytoplankton groups identified by this project are capable of utilizing a variety of nutrient sources, both inorganic (*e.g.* nitrate, ammonia) and organic (*e.g.* dissolved organic nitrogen compounds). The naturally high concentrations of humic compounds mean that even as nutrient inputs from fertilizers or WRF effluent are controlled and reduced by management activities, phytoplankton (*i.e.* concentration of chlorophyll) will remain abundant for some time. Given the observations of seasonal differences in water color made by the City of Sanibel‘s ambient water quality monitoring program, further analysis of the relationship between humic substances and other components of water quality would be useful.

There is some debate as to whether nutrient reduction programs intended to reduce phytoplankton abundance should focus on phosphorus, nitrogen, or both nutrients (refs. in Smith 2006; Paerl *et al..*

2007, 2008). Although phytoplankton in the Sanibel River seem to assimilate more ammonia than nitrate, chlorophyll *a* concentrations seem to be more closely related to total phosphorus than total nitrogen, at least in the eastern sub-basin. These conditions are similar to 300 coastal locations around Florida (Hoyer *et al.* 2002) and 354 Florida lakes (Brown *et al.* 2000). Phosphorus is naturally abundant in many Florida soils, and Sanibel's fertilizer ordinance recognizes this by including a restriction against fertilizers containing more than 2% phosphorus by weight. However, the distribution of total phosphorus along the Sanibel River is not random, and seems to increase from west to east, reaching its peak at SAN-WQ8 (Fig. 3), well within that part of the eastern sub-basin that has experienced the most urban development. This suggests an anthropogenic source of phosphorus.

As pointed out earlier, the same nutrient management actions taken by the City of Sanibel that are intended to reduce nitrogen should also reduce the delivery of new phosphorus to the Sanibel River. However, a study of management actions intended to reduce the concentration of chlorophyll focused solely on the control and ambient concentration of nitrogen-nutrients will underestimate (or miss) the influence of phosphorus on phytoplankton, which may lead to an unfavorable shift in phytoplankton community composition over time (Paerl *et al.* 2008).

Therefore, the obvious patterns in phytoplankton community composition identified by this project are important to recognize. In addition to the availability of nutrients and light, another control on phytoplankton abundance (as concentration of chlorophyll) is the rate of grazing by zooplankton. Upstream stations exhibit dominance by chrysophytes, which are among the phytoplankton taxa that are preferred by zooplankton, and so contribute to a healthy aquatic ecosystem (after Paerl *et al.* 2003, 2007). However, zooplankton do not consume all types of phytoplankton evenly; diatoms, chrysophytes, and cryptophytes are generally favored over dinoflagellates and cyanophytes.

Dinoflagellates and cyanophytes are proportionately more abundant at the downstream stations in each sub-basin, and grazing by zooplankton may exert less control over phytoplankton abundance in these locations. For example, Livingston (2007) found an association between high dinoflagellate abundance and low invertebrate (*e.g.* zooplankton) and fish populations in a northern Florida estuary, an observation with obvious implications in the context of the IWR (Impaired Waters Rule). In addition, cyanophytes (a.k.a. blue-green algae, or cyanobacteria) have the unusual capacity to acquire nutrient-nitrogen from atmospheric nitrogen. Therefore, in cases where low ammonia or nitrate concentrations (relative to phosphorus) may limit the growth of other types of phytoplankton, cyanophytes may access a limitless supply of nitrogen, become the dominant phytoplankton taxa, insensitive to nutrient reduction actions focused on nitrogen alone (*e.g.* Paerl *et al.* 2008). This may be the case in at least one station along the Sanibel River, WQ5, and the high phosphorus concentration observed at WQ8 suggests more potential for this scenario.

Therefore, although nutrient reduction measures may successfully decrease the amount of nutrients in the Sanibel River, phytoplankton abundance may remain high for several reasons. First, if the phytoplankton community shifts to dominance by cyanophytes (a group that is less dependant on nitrogen nutrients), chlorophyll concentration will remain high. Next, internal cycling of naturally occurring organic matter (as indicated by color, CDOM, etc) will continue to provide organic nutrients for most of the phytoplankton groups detected in the Sanibel River. Finally, dominance by less palatable phytoplankton taxa, *e.g.* dinoflagellates and cyanophytes, suggests less grazing by zooplankton.

To address these concerns, the results of our "nutrient source – phytoplankton community" paired analyses may be applied to a number of future monitoring topics. To begin with, collecting samples representing seasonal differences in water quality conditions should greatly extend the predictive capacity that can be associated with management actions targeted at nutrient reduction. For example, three specific research topics that may help address this goal of predictability include: (a) wet v dry season persistence of nutrient availability, especially with respect to fertilizer use; (b) the influence of storm events (*i.e.* first flush) on nutrient availability and subsequent use by phytoplankton groups (*i.e.* fast vs. slow growing taxa); and (c) the influence of specific habitats or landforms comprising upstream areas (*e.g.* light residential development, marsh lands, restored stream banks, conservation areas) on downstream water quality variables, including inorganic and total nutrients; phytoplankton abundance;

dissolved organic matter; and dissolved oxygen (the possible relationship between these latter two variables is discussed below).

A number of other important questions may be addressed by future phytoplankton community analysis. For instance, does the phytoplankton community change with increased chlorophyll concentration in the Sanibel River, which almost doubles from dry season to wet season? Does the phytoplankton community change with concentration of total nitrogen and total phosphorus, which increase by 50% - 75% (respectively) from dry to wet seasons? Nutrient-isotope-algae studies can be designed to distinguish between phytoplankton size classes (*sensu* Hollander and Peebles 2004), and a size-fractionated version of our pilot project could be easily adapted to locations along the Sanibel River. Therefore, to provide a stronger case for predicting the efficacy of nutrient reduction actions, we recommend repeating this project in different months or seasons during the year – May or June, for example, to represent the end of the dry season and the end of the "fertilizer" season; and October, to represent the end of the wet season, when very little of the nitrogen-nutrients should be traced to fertilizer.

Finally, dissolved oxygen levels were below the state standard of 5 mg/L at all but one station during our sampling event (Table 2a). Given the preceding discussion of nutrients, phytoplankton and dissolved organic matter, an additional topic regarding the water quality issues raised by the IWR should be addressed: how does dissolved organic matter influence the concentrations of total nitrogen, total phosphorus, and dissolved oxygen? Chlorophyll concentration in the Sanibel River is very high, implying a great capacity to produce oxygen, but photosynthesis may be limited by color (*e.g.* tannins), turbidity from non-algal particles, or by the abundant phytoplankton cells themselves. The question then becomes one of the oxygen required by microbes, bacteria, and several of the phytoplankton taxa identified by this project when decomposing organic matter.

The decomposition of CDOM into nutrients and substrate for bacteria (Carlsson and Graneli 1993) consumes oxygen. However, a portion of CDOM originating from a watershed is flocculated (becomes particulate) by salinity (Hall 2004), perhaps removing it from use by bacterial and phytoplankton, and conceivably reducing the demand for dissolved oxygen. We may have observed this: our lowest CDOM in the western sub-basin occurred at WQ5, corresponding with the highest salinity (6.4 g/kg) and highest dissolved oxygen (5.4 mg/L) for all stations. The interaction between CDOM and salt water may have influenced dissolved oxygen concentration (though nutrients did not decrease). McCallister *et al*. (2006a and 2006b) describe methods used to determine the processes involving particulate and dissolved organic matter in estuaries using a combination of stable isotope analyses similar to the present study in combination with other biochemical techniques. While these topics may appear removed from "water quality" as defined by the IWR, the relationships between nitrogen and phosphorus, phytoplankton, and dissolved organic matter that have been illustrated by this project are complex, and will require additional study.

In closing, it should be noted that the Sanibel River is managed for several purposes in addition to the IWR mandate describing "recreation, and propagation and maintenance of a healthy, well-balanced population of fish and wildlife". For example, water levels in the river are monitored at least once per week, and manipulated to reduce the potential for flooding during the rainy season. Therefore, we believe future studies of Sanibel River water quality issues should be made in the context of the integrated management of surface water nutrients in conjunction with other water quality variables.

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FIGURES AND TABLES

Figure 1.
Sanibel Island, with selected sections of the Sanibel River (blue outline), and 11 sampling locations identified. From west to east: SANWQ3 (at Gulf Pines), Dimmick Dr., SANWQ4 (at Rabbit Rd), SANWQ5 (at Tarpon Bay Weir), Sanibel River at Tarpon Bay Road ("SRTBR"), two storm water swales along Periwinkle Way ("PERIWK" and "CHURCH", see text), SANWQ6 (at Casa Ybel Rd), Beachview Golf Course ("GOLF"), SANWQ7 (near Donax Rd WRF), and SANWQ8 (at Beach Road Weir).

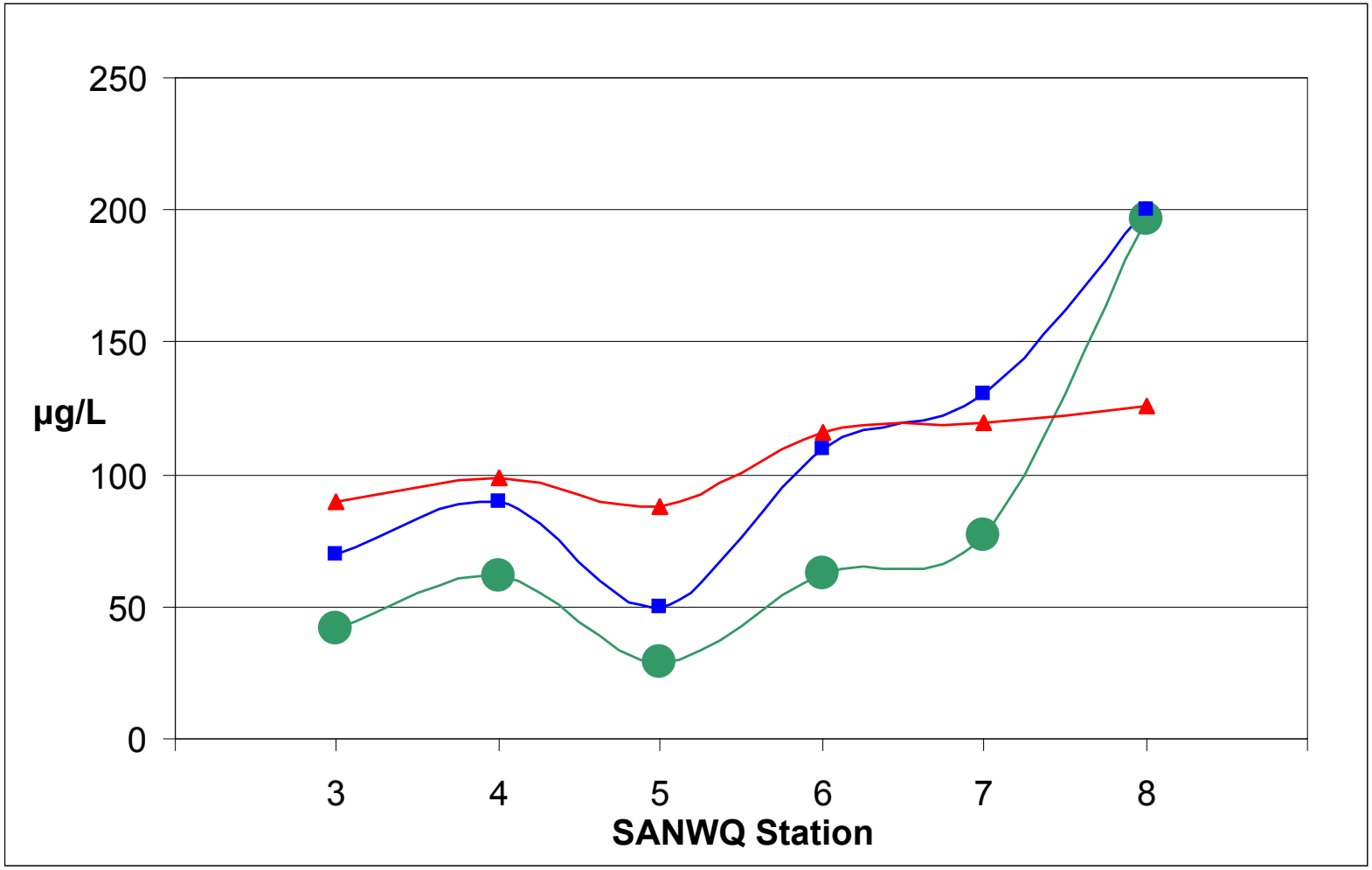

Figure 2.
Relationships among concentrations of total nitrogen (▲, TN*50), total phosphorus (■, TP*1000), and chlorophyll a (●) collected at ambient monitoring stations along the Sanibel River. Stations 3, 4, and 5 are in the western sub-basin; stations 6, 7, and 8 are in the eastern sub-basin.

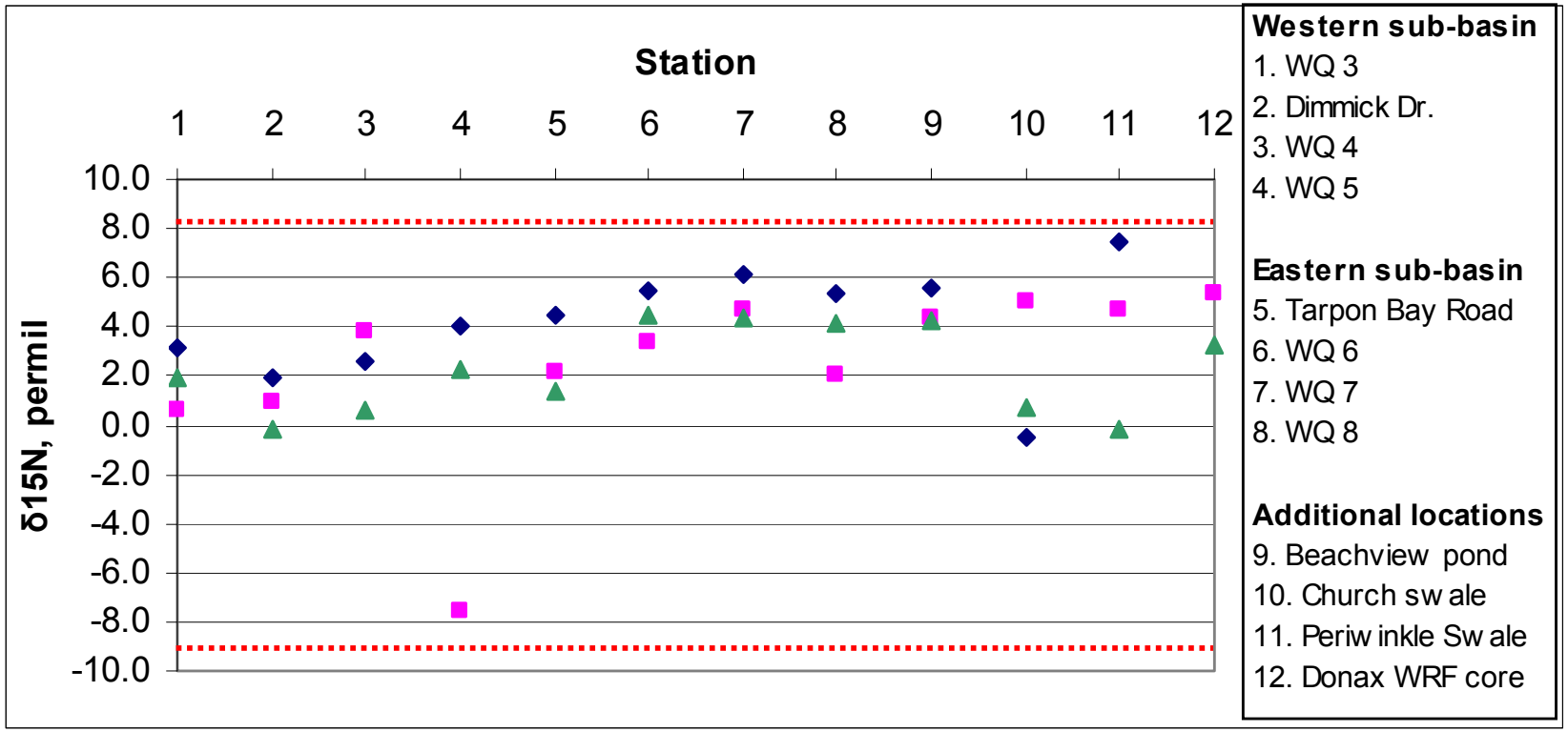

Figure 3.
Mean stable nitrogen isotope ratios for ammonia (♦), nitrate (■), and SPOM (▲) measured at Sanibel River stations (1-8), and 4 additional locations (9-12). Dashed red lines (-------) indicate heavy and light bounds on potential $\delta^{15}N$ as defined by analysis of fertilizer and reclaimed water.

Table 1.
Station names, general locations, and types of samples collected at each.

Station/ Source	Water Quality		Stable Nitrogen Isotope, $\delta^{15}N$			Phytoplankton Community
	Ambient	RECON	Ammonia	Nitrate	SPOM	HPLC - ChemTax
Western Sub-basin						
Sanibel River WQ3	x	x	x	x	x	x
Sanibel River at Dimmick Dr.		x	x	x	x	x
Sanibel River WQ4	x	x	x	x	x	x
Sanibel River WQ5	x	x	x	x	x	x
Eastern Sub-basin						
Sanibel River at Tarpon Bay Rd		x	x	x	x	x
Rhizophora mangle					x	
Conocarpus erectus					x	
Typha sp					x	
Filamentous algae					x	
Sediment organic matter					x	
Sanibel River WQ6	x	x	x	x	x	x
Sanibel River WQ7	x	x	x	x	x	x
Sanibel River WQ8	x	x	x	x	x	x
Additional locations						
Donax WRF core			x	x	x	
Beachview Golf Course pond			x	x	x	
Beachview Golf Course SAV					x	
Church swale			x	x	x	
Periwinkle swale			x	x	x	
Nutrient Sources						
Donax WRF AFBC			x	x	x	
Donax WRF AFAC			x	x	x	
15 0 15 fertilizer			x			
6 6 6 fertilizer			x			

Table 2a.
Selected Sanibel River ambient surface water quality monitoring data collected July 28, 2008. [a] station and/or physical data collected by RECON; [b] nutrient data analyzed by Lee Count Environmental Laboratory. Abbreviations: Temp, temperature; Sal., salinity; Sp. Cond., specific conductivity; DO, dissolved oxygen; TN, total nitrogen; TKN, total Kjeldahl nitrogen; NH4-N, ammonium ion nitrogen; NO23-N, nitrate-nitrite nitrogen; TP, total phosphorus; Ortho-P, orthophosphorus; Chl.a, chlorophyll a; TOC, Total organic carbon; TSS, total suspended solids; TURB, turbidity.

Station	Temp. [a] (°C)	Sal. (G/KG)	Sp. Cond. (umhos/cm)	DO [a] (mg/L)	CDOM [a] (QSDE)	TN (mg/L)	TKN (mg/L)	NH4-N (mg/L)	NO23-N (mg/L)	TP (mg/L)	Ortho-P (mg/L)	Chl. *a* (mg/m^3)	TOC (mg/L)	TSS (mg/L)	TURB (NTU)
WQ3	28.9	2.1	3650	0.9	144.4	1.8	1.8	0.063	BDL	0.067	0.02	41.6	33.76	5.6	3.4
Dimmick Dr. [a]	29.3	2.6	5197	1.1	147.2			0.022 [b]	BDL [b]			29.4			4.5
WQ4	30.5	2.8	4810	2.5	140.5	1.98	1.98	0.076	BDL	0.088	0.029	61.9	47.5	9.6	3.5
WQ5	29.7	6.4	10600	5.4	127.7	1.75	1.75	0.062	BDL	0.047	0.027	29.1	43.2	3.2	2.5
Tarpon Bay Rd [a]	28.1	1.8	3600	0.1	110.6			BDL [b]	BDL [b]			25.2			19.4
WQ6	29.4	1.7	3110	0.9	108.6	2.32	2.32	0.073	BDL	0.109	0.035	62.7	36.7	10.1	6
WQ7	30.7	1.6	2960	1.1	109.9	2.39	2.39	0.066	BDL	0.129	0.04	76.7	32	10.7	6.7
WQ8	29.0	1.7	3080	0.8	110.4	2.52	2.52	0.109	BDL	0.204	0.038	197	31.9	13.7	7.9

Table 2b.
Mean stable nitrogen isotope ratios ($\delta^{15}N$) of ammonia (NH_4), nitrate (NO_3), and suspended particulate organic matter (SPOM) collected by location on July 28, 2008.

Station/ Source	$\delta^{15}NH_4$ (‰)	$\delta^{15}NO_3$ (‰)	$\delta^{15}N$ SPOM (‰)
Western Sub-basin			
Sanibel River WQ3	3.2	0.63	1.9
Sanibel River at Dimmick Dr.	1.9	0.99	-0.1
Sanibel River WQ4	2.6	3.8	0.6
Sanibel River WQ5	4.0	-7.5	2.2
Eastern Sub-basin			
Sanibel River at Tarpon Bay Rd	4.5	2.1	1.4
Rhizophora mangle			-0.04
Conocarpus erectus			2.5
Typha sp			0.6
Filamentous algae			2.3
Sediment organic matter			-0.2
Sanibel River WQ6	5.4	3.4	4.5
Sanibel River WQ7	6.1	4.7	4.3
Sanibel River WQ8	5.3	2.0	4.1
Additional locations			
Donax WRF core		5.4	3.2
Donax WRF sediment OM			5.6
Beachview Golf Course pond	5.6	4.4	4.3
Beachview Golf Course SAV			0.5
Church swale	-0.5	5.0	0.7
Periwinkle swale	7.5	4.7	-0.1
Nutrient Sources			
Donax WRF AFBC	30.4		
Donax WRF AFAC	8.0		6.8
15 0 15 fertilizer	-8.9		
6 6 6 fertilizer	-10.6		

Table 3.
Mass balance table for ammonia based on mean $\delta^{15}N$-ammonia at each station in the Sanibel River.

Sample Location	Observed ammonia		Predicted fractions		Predicted contribution to concentration	
	$\delta^{15}N$, ‰	Concentration, µg/l	% ammonia from fertilizer	% ammonia from organic sources	µg/l ammonia from fertilizer	µg/l ammonia from organic sources
Western sub-basin						
WQ3	3.2 ‰	63 µg/l	28%	72%	18 µg/l	45 µg/l
Dimmick Dr.	1.9	22	36	64	8	14
WQ4	2.6	76	32	68	24	52
WQ5	4.0	62	24	76	15	47
Eastern sub-basin						
Tarpon Bay Road	4.5	BDL				
WQ6	5.4	73	15	85	11	62
WQ7	6.1	66	11	89	7	59
WQ8	5.3	109	16	84	17	92

Table 4.
Proportional contributions to chlorophyll a concentration made by eight phytoplankton taxa.

Sample Location	Diatoms	Haptophytes	Euglenophytes	Cryptophytes	Chrysophytes	Chlorophytes	Dinoflagellates	Cyanophytes
Western sub-basin								
WQ 3	0	0	9.5	15.6	47.7	7	2.2	18
Dimmick Dr.	0	3.7	16.8	8.8	58.8	1.5	3.2	7.2
WQ 4	0	12.4	8.7	6.5	63.8	0.9	2.2	5.4
WQ 5	10.4	0	0.3	30.2	6.8	11.6	7.2	33.4
Eastern sub-basin								
Tarpon Bay Rd.	0	4.5	0	2.3	88.1	5	0.2	0
WQ 6	0	3	3.1	10.2	33.4	23	7.7	19.6
WQ 7	0	2.2	3.3	9.3	22.7	17.7	30.2	14.6
WQ 8	0	0.1	2.9	12	15.4	20.3	38.8	10.4