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Responses of nitrogen stable isotopes in fish to phosphorus limitation in freshwater wetlands

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Abstract – Human-induced eutrophication has altered ecological processes in aquatic ecosystems. Detection of ecological changes is a prerequisite for protecting ecosystems from degradation. In this study, nitrogen stable isotopes ($\delta^{15}N$) in fish are evaluated as indicators of environmental changes in south Florida wetlands. Stable nitrogen isotope ($\delta^{15}N$) data of select fish species and water quality collected from the Florida Everglades between the 1990s and 2000s were used to assess the relationship between total phosphorus concentrations and $\delta^{15}N$ ratios. The $\delta^{15}N$ ratios in nine of ten select fish species increase significantly as total phosphorus concentration in the surface water increases. There were significant relationships between total nitrogen concentration in the surface water and $\delta^{15}N$ ratios in several fish species. The pattern of changes in $\delta^{15}N$ ratios along nutrient gradients suggests that increased eutrophication is recorded as the $\delta^{15}N$ ratios in fish. The lack of human wastewater loading, the dominance in agricultural runoff and the high TN:TP ratio suggest that phosphorus is the limiting factor driving ecosystem productivity and the changes of $\delta^{15}N$ ratios in fish. Results from this analysis demonstrate that $\delta^{15}N$ ratios in fish integrate biotic responses to eutrophic process over time and could be a robust indicator for early ecological changes.

Keywords: Eutrophication / Everglades / Fish / Nitrogen / Nutrient gradient / Phosphorus / Stable nitrogen isotopes / Wetlands

Résumé – Réponses des isotopes stables de l'azote chez les poissons à la limitation du phosphore dans les zones humides d'eau douce. L'eutrophisation d'origine humaine a altéré les processus écologiques des écosystèmes aquatiques. La détection des changements écologiques est une condition préalable à la protection des écosystèmes contre la dégradation. Dans cette étude, les isotopes stables de l'azote ($\delta^{15}N$) chez les poissons sont évalués comme indicateurs des changements environnementaux dans les zones humides du sud de la Floride. Les données sur les isotopes stables de l'azote ($\delta^{15}N$) de certaines espèces de poissons et sur la qualité de l'eau recueillies dans les Everglades de Floride entre les années 1990 et 2000 ont été utilisées pour évaluer la relation entre les concentrations de phosphore total et les rapports $\delta^{15}N$. Les rapports $\delta^{15}N$ de neuf des dix espèces de poissons sélectionnées augmentent de manière significative lorsque la concentration de phosphore total dans les eaux de surface augmente. On a constaté des relations significatives entre la concentration d'azote total dans les eaux de surface et les rapports $\delta^{15}N$ chez plusieurs espèces de poissons. Le schéma des changements des rapports $\delta^{15}N$ le long des gradients de nutriments suggère qu'une eutrophisation accrue est enregistrée dans les rapports $\delta^{15}N$ chez les poissons. L'absence de charge en eaux usées, la prédominance du ruissellement agricole et le rapport élevé TN:TP suggèrent que le phosphore est le facteur limitant qui détermine la productivité des écosystèmes et les changements des rapports $\delta^{15}N$ chez les poissons. Les résultats de cette analyse montrent que les rapports $\delta^{15}N$ chez les poissons intègrent les réponses biotiques au processus d'eutrophisation au fil du temps et pourraient être un indicateur robuste des premiers changements écologiques.

Mots-clés : Eutrophisation, Everglades, Poissons / Azote, Gradient de nutriments / Phosphore / Isotopes d'azote stables / Zones humides

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1 Introduction

A number of environmental indicators have been used to assess ecological processes in aquatic ecosystems. Current indicators of environmental changes for freshwater include nutrient loading such as soil and water total phosphorus (TP), total nitrogen (TN) concentrations, biodiversity and primary productivity (Jeppesen and Sammalkorpi, 2002). These indicators often reveal the changes in the impacted systems at the basal resource levels (nutrients and primary producers). Identification of signs of environmental change along higher trophic levels in aquatic food webs is critical for the restoration of disturbed systems and wildlife protection (Vander Zanden *et al.*, 2005).

Nitrogen stable isotope compositions of organic matter may be a complementary means to detect environmental changes in aquatic ecosystems. The ratios of $^{15}N/^{14}N$ (defined as $\delta^{15}N$) may provide insight into the sources, sinks and cycling of nitrogen in biota that interact with their physical and chemical environments (Peterson and Fry, 1987). The use of $\delta^{15}N$ as an indicator of aquatic eutrophication is based on the fact that increases in ecosystem productivity controlled by nutrient enrichments will lead to decreases in isotope fractionation by primary producers and the transfers of organic matter from one trophic level to another will result in predictable isotope enrichment along food chain (Post, 2002; Vander Zanden *et al.*, 2015). At present, the use of consumer $\delta^{15}N$ largely focuses on the source of nitrogen contaminations (Lake *et al.*, 2001; Vander Zanden *et al.*, 2005; Schlacher *et al.*, 2005; Robinson *et al.*, 2016; Souza *et al.*, 2018) and trophic interactions (Post, 2002; Vander Zanden *et al.*, 2015; Wang *et al.*, 2018). Very few studies link consumer isotope composition to primary productivity in freshwater ecosystems (Woodland *et al.*, 2012; Hou *et al.*, 2013).

In this study, we compared $\delta^{15}N$ ratios in 10 species of freshwater fish collected along a nutrient gradient in the Everglades, Florida, USA. The purposes of this study were (1) to understand the responses and mechanisms controlling the isotope variations along the nutrient gradient, and (2) to evaluate if fish $\delta^{15}N$ is a reliable and feasible candidate for human-induced eutrophication in freshwater wetlands. This study provides insight into isotopic responses to changing water quality.

2 Materials and methods

2.1 Site description

The Everglades is the largest subtropical peatland in the United States with its historic geochemistry and biological community characterizing an oligotrophic ecosystem (Wright *et al.*, 2008; Richardson, 2010). Since human settlement, a large portion of the Everglades peatland immediately south of Lake Okeechobee was converted into farmlands, *i.e.*, Everglades Agricultural Area (EAA). The remaining Everglades has been divided by drainage canals, levees and water control structures into three Water Conservation Areas (WCA-1, WCA-2 and WCA-3), and Everglades National Park (ENP). Discharge of EAA runoff which contains high concentrations of total phosphorus (TP) and total nitrogen (TN) has led to cattail (*Typha* spp.) invasion and replacement of the native macrophytes and periphyton (Sklar *et al.*, 2005). Increased P loads in surface water runoff have shifted portions of the ecosystem from oligotrophic to eutrophic states. As a result, TP and TN concentrations in the water column and soil near the inflow regions are elevated (Wright *et al.*, 2008).

2.2 Sources of data

Stable isotope data on fish collected from 1994 to 1999 were downloaded from the United States Geological Survey South Florida Information Access (Appendix Tab. A1). A total of 16 sites, with three sites in Stormwater Treatment Area-1 West (STA-1W) and 13 sites in the WCAs and ENP (Fig. 1) were sampled, often on multiple field trips. These sites include canals, near levee inflow and outflow structures and interior marshes. Fish were caught randomly and brought to a laboratory where muscle tissue from each fish was removed, dried and ground to fine powder for stable isotopes analysis. Additional samples of mosquitofish were collected along the nutrient gradient in WCA-2A in 2007 (Fig. 1 and Tab. 1). Muscle tissue from 3 to 5 mosquitofish was composed into a single sample per site and processed as above prior to stable isotope analysis.

Select environmental data were downloaded from DBHYDRO, a hydrometeorologic, water quality, and hydrogeologic data retrieval system managed by the South Florida Water Management District (West Palm Beach Florida, USA). Water quality data recorded one year before fish collection date were averaged to reflect environmental conditions of each habitat. When environmental data were not available from the same fish collection site, data from closely located sites were used.

Fish samples collected in the 1990s were analyzed for stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) using a Micromass Optima continuous flow mass spectrometer coupled to a Carlo Erba elemental analyzer at US Geological Survey. Results are reported in the usual delta notation relative to V-PDB for ^{13}C and air for ^{15}N. Analytical precision (1σ) based on repeat analysis of both samples was generally in the range of 0.1−0.2‰ for both C and N, but for some samples replication was no better than ±0.5 ‰ due to sample heterogeneity (Kendall *et al.*, 2005). Mosquitofish samples collected in 2007 were analyzed using a Carlo Erba Elemental Analyzer interfaced to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer (IRMS) at Florida State University. The precision of the C and N isotope analysis was ±0.2‰ (1σ) or better on the basis of repeated analysis of different laboratory standards.

2.3 Statistics

Because not all fish were found in each site, only fish found in at least 6 sites were used in this analysis. Ten species belonging to different trophic levels met this criterion. The $\delta^{15}N$ ratios of select fish species from different years of collection were pooled from a given site (Appendix Tab. A1). Spearman Rank Correlation analysis was used to establish relationships between TP, TN and molar TN:TP ratios and $\delta^{15}N$ of each fish species. All statistics were performed using SAS JMP (Version 7, SAS Institute). Statistics were considered significant at $P < 0.05$.

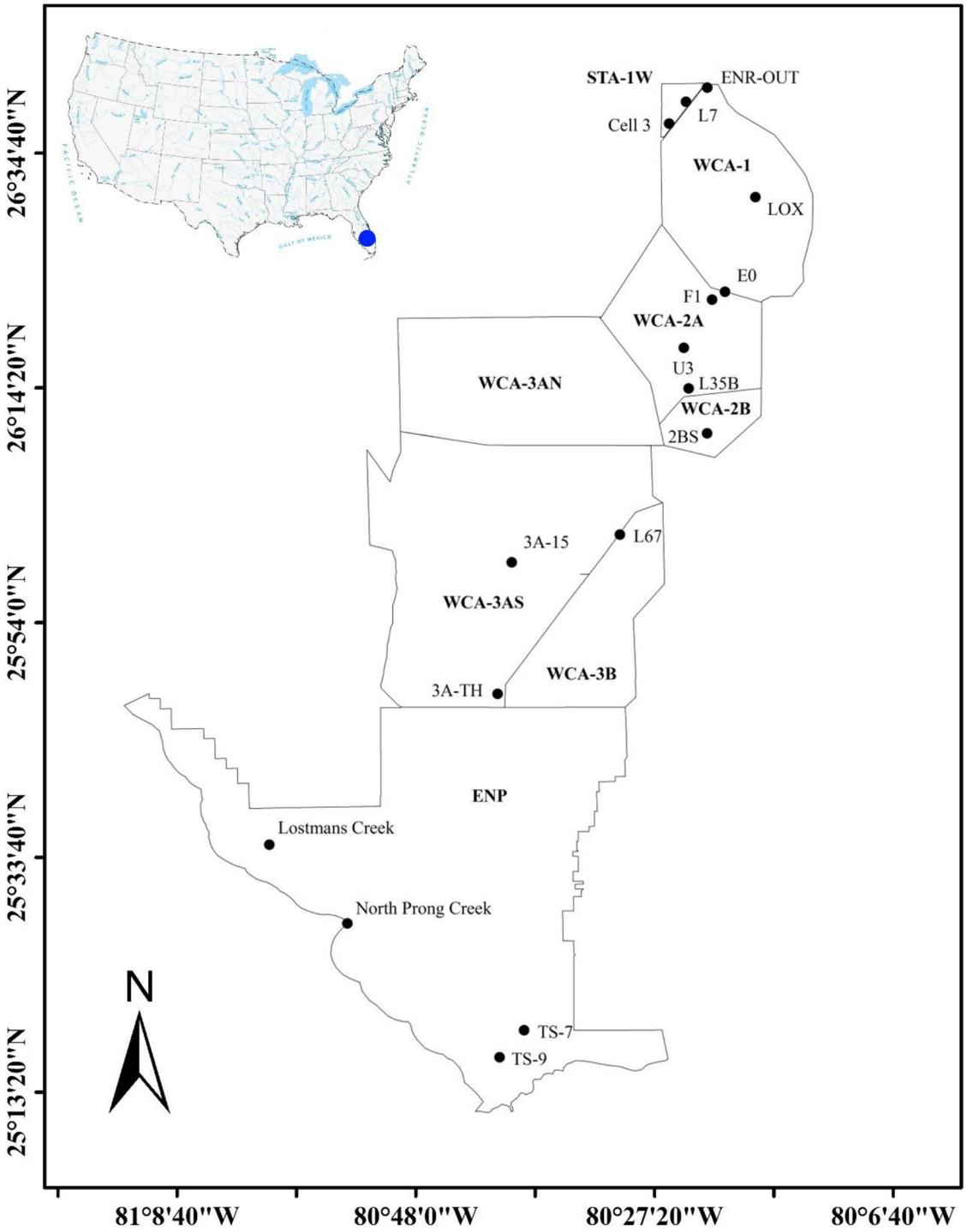

Fig. 1. Map showing sampling sites in Stormwater Management Area 1 West (STA-1W), Water Conservation Areas (WCAs) and Everglades National Park (ENP) of south Florida. The insert indicates location of the study site in the USA.

3 Results

3.1 Environmental conditions

The 16 study sites received water with considerably different concentrations of nutrients and other chemical compounds (Tab. 1). Study sites at Cell 3 and Cell 4 of the STA-1W, and E0 and F1 of WCA-2A, which received direct EAA discharges are highly enriched with TP ($>40\ \mu g\ L^{-1}$). Study sites located immediately downstream of STA-1W, WCAs or close to interior canals are moderately enriched with TP (>10 and $<30\ \mu g\ L^{-1}$). Interior marshes in WCAs and ENP typically maintain the oligotrophic state indicated by a low TP concentration ($<10\ \mu g\ L^{-1}$). Total nitrogen (TN) and dissolved inorganic nitrogen (DIN) concentrations were also higher at the near inflow sites than the marsh interior sites, except for

Table 1. Averages of environmental variables for each study site during the study period. Sites are generally listed from north to south.

Site	pH SU	DO mg L^{-1}	TP μg L^{-1}	SRP μg L^{-1}	NH_4^+ mg L^{-1}	NO_x^- mg L^{-1}	TN mg L^{-1}	TN:TP Molar ratio
STA-W Cell 3*	7.4	2.8	42	16	0.13	0.051	2.049	108
STA-W Cell 4*	7.1	1.5	46	12	0.169	0.026	2.071	99
ENR outflow*	7.4	3.6	24	8	0.047	0.061	2.271	206
L7	7.4	3.6	24	8	0.047	0.061	2.271	206
LOX*	6.4	3.1	8	1	0.023	0.007	1.208	322
E0	7.1	2.0	74	41	0.186	0.29	2.97	89
F1	7.2	1.7	70	24	0.575	0.061	2.861	91
U3*	7.5	4.8	5	2	0.269	0.006	2.177	964
L35B	7.2	4.1	13	5	0.017	0.038	1.748	298
2BS	7.3	3.9	21	4	0.049	0.058	1.541	162
L67*	7.0	2.5	15	5	0.34	0.086	1.648	238
3A15*	7.1	2.9	6	1	0.036	0.008	1.016	401
3ATH	7.2	3.6	13	4	0.032	0.063	1.377	242
TS-7	7.7	7.0	4	3	0.058	0.02	0.775	446
TS-9	7.7	7.0	4	3	0.058	0.02	0.775	446
North Prong Creek	7.4	4.0	9	3	0.029	0.043	1.327	326

Note: DO: dissolved oxygen. TP: total phosphorus. SRP: soluble reactive phosphorus; TN: total nitrogen. Averages are calculated from weekly to biweekly samples taken between 1990 and 1999.

Table 2. List of fish species and mean $\delta^{15}N$ ratios used in this analysis.

Common name	Scientific name	Mean	SD	N
Sailfin molly	*Poecilia latipinna*	8.4	2.4	49
Golden topminnow	*Fundulus chrysotus*	9.3	2.9	43
Mosquitofish	*Gambusia holbrooki.*	10.6	2.8	473
Bluefin killifish	*Lucania goodei*	10.6	2.8	72
Least killifish	*Heterandria formosa*	10.8	3.0	171
Spotted sunfish	*Lepomis punctatus*	9.3	1.1	81
Redear sunfish	*Lepomis microlophus*	8.8	1.5	76
Bluegill	*Lepomis macrochirus*	9.8	1.5	88
Largemouth bass	*Micropterus salmoides*	11.1	1.5	576
Florida gar	*Lepisosteus platyrhincus*	11.6	1.3	56

U3, the interior site of WCA-2A, which is enriched with ammonium and TN. Theses study sites are also generally characterized by above-neutral pH, high alkalinity, and low dissolved oxygen (DO) concentrations, with an exception of an interior site in WCA-1. This is a rain-driven system, where pH values are low (Tab. 1).

3.2 Fish ecology and $\delta^{15}N$ ratios

Species described in Table 2 represent major fish assemblage in the subtropical wetlands. They are either omnivorous, feeding on both algae, aquatic macrophytes and invertebrates (killifish, golden topminnow, mosquitofish and sailfin molly), primary consumers, feeding on aquatic insects (bluegill and spotted sunfish), snails (redear sunfish) or piscivores (largemouth bass and Florida gar).

Average $\delta^{15}N$ ratios for each species collected from multiple sites and years ranged from 8.4‰ in sailfin molly to 11.6‰ in Florida gar. In general, $\delta^{15}N$ ratios reflect the trophic position of each species. For example, both the Florida gar and largemouth bass which are piscivores displayed higher $\delta^{15}N$ ratios than all other species preying on lower trophic levels. Omnivorous species depending on both primary producers and invertebrates typically show low $\delta^{15}N$ ratios. It is surprising that the least killifish, bluefin killifish and mosquitofish which are reported depending partially on primary producers had higher $\delta^{15}N$ ratios than those reportedly true primary consumers such as the three sunfish species.

3.3 Patterns of fish $\delta^{15}N$ along the nutrient gradient

Fish $\delta^{15}N$ ratios selected in this analysis generally increased with the increases in TP concentrations (Figs. 2 and 3). Except for the golden topminnow, all other fish had significant correlation between water column TP concentrations and $\delta^{15}N$ ratios (Tab. 3). Significant correlation between TN concentrations and fish $\delta^{15}N$ ratios were found in five species (Tab. 3). Nearly all fish displayed a decline in $\delta^{15}N$ ratios with increases in molar TN/TP ratio although only seven species displayed significant correlation (Tab. 3). Highly significant correlations ($p < 0.001$) between TP, TN, TN/TP ratio and $\delta^{15}N$ ratios were found in the sailfin molly,

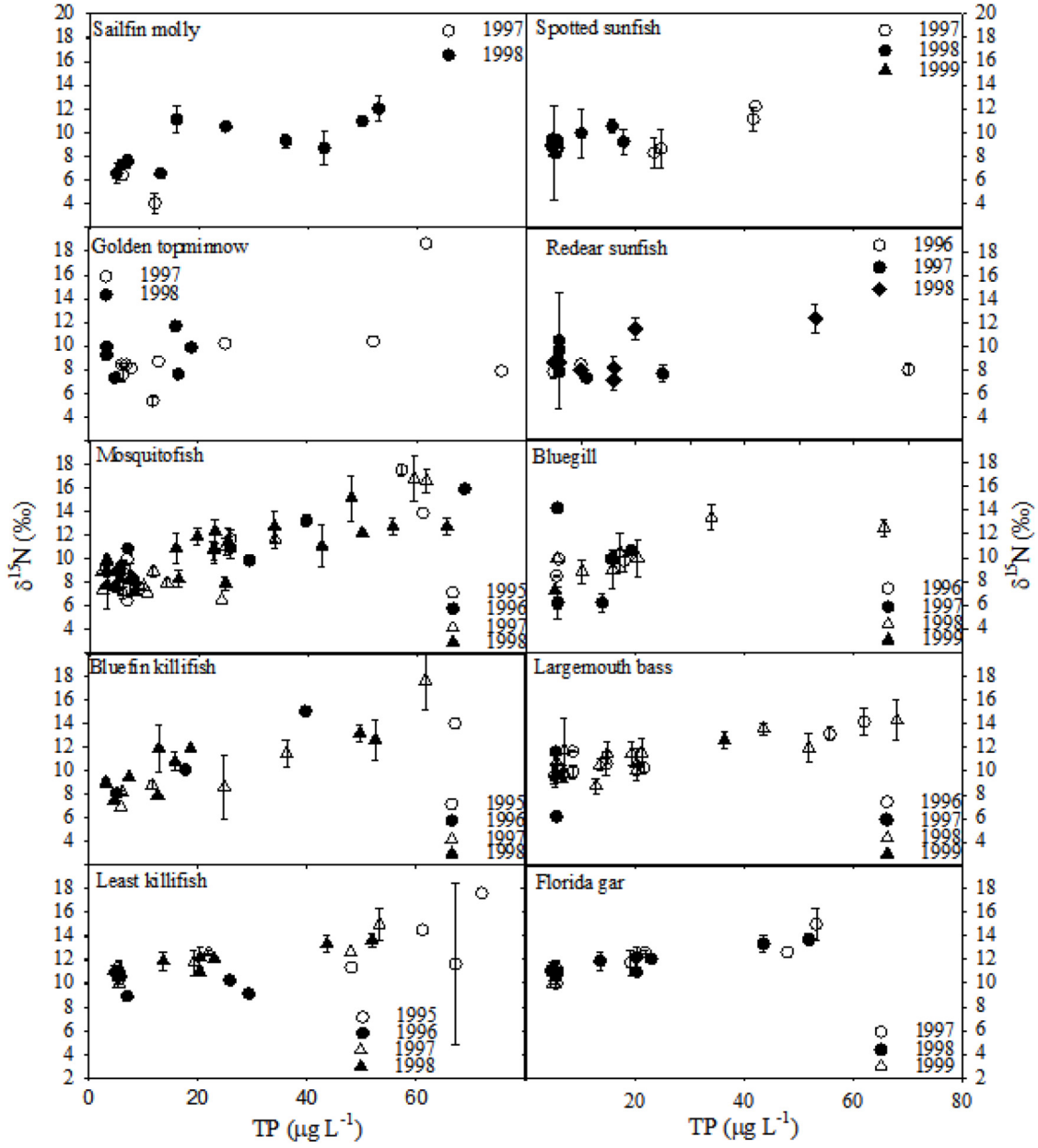

Fig. 2. $\delta^{15}N$ ratios (mean ± SD) of ten fish species along the TP concentration gradient in the Everglades wetlands. Each dot represents data from a specific date of sample collection in the 1990s.

mosquitofish, least killifish, largemouth bass and Florida gar (Tab. 3). The $\delta^{15}N$ ratios of mosquitofish samples collected from seven study sites in 2007 were plotted against TP concentrations (Fig. 4). A highly significant relationship between nutrients and $\delta^{15}N$ ratios ($p < 0.001$) was also found in these samples.

The $\delta^{15}N$ ratios of mosquitofish and least killifish were available in all monitoring sites from WCA-2 (Fig. 1) and plotted along with TP concentrations collected for each site (Fig. 5). The $\delta^{15}N$ ratios of both fish and TP concentrations were considerably higher in both inflow (E0) and near inflow (F1) sites than those in the interior site (U3) of WCA-2A and near a canal site (L35B) and interior (2BS) WCA-2B which displayed similar $\delta^{15}N$ ratios but variously low TP concentrations.

4 Discussion

Findings from this analysis are consistent with previous studies, which reveal positive relationship between nutrient concentrations and biota $\delta^{15}N$ ratios (Cole *et al.*, 2004; Inglett and Reddy, 2006; Gu *et al.*, 2009). However, the results from this analysis may be complicated by several factors, including study design and variability of nutrient data selected for this analysis. For example, biological characteristics of fish including age, size, gender, growth rate and feeding habits at each site will certainly introduce additional variations. Without data for fish age and tissue turnover time, we used the average TP concentrations measured one year prior to fish collection, which may not accurately reflect the growth condition of fish.

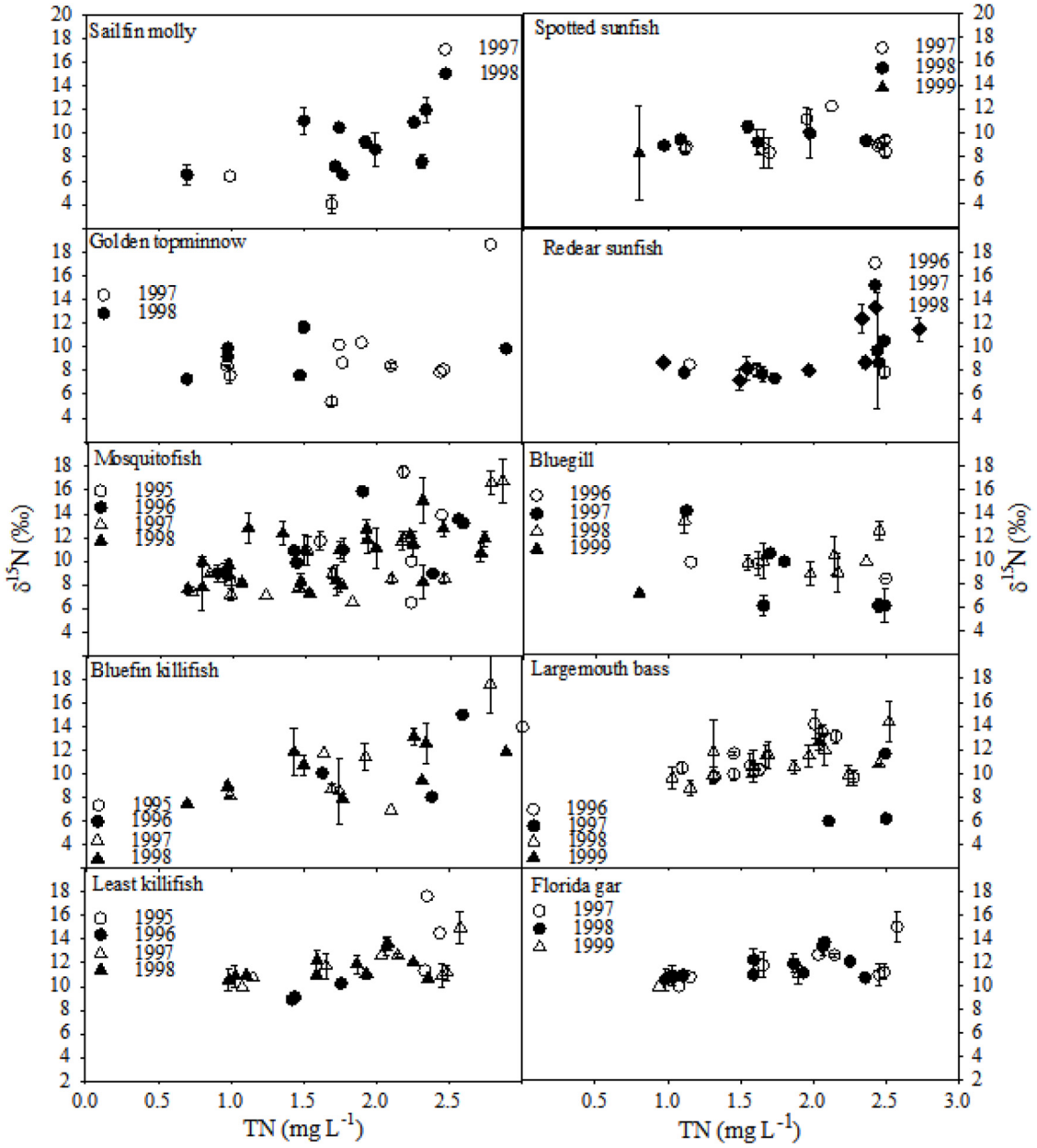

Fig. 3. $\delta^{15}N$ values (mean ± SD) of ten fish species along the TN concentration gradient in the Everglades wetlands. Each dot represents data from a specific date of sample collection in the 1990s.

Half of the species also had significant relationship between TN concentrations and $\delta^{15}N$ ratios. Nitrogen from human and animal wastes is often enriched in ^{15}N (McClelland *et al.*, 1997). Therefore, the increasing pattern of $\delta^{15}N$ ratios along the nutrient gradient may also be caused by increases in wastewater loading. Enriched $\delta^{15}N$ of various flora and fauna has been used as an indicator for sewage influence in the freshwater and coastal marine environments (Cole *et al.*, 2004; Rožič *et al.*, 2014; Souza *et al.*, 2018; de Carvalho *et al.*, 2019). There have been no reports of any significant wastewater contributions from human or animal sources to the Everglades. Inglett *et al.* (2005) reported that the $\delta^{15}N$ ratios of porewater NH_4^+ (the dominant N species in reduced soils) is similar at both the eutrophic and nonaffected WCA-2A sites. The high $\delta^{15}N$ at the impacted sites is unlikely the result of the uptake of wastewater enriched with ^{15}N and subsequent transfers to the consumer community (Cabana and Rasmussen, 1995).

Other nitrogen cycling processes (nitrification, denitrification and volatilization) may impact on the isotope composition of DIN in natural wetlands. However, ammonium, not nitrate, was the dominant species of DIN in this region (Tab. 1). Under low DO concentrations in this shallow wetland, nitrification is not likely the main process. Denitrification occurs under low DO concentrations and may result in significant changes in isotope composition the substate and products. However, nitrate was not the major form of DIN in the Everglades. Finally, volatilization occurs only under high pH and the nearly neutral pH found in the south Florida wetlands (Tab. 1) makes this process highly unlikely. Along with the findings from a previous study showing the similar N signatures in both

Table 3. Result of Spearman Rank Correlation between fish $\delta^{15}N$ ratios, water-column TP and TN concentrations and molar TN/TP ratios in this study.

Species name	TP	TN	TN:TP	N
Sailfin molly	0.76^{***}	0.55	-0.50^{**}	12
Golden topminnow	0.46	0.29	−0.31	16
Mosquitofish	0.76^{***}	0.55^{***}	-0.72^{***}	56
Bluefin killifish	0.77^{***}	0.50^{*}	-0.72^{***}	21
Least killifish	0.72^{***}	0.64^{***}	-0.73^{***}	33
Spotted sunfish	0.69^{***}	0.23	−0.12	17
Redear sunfish	0.52^{*}	0.44	0.17	16
Bluegill	0.53^{*}	0.38	-0.51^{*}	21
Largemouth bass	0.69^{***}	0.46^{***}	-0.57^{***}	40
Florida gar	0.80^{***}	0.63^{***}	-0.64^{***}	21

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

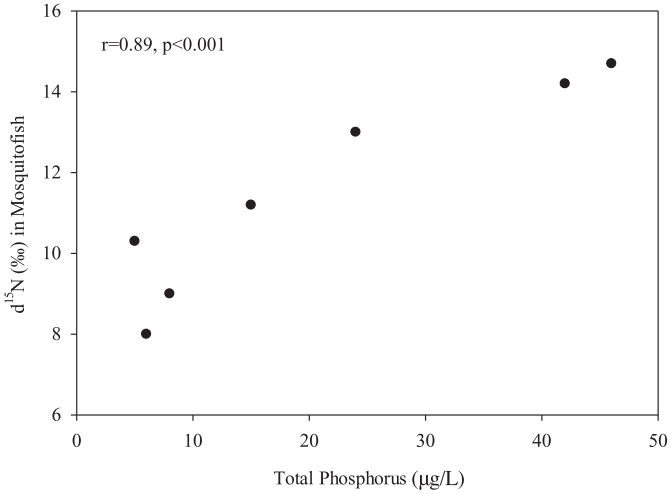

Fig. 4. Relationship between total P (TP) concentration and $\delta^{15}N$ ratios in mosquitofish collected from the Everglades Protection Area in 2007.

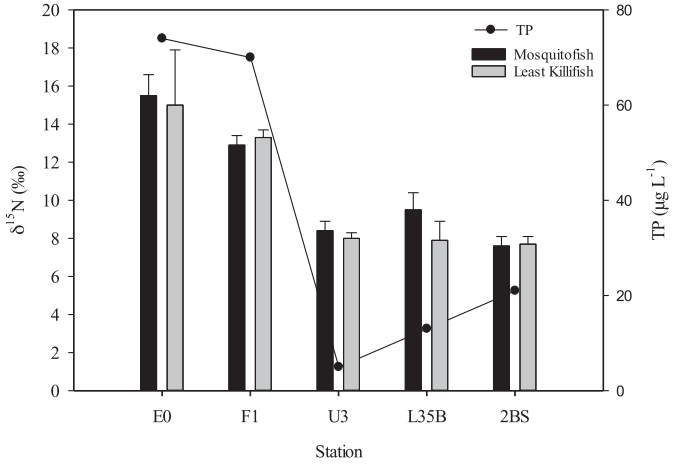

Fig. 5. Changes in the $\delta^{15}N$ ratios of mosquitofish and least killifish and TP concentrations along the nutrient gradient in the WCA-2.

affected and unaffected area of Everglades (Inglett *et al.*, 2006), site-specific N transformation would not likely be the major process leading to the differences in fish $\delta^{15}N$.

Nitrogen concentration may also influence biota $\delta^{15}N$ ratios through a substrate-mediated isotope effect (Peterson and Fry, 1987). When N is not a limiting nutrient in a system, an increase in N concentration will normally cause an increase in isotopic fractionation and therefore a decrease in $\delta^{15}N$ ratios. This would be evidenced by a negative correlation between TN concentration and fish $\delta^{15}N$ ratios in this study. Instead, the majority of the fish in this study displayed positive relationship between TN and $\delta^{15}N$ ratios. This implies that N is not a limiting nutrient to plant growth in the Everglades. This is also supported by the high molar ratios of TN:TP ratios. Some negative correlation between the water column TN:TP ratio and the fish $\delta^{15}N$ ratios also indicate that P, not N, is the limiting nutrient in the Everglades.

The major external source of N in the Everglades is agricultural runoff (Richardson, 2010). Because manufactured fertilizers are depleted in ^{15}N (Kohl *et al.*, 1971), assimilation of this ^{15}N-depleted N will not result in ^{15}N enrichment in the impacted sites. We conclude that the ^{15}N enrichment in the Everglades is the result of increased primary production stimulated by P availability. The average $\delta^{15}N$ ratios for fish increase progressively along the TP gradient. The low $\delta^{15}N$ ratios in fish at the unimpacted sites was the result of low TP concentration and large ^{15}N fractionation by primary producers during DIN uptake under P stress. The high N availability at the unimpacted sites also allowed selective assimilation of ^{14}N by aquatic plants. In contrast, the high $\delta^{15}N$ ratios at the impacted sites were due to P enrichment which leads to high N demand and low ^{15}N fractionation. Many studies have demonstrated that P is the limiting nutrient in the Everglades (*e.g.*, Newman *et al.*, 1996). Recent studies using stable isotopes found a positive relationship between the TP and $\delta^{15}N$ ratios of periphyton, sawgrass and cattail in WCA-1 and WCA-2A, which was attributed to P-driven plant growth and reduced isotope fractionation (Inglett and Reddy, 2006; Chang *et al.*, 2009; Wang *et al.*, 2015).

The widespread significant correlation between TP concentrations and fish $\delta^{15}N$ ratios is the consequence of the transfers of plant protein and the associated $^{15}N/^{14}N$ signal to the consumers, with ^{15}N enrichment along the food chain. The consumer $\delta^{15}N$ ratios increases along the TP gradient, which is consistent with the pattern of increases in the primary

producers. Furthermore, the relationship between TP concentration and fish $\delta^{15}N$ ratios seems to improve as the trophic level increases. For example, Florida gar, which is positioned at the highest trophic level in our samples, had the highest correlation coefficient ($r = 0.80$) with a moderate sample size ($n = 21$) among fish selected for this study. In general, fish $\delta^{15}N$ is a better indicator of the eutrophication process because they integrate temporal and spatial variations in source $\delta^{15}N$ ratios over longer time periods (Cabana and Rasmussen, 1996; Vander Zanden *et al.*, 2005).

5 Conclusions

The eutrophication process resulting from excessive P loading from the agricultural runoff to the Everglades is demonstrated using the nitrogen stable isotopic ratios of fish. The $\delta^{15}N$ ratios of nearly all fish species responded positively to the increases in TP concentration. This is considered to be caused by increasing the N uptake and decreasing the ^{15}N fractionation by primary producers stimulated by P enrichment. The ^{15}N enrichment along the nutrient gradient is evident in fish that reliably transfer the isotope signals from primary producers along the trophic level. The significant correlations between TP concentration and $\delta^{15}N$ ratios in mosquitofish in the 1990s and 2007 suggest that the eutrophication trend along the nutrient gradient persisted after almost two decades. Results from this study indicate that aquatic consumers such as fish are the better environmental indicators because they are capable of integrating biogeochemical changes over time.

Compliance with ethical standards

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Appendix

Table A1. Stable isotope data used in this analysis.

1. Data from 1990s Site	DATE	Common Name	δ^{15}N mean	δ^{15}N SD	N
TS-7	Jan-1998	Golden Topminnow	9.92	0.26	4
TS-9	Jan-1998	Golden Topminnow	9.22	0.45	3
3A15	Jan-1998	Golden Topminnow	7.29	0.27	7
U3	Sep-1997	Golden Topminnow	8.39	0.19	3
3A15	Sep-1997	Golden Topminnow	7.58	0.65	2
3A15	Jun-1998	Golden Topminnow	8.40	0.12	2
U3	Jul-1997	Golden Topminnow	8.10		1
L35B	Sep-1997	Golden Topminnow	5.36	0.36	3
2BS	Jul-1997	Golden Topminnow	8.68		1
L67	Jan-1998	Golden Topminnow	11.68	0.46	3
2BS	Jan-1998	Golden Topminnow	7.61	0.42	9
ENR-OUT	Jan-1998	Golden Topminnow	9.86		1
L67	Sep-1997	Golden Topminnow	10.20		1
Cell 3	Jun-1997	Golden Topminnow	10.38		1
E0	Jul-1997	Golden Topminnow	18.66		1
F1	Sep-1997	Golden Topminnow	7.87		1
3A15	Jan-1998	Sailfin Molly	6.52	0.85	4
U3	Jun-1998	Sailfin Molly	7.23	0.46	4
3A15	Sep-1997	Sailfin Molly	6.39		1
L35B	Jan-1998	Sailfin Molly	7.59	0.56	2
L35B	Sep-1997	Sailfin Molly	4.02	0.84	4
2BS	Jan-1998	Sailfin Molly	6.52	0.34	5
L67	Jan-1998	Sailfin Molly	11.08	1.10	5
L67	Jun-1998	Sailfin Molly	10.49		1
Cell 3	Jun-1997	Sailfin Molly	9.28	0.55	6
Cell 4	Jun-1998	Sailfin Molly	8.66	1.37	7
Cell 3	Jun-1998	Sailfin Molly	10.95	0.19	6
Cell 3	Jan-1998	Sailfin Molly	11.99	1.09	4
Cell 3	Jun-1997	Least Killifish	11.04	0.62	12
Cell 3	Jan-1998	Least Killifish	13.44	0.54	9
Cell 3	Jun-1998	Least Killifish	13.02	0.59	10
LOX	Jan-1998	Least Killifish	8.63	0.34	5
E0	Dec-1995	Least Killifish	11.66	6.89	2
E0	Jul-1995	Least Killifish	14.51		1
E0	Jun-1998	Least Killifish	15.97	0.62	9
E0	Sep-1997	Least Killifish	16.77	1.57	5
F1	Mar-1995	Least Killifish	17.64		1
F1	Jul-1995	Least Killifish	11.35		1
F1	Jun-1996	Least Killifish	15.54		1
F1	Dec-1996	Least Killifish	13.71	0.57	2
F1	Sep-1997	Least Killifish	11.01	0.33	4
F1	Jan-1998	Least Killifish	12.64	0.47	6
U3	Jul-1997	Least Killifish	8.43	0.06	2
U3	Sep-1997	Least Killifish	8.27	0.60	2
L35B	Sep-1997	Least Killifish	6.71	1.07	5
2BS	Jan-1998	Least Killifish	7.68	0.47	11
L67	Jun-1996	Least Killifish	9.10		1
L67	Dec-1996	Least Killifish	10.25		1
L67	Sep-1997	Least Killifish	12.57		1

Table A1. (continued).

1. Data from 1990s Site	DATE	Common Name	δ^{15}N mean	δ^{15}N SD	N
L67	Jan-1998	Least Killifish	12.28	0.93	8
L67	Jun-1998	Least Killifish	12.00	2.03	8
3A15	Jun-1996	Least Killifish	8.90		1
3A15	Jul-1997	Least Killifish	8.39	0.55	3
3A15	Sep-1997	Least Killifish	7.88	0.54	5
3A15	Jan-1998	Least Killifish	7.98	0.33	15
3A15	Jun-1998	Least Killifish	7.80	0.43	12
3A-Th	Sep-1997	Least Killifish	7.38	0.33	3
3A-Th	Jan-1998	Least Killifish	7.87	0.37	15
TS-7	Jan-1998	Least Killifish	9.49	1.04	2
TS-9	Jan-1998	Least Killifish	9.63	0.30	4
TS-9	Jun-1998	Least Killifish	8.30	0.56	4
2BS	Jul-1997	Mosquitofish	7.95	0.35	5
2BS	Jan-1998	Mosquitofish	8.30	0.69	11
2BS	Apr-1997	Mosquitofish	6.53		1
2BS	Jun-1998	Mosquitofish	7.93	0.54	12
3A15	Jan-1998	Mosquitofish	7.64	0.38	14
3A15	Dec-1996	Mosquitofish	8.97	0.68	5
3A15	Jul-1997	Mosquitofish	8.31	1.40	5
3A15	Sep-1997	Mosquitofish	7.16	0.52	5
3A15	Apr-1997	Mosquitofish	9.05	0.81	9
3A15	Jun-1996	Mosquitofish	10.86		1
3A-Th	Jan-1998	Mosquitofish	7.28	0.25	15
3A-Th	Sep-1997	Mosquitofish	7.69	0.19	5
3A-Th	Jul-1997	Mosquitofish	7.12		1
Cell 3	Jun-1998	Mosquitofish	12.77	1.27	20
Cell 3	Jun-1997	Mosquitofish	11.68	0.77	21
Cell 3	Jan-1998	Mosquitofish	12.73	0.71	15
Cell 4	Jun-1998	Mosquitofish	11.09	1.75	8
E0	Dec-1996	Mosquitofish	13.20	0.48	5
E0	Jun-1998	Mosquitofish	15.12	1.92	18
E0	Mar-1995	Mosquitofish	17.52	0.51	2
E0	Sep-1997	Mosquitofish	16.77	1.90	5
E0	Jul-1995	Mosquitofish	13.89		1
E0	Jul-1997	Mosquitofish	16.61	1.03	5
E0	Jun-1996	Mosquitofish	15.89		1
ENR-OUT	Jan-1998	Mosquitofish	11.89	0.67	9
ENR-OUT	Jun-1998	Mosquitofish	10.71	0.76	9
F1	Feb-1998	Mosquitofish	12.20		1
F1	Jun-1998	Mosquitofish	12.71	0.74	14
F1	Dec-1996	Mosquitofish	13.55	0.11	2
F1	Jun-1996	Mosquitofish	16.64		1
F1	Sep-1997	Mosquitofish	11.94	0.35	5
F1	Jan-1998	Mosquitofish	11.77	1.09	10
F1	Jul-1995	Mosquitofish	11.62		1
L35B	Jan-1998	Mosquitofish	8.22	1.44	19
L35B	Sep-1997	Mosquitofish	8.96	0.43	4
L35B	Jun-1998	Mosquitofish	11.45	1.08	20
L67	Jan-1998	Mosquitofish	10.91	1.25	20
L67	Jul-1997	Mosquitofish	10.88	1.25	8
L67	Jun-1998	Mosquitofish	12.35	0.98	40
L67	Sep-1997	Mosquitofish	10.98	0.72	10

Table A1. (continued).

1. Data from 1990s Site	DATE	Common Name	δ^{15}N mean	δ^{15}N SD	N
L67	Jul-1995	Mosquitofish	11.68	0.76	4
L67	Dec-1996	Mosquitofish	10.92	0.96	12
L67	Jun-1996	Mosquitofish	9.86		2
LOX	Jan-1998	Mosquitofish	8.18	0.35	6
TS-7	Jul-1997	Mosquitofish	8.92	0.13	2
TS-7	Jan-1998	Mosquitofish	9.71	0.36	10
TS-7	Jun-1998	Mosquitofish	9.94	0.41	10
TS-9	Jul-1997	Mosquitofish	7.35		1
TS-9	Jan-1998	Mosquitofish	8.88	0.46	20
TS-9	Jun-1998	Mosquitofish	7.78	1.95	16
U3	Dec-1996	Mosquitofish	8.93	0.15	2
U3	Jun-1998	Mosquitofish	8.40	1.29	13
U3	Sep-1997	Mosquitofish	8.48	0.33	5
U3	Mar-1995	Mosquitofish	9.98		1
U3	Jul-1995	Mosquitofish	6.49		1
U3	Jul-1997	Mosquitofish	8.50	0.40	5
Cell 3	Jun-1997	Bluefin Killifish	11.41	1.16	12
Cell 3	Jan-1998	Bluefin Killifish	12.60	1.72	4
Cell 3	Jun-1998	Bluefin Killifish	13.14	0.71	5
ENR-OUT	Jan-1998	Bluefin Killifish	11.83	0.00	2
E0	Dec-1995	Bluefin Killifish	13.97		1
E0	Dec-1996	Bluefin Killifish	15.00		1
E0	Sep-1997	Bluefin Killifish	17.61	2.47	7
F1	Sep-1997	Bluefin Killifish	11.74		1
U3	Dec-1996	Bluefin Killifish	8.06		1
U3	Sep-1997	Bluefin Killifish	6.93		1
L35B	Sep-1997	Bluefin Killifish	8.76	0.35	3
L35B	Jan-1998	Bluefin Killifish	9.45		1
2BS	Jan-1998	Bluefin Killifish	7.90		1
L67	Jun-1996	Bluefin Killifish	10.06		1
L67	Sep-1997	Bluefin Killifish	8.59	2.77	3
L67	Jan-1998	Bluefin Killifish	10.77	0.83	8
L67	Jun-1998	Bluefin Killifish	11.87	2.04	6
3A15	Sep-1997	Bluefin Killifish	8.14	0.10	2
3A15	Jan-1998	Bluefin Killifish	7.47	0.11	5
TS-7	Jan-1998	Bluefin Killifish	8.85	0.26	2
TS-9	Jan-1998	Bluefin Killifish	8.98	0.38	5
Cell 3	Apr-1997	Spotted Sunfish	11.12	0.97	6
Cell 3	Oct-1997	Spotted Sunfish	12.21		1
U3	Oct-1996	Spotted Sunfish	8.36	0.47	5
U3	Sep-1997	Spotted Sunfish	8.86	0.24	5
U3	Oct-1997	Spotted Sunfish	9.36	0.28	2
U3	Nov-1997	Spotted Sunfish	9.09		1
U3	Jan-1998	Spotted Sunfish	9.29	0.42	3
L35B	Oct-1996	Spotted Sunfish	9.18	1.12	15
L35B	Jan-1998	Spotted Sunfish	9.90	2.03	14
L67	Feb-1997	Spotted Sunfish	8.60	1.67	4
L67	Jun-1997	Spotted Sunfish	8.28	1.25	3
L67	Jan-1998	Spotted Sunfish	10.51	0.52	4
3A15	Sep-1997	Spotted Sunfish	8.62	0.56	5
3A15	Oct-1997	Spotted Sunfish	8.80	0.48	5
3A15	Jan-1998	Spotted Sunfish	9.42	0.45	4

Table A1. (continued).

1. Data from 1990s Site	DATE	Common Name	δ^{15}N mean	δ^{15}N SD	N
3A15	Mar-1998	Spotted Sunfish	8.88	0.34	3
3A15	Jan-1999	Spotted Sunfish	8.22		1
Cell 3	Jan-1998	Redeared Sunfish	12.40	1.18	4
ENR-OUT	Jan-1998	Redeared Sunfish	11.51	0.97	9
U3	Oct-1996	Redeared Sunfish	7.88	0.53	5
U3	Sep-1997	Redeared Sunfish	9.70	4.94	6
U3	Oct-1997	Redeared Sunfish	10.53		1
U3	Nov-1997	Redeared Sunfish	8.66	0.06	3
U3	Jan-1998	Redeared Sunfish	8.70	0.43	6
L35B	Oct-1996	Redeared Sunfish	8.07	0.52	9
L35B	Jun-1997	Redeared Sunfish	7.36	0.38	2
L35B	Jan-1998	Redeared Sunfish	8.04	0.41	5
2BS	Jan-1998	Redeared Sunfish	7.17	0.86	2
L67	Feb-1997	Redeared Sunfish	7.70	0.69	10
L67	Jan-1998	Redeared Sunfish	8.21	0.97	8
3A15	Dec-1996	Redeared Sunfish	8.52		1
3A15	Sep-1997	Redeared Sunfish	7.84	0.19	4
3A15	Mar-1998	Redeared Sunfish	8.70		1
3A15	Jan-1999	Bluegill	7.24		1
U3	Oct-1996	Bluegill	8.46	0.03	2
U3	Jan-1998	Bluegill	9.91		1
3A15	Oct-1997	Bluegill	9.06	1.07	2
3A15	Nov-1997	Bluegill	9.77		1
U3	Oct-1997	Bluegill	8.77	0.27	4
U3	Sep-1997	Bluegill	9.70	2.99	9
3A15	Dec-1996	Bluegill	9.86		1
L35B	Jan-1998	Bluegill	8.83	0.99	5
L67	Jun-1997	Bluegill	11.36		1
L35B	Feb-1997	Bluegill	8.85	1.03	8
L35B	Jun-1998	Bluegill	8.97	1.62	4
L67	Jan-1998	Bluegill	9.81	0.63	2
L35B	Sep-1998	Bluegill	10.41	1.64	9
L35B	Oct-1996	Bluegill	9.74	0.93	11
L67	Nov-1997	Bluegill	9.70	0.19	3
L67	Sep-1998	Bluegill	9.96	1.53	2
L67	Feb-1997	Bluegill	8.06	0.93	9
Cell 3	Jun-1998	Bluegill	13.38	1.04	5
Cell 3	Jun-1997	Bluegill	12.01	0.62	2
Cell 3	Jan-1998	Bluegill	12.53	0.73	6
LOX	Dec-1996	Laregmouth bass	9.70	0.36	36
LOX	Sep-1997	Laregmouth bass	9.28	0.77	6
LOX	Oct-1998	Laregmouth bass	8.76	0.63	20
Cell 3	Oct-1996	Laregmouth bass	13.13	0.58	34
Cell 3	Apr-1997	Laregmouth bass	12.84	0.41	8
Cell 3	Oct-1997	Laregmouth bass	12.74	1.24	26
Cell 3	Jan-1998	Laregmouth bass	11.98	1.25	2
Cell 3	Aug-1998	Laregmouth bass	13.61	0.49	20
Cell 4	Jan-1999	Laregmouth bass	12.61	0.70	6
L-7	Oct-1996	Laregmouth bass	14.17	1.16	32
L-7	Sep-1997	Laregmouth bass	15.05	1.47	20
L-7	Sep-1998	Laregmouth bass	14.32	1.70	20
U3	Oct-1996	Laregmouth bass	9.62	0.68	12

Table A1. (continued).

1. Data from 1990s Site	DATE	Common Name	δ^{15}N mean	δ^{15}N SD	N
U3	Sep-1997	Laregmouth bass	10.43	0.27	6
U3	Oct-1997	Laregmouth bass	10.21	0.30	13
U3	Nov-1997	Laregmouth bass	10.33	0.48	4
U3	Jan-1998	Laregmouth bass	10.77	0.11	2
U3	Mar-1998	Laregmouth bass	9.86	0.83	7
L35B	Oct-1996	Laregmouth bass	10.64	0.98	35
L35B	Jan-1998	Laregmouth bass	10.54	0.54	11
L35B	Sep-1998	Laregmouth bass	11.49	0.93	20
2BS	Jan-1998	Laregmouth bass	10.11	0.50	10
L67	Dec-1996	Laregmouth bass	10.30	0.46	35
L67	Jun-1997	Laregmouth bass	10.47	1.82	5
L67	Sep-1997	Laregmouth bass	11.75	1.18	20
L67	Nov-1997	Laregmouth bass	10.67	1.24	8
L67	Jan-1998	Laregmouth bass	10.61	1.36	15
L67	Sep-1998	Laregmouth bass	11.57	1.14	10
L67	Oct-1998	Laregmouth bass	11.47	0.97	10
3A15	Dec-1996	Laregmouth bass	10.47	0.52	12
3A15	Sep-1997	Laregmouth bass	9.76	0.50	18
3A15	Oct-1997	Laregmouth bass	8.94	1.31	5
3A15	Nov-1997	Laregmouth bass	10.55		1
3A15	Mar-1998	Laregmouth bass	9.60	0.95	6
LMC	Dec-1996	Laregmouth bass	9.92	0.51	33
LMC	Aug-1997	Laregmouth bass	10.64	0.16	2
LMC	Mar-1998	Laregmouth bass	9.84	0.63	10
NPC	Dec-1996	Laregmouth bass	11.68	0.11	5
NPC	Aug-1997	Laregmouth bass	12.46	0.38	14
NPC	Mar-1998	Laregmouth bass	11.80	2.66	16
Cell 3	Oct-1997	Florida Gar	12.63		1
Cell 3	Jan-1998	Florida Gar	13.67	0.54	2
Cell 3	Aug-1998	Florida Gar	13.32	0.71	4
ENRout	Oct-1997	Florida Gar	12.60	0.16	2
ENRout	Jan-1998	Florida Gar	12.06	0.05	2
E0	Sep-1997	Florida Gar	14.99	1.34	3
U3	Oct-1997	Florida Gar	11.17	0.64	2
U3	Nov-1997	Florida Gar	10.95	0.97	2
U3	Jan-1998	Florida Gar	10.69	0.20	2
U3	Mar-1998	Florida Gar	11.09	0.38	2
U3	Jan-1999	Florida Gar	11.00	0.77	3
L35B	Jan-1998	Florida Gar	11.88	0.78	5
2BS	Jan-1998	Florida Gar	10.95		1
L67	Nov-1997	Florida Gar	11.75	1.05	5
L67	Jan-1998	Florida Gar	12.21	0.91	6
3A15	Oct-1997	Florida Gar	9.97		1
3A15	Nov-1997	Florida Gar	10.74		1
3A15	Jan-1998	Florida Gar	10.91	0.33	4
3A15	Mar-1998	Florida Gar	10.88	0.90	3
3A15	May-1998	Florida Gar	10.57	0.92	4
3A15	Jan-1999	Florida Gar	9.95		1

Table A1. (continued).

1. Data from 1990s Site	DATE	Common Name	$\delta^{15}N$ mean	$\delta^{15}N$ SD	N
2. Data from 2007					
TP (ug/L)	$\delta^{15}N$ (‰)				
4.9	10.2				
7	8				
8.5	9.1				
15	11.3				
24.5	13.1				
42.1	14.2				
46.5	14.5				