Appendix 5C-1: Estimating Phosphorus and Nitrogen Excretion in Fishes in the Everglades Stormwater Treatment Areas

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SUMMARY

The rates at which nitrogen (N) and phosphorus (P) forms are contributed by fish through excretion and egestion to the water column in the Everglades Stormwater Treatment Areas (STAs) were estimated using short-term incubation experiments. This was done during daytime and nighttime periods for six of the most abundant species (3 large and 3 small) found in the STAs. All three small fish species excreted N and P at a faster rate per gram of biomass than all three large fish species. The three small species excreted more during the day, while all three large species excreted more at night. Since fish are poikilotherms, we assumed that excretory rate would be correlated with temperature. We attempted to limit experimental temperature within the time of day (day and night), but the small variation recorded was positively correlated with excretion rate in a few cases; the greatest variation in temperature was between day and night, which obscured any diurnal patterns of excretory rates. Since large species consistently excreted more in the generally cooler night trials, we believe these inter-specific differences arise from physiological and/or behavioral adaptations rather than simple temperature-metabolic effects.

Using areal biomass estimates for the six fish species in each SAV cell of STA-2 (Cell 3, 4, 5, and 6), STA-1 East (STA-1E; Cell 2, 4N, 4S, and 6), and STA 1-West (STA-1W; Cell 1B, 2B, 3, 4, and 5B), we estimated mass of N and P excreted per day by fish. Based on known inflow N and P loads of STA-2, STA-1E, and STA-1W, we estimated that fish excretion in the SAV cells can recycle 6, 9, and 1%, respectively of the daily total nitrogen entering the system in the water column, and 53, 12, and 1%, respectively, of the daily P entering the system. We believe altering fish abundance or species composition has potential to affect the efficient removal of nutrients in STA waters, with implications for achieving the state and federally mandated STA discharge limits. Ongoing research is addressing the role of fishes in resuspending N and P from sediments through bioturbation. Future research will focus on addressing seasonal variation of excretion and incorporating these into STA nutrient budgets.

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INTRODUCTION

The ability of STAs to remove P is affected by fauna, which cycle P through various forms, fluxes, and transformations. Waterbirds, fish, and macroinvertebrates, are recognized as important contributors to nutrient cycling in aquatic ecosystems (Vanni 2002, Doughty et al. 2016). One source of internal nutrient loading is direct mobilization of benthic or particulate nutrients through feeding and excretion (Vanni et al. 2006). Through these processes, animals can change the availability of dissolved labile nutrients and the rate of transformations in dissolved and particulate nutrient forms in the water column (Vanni et al. 2002). Remobilization of nutrients from fish excretion are of similar magnitude to external nutrient loading sources in freshwater systems (Schindler et al. 2001, Gido 2004, Zimmer et al. 2006) and may play important roles supporting primary producers. Starling et al. (2002) showed that excretion from invasive Nile tilapia (*Oreochromis niloticus*) contributed 12% of the total phosphorus (TP) load in a hypereutrophic littoral area of a tropical reservoir. In the STAs, this source of internal nutrient loading has the potential to affect the efficiency of nutrient removal and the ability to attain the state and federally mandated STA discharge limits.

While the ecological literature points to a pivotal role of animals in STA nutrient cycling, the direct role of fish excretion on nutrient cycles has only recently been studied in STAs and knowledge of the effect on N and P transformations is rudimentary. To accurately assess the role of excreted nutrients in an ecosystem it is important to first understand the influence of varying conditions on excretion rates. Fish are poikilotherms (their internal temperature varies with ambient temperatures) and their metabolism and excretion rates are directly related to environmental conditions (Vanni et al. 2002). Excretion rates may have diel patterns, but these patterns vary by species and conditions. Oliveira-Cuhna et al. (2018) suggest that diurnal difference may be the result of environmental and behavioral factors including temperature and feeding patterns, and that weighted averages of diurnal and nocturnal excretion should be used to assess contributions to nutrient cycling. Seasonal changes in South Florida's conditions are likely to influence excretion rates, and it is important to understand these influences to assess N and P contributions from excretion in the STAs.

To understand these processes within the STAs and ultimately the effect of fish on water column total N and P concentrations in the STA outflow cells, this study estimated diurnal and nocturnal mass specific N and P excretion rates of the six abundant species. Biomass and excretion estimates are combined to estimate areal P (per cell) excretion by the aquatic animal assemblage in STA-2 as rates of excretory N and P released to the water column in micrograms per cell per hour (µg/cell/hr). This section summarizes the results of the winter 2019 excretion incubations.

METHODS

N and P excretion and egestion were measured for three common large fish species found in electrofishing surveys and three common small fish species found in throw-trap surveys. The large species (maximum length greater than 8 centimeter [cm] and multi-year life span) were blue tilapia (*Oreochromis aureus*), largemouth bass (*Micropterus salmoides*) and sailfin catfish (*Pterygoplichthys* spp.). The small fish (maximum length less than 8 cm and annual life span) were bluefin killifish (*Lucania goodei*), sailfin molly (*Poecilia latipinna*) and eastern mosquitofish (*Gambusia holbrooki*). All species were selected for this excretion study based on their high abundance in the STAs, except for sailfin catfish, which was selected because of their potential effect on P budgets through bioturbation. Diurnal and nocturnal TP, total dissolved phosphorus (TDP), TN, and ammonium nitrogen (NH_4-N) excretion rates were estimated via short-term incubations.

Diurnal incubations were carried out from January 14 to 30, 2019, and nighttime incubations from January 28 to February 11, 2019. Incubations followed methods similar to those employed by Torres and Vanni (2007), Whiles et al. (2009), and Capps and Flecker (2013). Methods were modified from Evans et al. (2019) to assure sample analyte concentrations were consistently above minimum detection limits (MDLs: NH_4-N = 10 microgram per liter [µg/L], TN = 40 µg/L, and TP and TDP = 4 µg/L). All fish and experiment chamber water were collected prior to the start of the experiment from STA-1E Cell 2, except sailfin catfish nighttime trials. Sailfin catfish were collected from STA-2 Cell 6 because of low abundance in STA-1E Cell 2. Fish were held in a pen that was suspended in STA waters for a maximum of 1 hour following collection to minimize stress, fluctuations in conditions, and release of unmeasured excretion.

For the small fish, each incubation consisted of a 2-mil 25 cm x 50 cm polyethylene bag filled with 260 milliliters (mL) of STA water that was pre-filtered through a 0.7-micron (µm) glass fiber filter to remove phytoplankton and particulates. Incubation chambers were rafted together in STA waters and shaded to control changes in temperature. Immediately after filling with water, a given species of fish was placed into the bag and incubated for 1 hour. Incubation biomass varied among trials by changing the number of individuals in each incubation. Individuals were selected from a representative sample of specimens collected in the sampling area. Multi-fish samples were used because individual fish were too small to yield detectable levels of excretion. At the end of the incubation period, water samples were taken and the water temperature was measured. Fish were euthanized with *Tricaine methanesulfonate* (MS-222), stored on ice and later measured in the laboratory for standard length and wet weight.

For large fish species a single fish was used in each incubation chamber consisting of a 6 mil 76 cm x 76 cm polyethylene pond liner filled with 8 liters (L) of prefiltered STA water. A range of sizes of specimens was used in the large-fish trials to be representative of fishes collected. Measurements of standard length and wet weight were taken immediately after the incubation of large fish and they were released back into the STA cell where they were collected.

Water samples collected from each chamber immediately prior to adding the fish and at the conclusion of the incubation were analyzed to determine the change in nutrient concentrations due to fish. Additionally, fishless control bags were incubated in the same manner to determine non-fish nutrient transformations. For each species, 20 daytime and 20 nighttime incubations were conducted. Water samples were processed and preserved according to the SFWMD protocol (SFWMD 2017) and analyzed for TP, TN, TDP, and NH_4-N. The pilot study (Evans et al. 2019) also included soluble reactive phosphorus (SRP) and total dissolved nitrogen (TDN) to quantify all forms of N and P related to excretion; however, the reduced volume of incubation chambers used reduced the number of analytes possible from each trial. NH_4-N and TDP represent recently excreted dissolved N and P, respectively, whereas TN and TP also include egested solid forms and subsequent transformations following NH_4-N and TDP (Vanni et al. 2002).

STATISTICAL ANALYSES

Excreted mass was estimated as the difference between the concentration of each analyte at the end and beginning of an incubation. The excreted mass was compared to fishless controls using Student's t-test. Diurnal and nocturnal nutrient excretion rates were estimated by regressing the excreted mass against the biomass of fish present in the incubation bags. Analysis of covariance (ANCOVA) tested the effect of time of day (day versus night) on excretion rates (in milligrams per gram wet weight per hour [mg/g ww/hr]), where the mass excreted for the one-hour experiment was the dependent variable and fish biomass and time of day were covariates. A significant effect of the interaction variable (biomass · time of day) indicated that diurnal and nocturnal excretion rate per unit fish mass differed. Linear regressions were used to assess the effect of temperature on excretion rates for diurnal and nocturnal trials.

Areal biomass estimates (**Table 1**; Evans et al. 2019) were combined with species-specific excretion rates to generate areal estimates of total-assemblage excretion for each of the cells surveyed. Average daily excretion of a species in a cell were estimated using the linear relationships between total excreted mass and total biomass for diurnal and nocturnal trials:

$$\text{Average daily excretion} = ((m_d x + c_d) * h_d) + ((m_n x + c_n) * h_n) \quad \textbf{(1)}$$

Where the m_d and m_n, and c_d and c_n are the slope and y-intercept of the diurnal and nocturnal linear models, respectively; x is the biomass estimate for the cell; and h_d and h_n are the average number of hours of daytime and nighttime, respectively.

Table 1. Estimates of mean fish biomass in kilograms per cell (kg/cell) for each surveyed cell in STA-2, STA-1E, and STA-1W. This table is derived from data reported by Evans et al. (2019). (Note: #N/S – not sampled.)

STA	Cell	Species					
		Eastern Mosquitofish	Bluefin Killifish	Sailfin Molly	Blue Tilapia	Largemouth Bass	Sailfin Catfish
STA-2	3	2,200	6,100	13,800	600	6,400	1,500
	4	8,200	7,300	22,700	1,600	3,400	0
	5	6,000	2,700	1,300	2,400	3,700	<50
	6	3,200	5,400	1,600	400	2,400	<50
STA-1E	2	#N/S	#N/S	#N/S	0	500	<50
	4N	#N/S	#N/S	#N/S	0	2,100	100
	4S	#N/S	#N/S	#N/S	0	600	200
	6	#N/S	#N/S	#N/S	0	3,300	2,300
STA-1W	3	#N/S	#N/S	#N/S	0	700	100
	4	#N/S	#N/S	#N/S	0	0	200
	1B	#N/S	#N/S	#N/S	0	<50	0
	2B	#N/S	#N/S	#N/S	0	0	0
	5B1	#N/S	#N/S	#N/S	0	700	0
	5B2	#N/S	#N/S	#N/S	0	0	0

RESULTS

Nutrient concentrations in equipment blanks and fishless controls were below detection limits for all samples and analytes (**Table 2**). The modification of methods from Evans et al. (2019) succeeded in increasing nutrient concentrations in the incubation chambers as the excretion effect sizes were greater than MDL for all N and P analytes across all species (**Table 2**). Excreted masses for each species, time of day (day versus night) and analyte combination were normally distributed (Shapiro-Wilks probability factor (p) > 0.05). Positive relationships between fish biomass and nutrient excreted mass were found for all species and all analytes (**Figures 1** through **4**). Diurnal and nocturnal excretion rates were shown to be significantly different for at least one analyte in each species (**Table 3**).

The fish biomass used in diurnal and nocturnal incubations were indistinguishable for each species except largemouth bass, where nighttime biomass was significantly higher than daytime (**Table 4**). To ensure that comparisons of diurnal and nocturnal excretion rates were appropriate, largemouth bass excretion rates were compared at overlapping mass ranges (< 100 grams per liter) between nocturnal and diurnal incubations.

We minimized inter-trial variation in temperature within nighttime and daytime experiments and excretion rate was generally unaffected by the small temperature variation we recorded. Excretion of ammonium within nighttime or daytime from blue tilapia (day: R squared [R^2] = 0.34, $p < 0.01$) and sailfin catfish (day: $R^2 = 0.16$, $p < 0.05$; night: $R^2 = 0.18$, $p < 0.05$) were the only trials where temperature had a significant effect on excretion rates (**Table 4**). In these cases, there was a positive relationship between excretion rate and temperature, though the small amount of variance explained in each case illustrates even those effects were weak. The effect of temperature on the difference in excretion rate between day and night could not be tested as temperature was confounded with time of day.

Hourly excretion rates were derived from the slope and intercept of the relationship between mass excreted and biomass (**Table 5**). Small fish had the highest daily excretion rates for all forms of P and N, with eastern mosquitofish always being the highest (TP = 1.39 gram per kilogram wet weight per day [g/kg ww/d]; TDP = 0.0.31 g/kg ww/d; TN = 5.51 g/kg ww/d; NH_4-N = 0.81 g/kg ww/d). Large fish always had the lowest daily excretion rates for all forms of N and P with largemouth bass consistently the highest (TP = 0.03 g/kg ww/d; TDP = 0.02 g/kg ww/d; TN = 0.30 g/kg ww/d; NH_4-N = 0.14 g/kg ww/d), and sailfin catfish the lowest (TP = 0.01; TDP = 0.008; TN = 0.19; NH_4-N = 0.07 g/kg ww/d). The hourly excretion rates were used to estimate daily areal excretion rates for each species in each surveyed cell based on an average of 10.9 hours of daylight during winter months (November to February) in South Florida (**Tables 6**, **7**, and **8**).

Table 2. Summary of treatment effect sizes. "Control" and "Treatment" in micrograms per liter per hour (µg/L/hr) are the means and standard deviations of the difference in nutrient concentrations in water samples taken before and after the fishless and fish-containing incubation, respectively. Note that three control samples had concentrations more than 2 standard deviations from the mean and were removed as outliers.

Fish Species	Analyte	Time of Day (Day/ Night)	Control (µg/L/hr)	Treatment (µg/L/hr)
Eastern Mosquitofish	NH_4	Day	0.2 ± 13.1	310.5 ± 216.5
		Night	4.2 ± 7.4	134.4 ± 104.2
	TDP	Day	0.4 ± 0.9	95.0 ± 62.0
		Night	0.2 ± 0.8	69.4 ± 61.8
	TN	Day	1.3 ± 17.8	2,716.5 ± 1,970.0
		Night	-0.8 ± 1.3	665.0 ± 720.0
	TP	Day	3.4 ± 3.6	985.1 ± 835.8
		Night	0.4 ± 0.5	95.2 ± 99.6
Bluefin Killifish	NH_4	Day	-1.6 ± 6.8	104.9 ± 54.4
		Night	-5.8 ± 10.9	67.5 ± 80.4
	TDP	Day	0.4 ± 0.5	50.8 ± 25.6
		Night	-0.2 ± 0.4	41.2 ± 35.6
	TN	Day	38.0 ± 41.6	63.4 ± 43.5
		Night	3.5 ± 41.5	334.0 ± 258.5
	TP	Day	0.8 ± 0.8	9.3 ± 66.5
		Night	0.4 ± 0.6	53.8 ± 47.6
Sailfin Molly	NH_4	Day	-1.0 ± 4.8	106.9 ± 69.4
		Night	-1.0 ± 9.5	126.5 ± 66.8
	TDP	Day	0 ± 0	56.1 ± 31.6
		Night	0.8 ± 1.5	52.8 ± 31.8
	TN	Day	30.0 ± 12.6	1,356.0 ± 853.7
		Night	-10.0 ± 27.5	706.5 ± 436.9
	TP	Day	2.2 ± 3.8	321.0 ± 253.1
		Night	3.2 ± 6.2	108.0 ± 82.1
Largemouth Bass	NH_4	Day	3.6 ± 7.5	276.0 ± 145.0
		Night	2.4 ± 1.5	448.5 ± 218.6
	TDP	Day	0.4 ± 0.9	16.1 ± 12.5
		Night	0.2 ± 0.4	71.7 ± 87.5
	TN	Day	-12.5 ± 43.2	500.0 ± 315.7
		Night	10.0 ± 12.2	916.3 ± 715.1
	TP	Day	0.4 ± 0.5	20.8 ± 15.9
		Night	0.2 ± 0.4	89.7 ± 108.9
Blue Tilapia	NH_4	Day	0.8 ± 1.6	251.0 ± 84.0
		Night	6.2 ± 11.3	448.6 ± 122.0
	TDP	Day	0.4 ± 0.5	52.0 ± 32.0
		Night	0.4 ± 0.5	124.0 ± 104.0
	TN	Day	-2.2 ± 3.6	829.0 ± 384.0
		Night	20.0 ± 27.0	1,773.0 ± 1,074.0
	TP	Day	0 ± 0	76.0 ± 48.0
		Night	0.6 ± 0.9	185.0 ± 152.0
Sailfin Catfish	NH_4	Day	-2.4 ± 6.9	269.2 ± 86.3
		Night	-0.6 ± 3.4	430.1 ± 171.0
	TDP	Day	0.2 ± 0.8	16.5 ± 9.7
		Night	0.8 ± 1.3	26.8 ± 18.0
	TN	Day	4.0 ± 23.0	402.0 ± 135.0
		Night	8.0 ± 25.0	701.0 ± 289.0
	TP	Day	-1.0 ± 1.9	246.0 ± 142.0
		Night	0.2 ± 0.8	41.0 ± 29.0

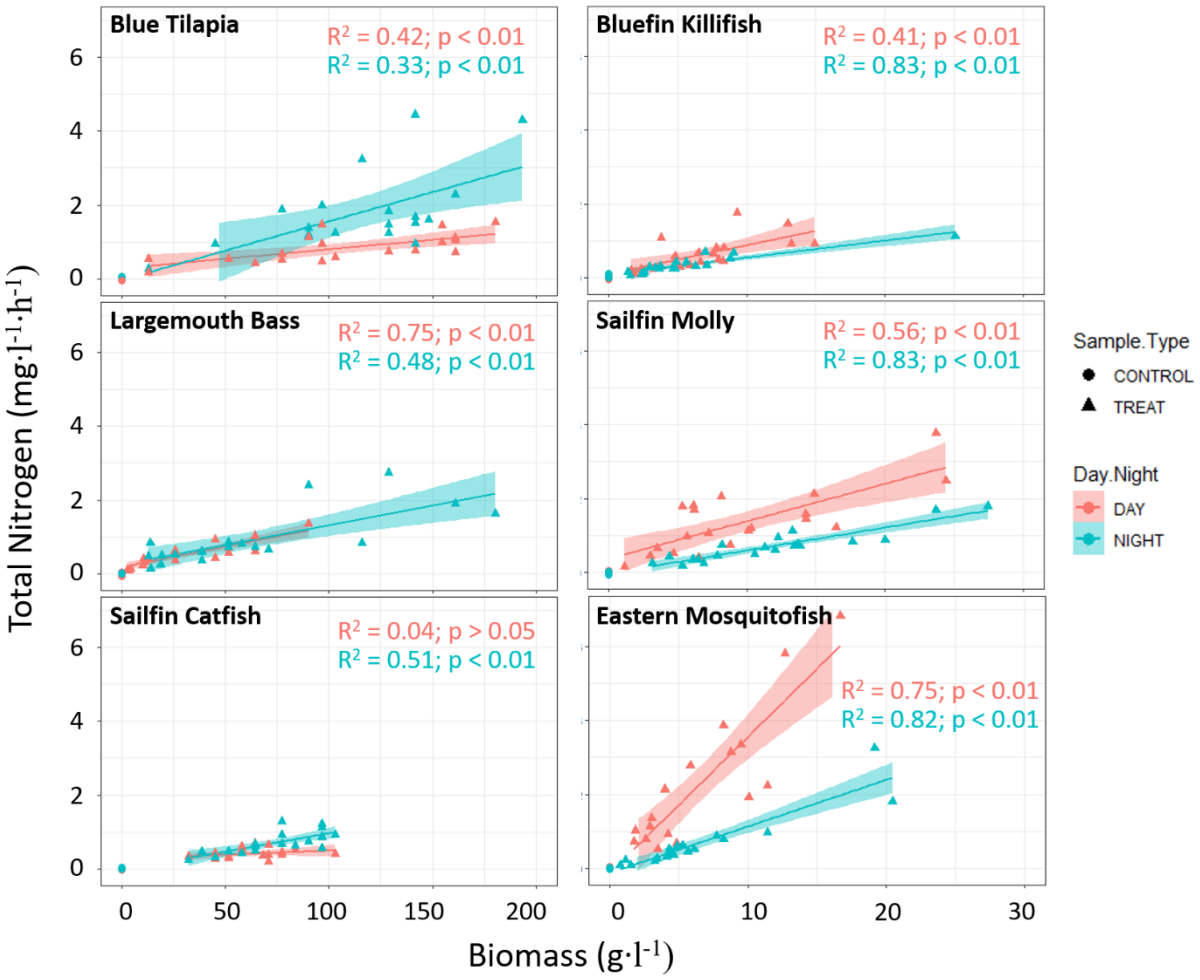

Figure 1. Relationships between total nitrogen excretion rate in milligrams per liter per hour ($mg \cdot L^{-1} \cdot hr^{-1}$) and biomass in grams per liter ($g \cdot L^{-1}$). Confidence intervals are 95%.

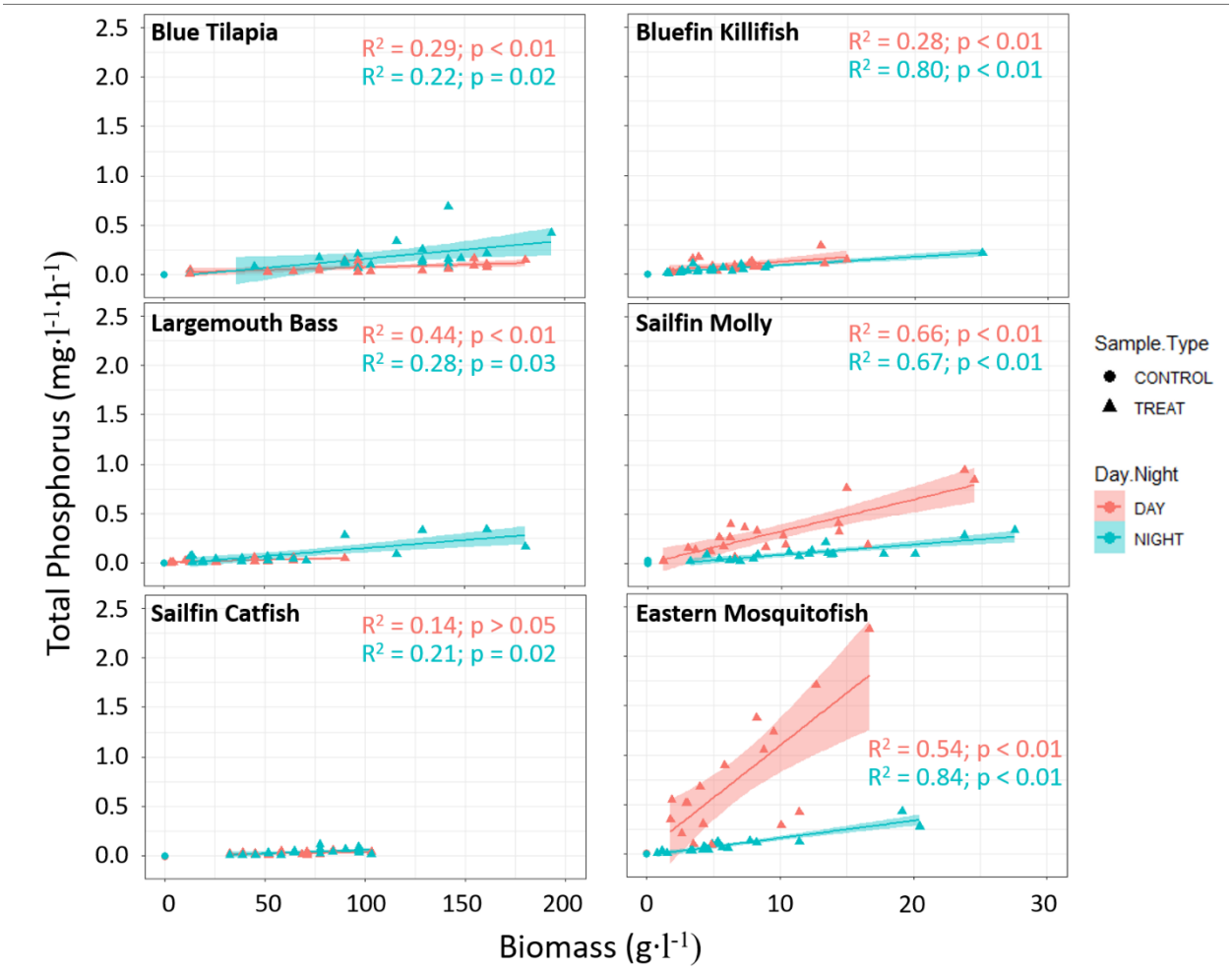

Figure 2. Relationship between TP excretion rate in milligrams per liter per hour ($mg \cdot L^{-1} \cdot hr^{-1}$) and biomass in grams per liter ($g \cdot L^{-1}$). Confidence intervals are 95%.

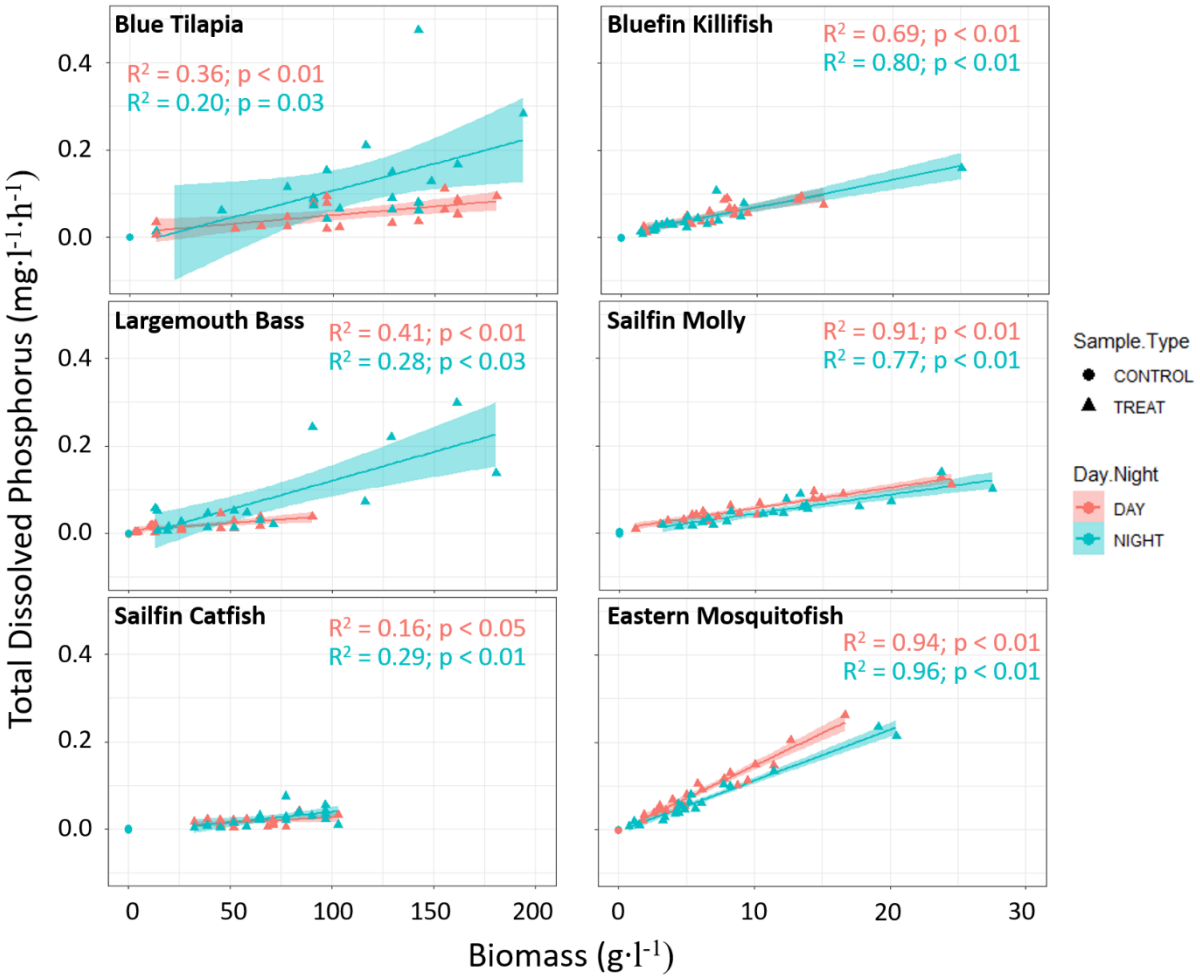

Figure 3. Relationship between TDP excretion rate in milligrams per liter per hour ($mg \cdot L^{-1} \cdot hr^{-1}$) and biomass in grams per liter ($g \cdot L^{-1}$). Confidence intervals are 95%.

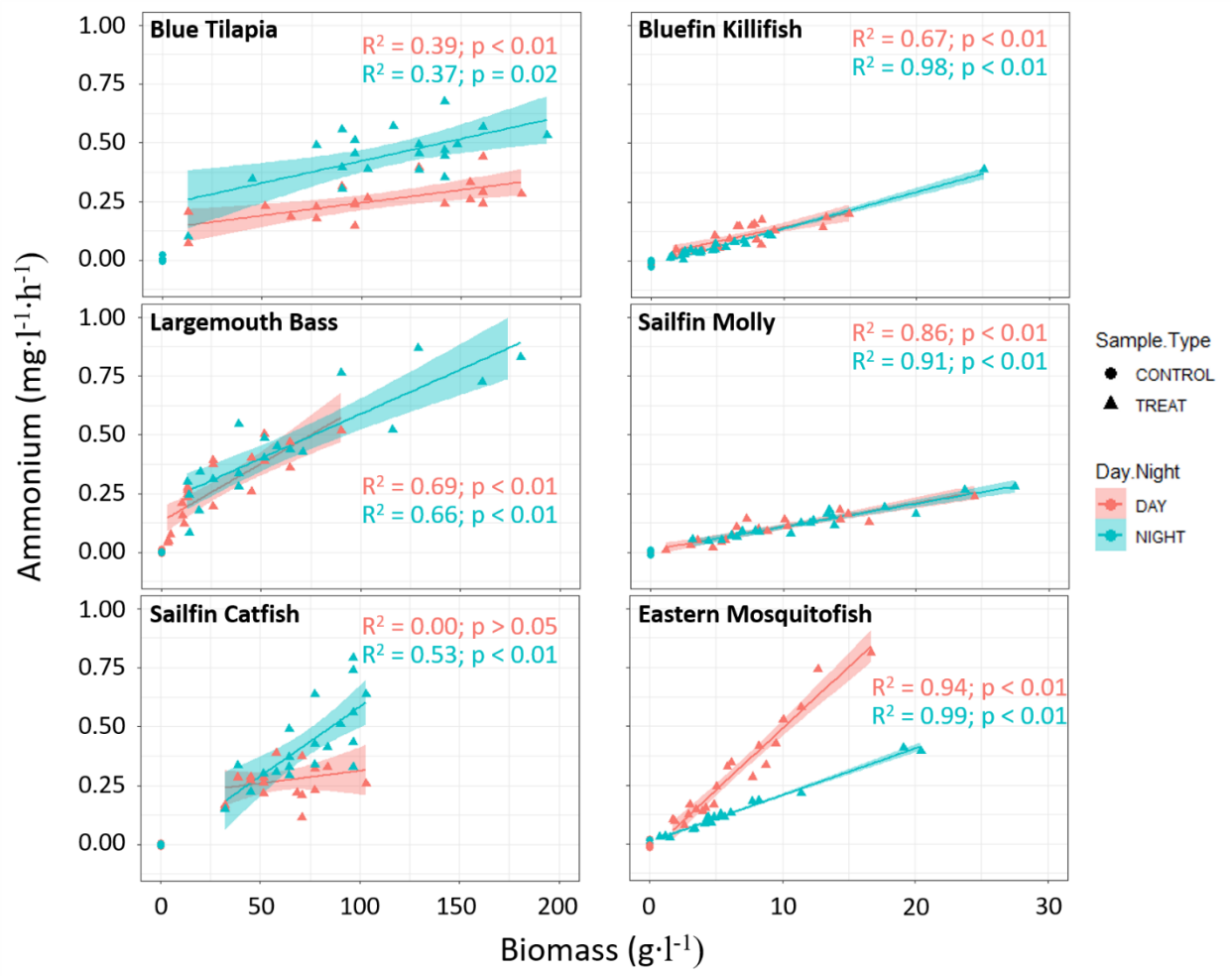

Figure 4. Relationship between ammonium excretion rate in milligrams per liter per hour ($mg \cdot L^{-1} \cdot hr^{-1}$) and biomass in grams per liter ($g \cdot L^{-1}$). Confidence intervals are 95%.

Table 3. Comparison of excretion rates between night and day. "Excretion" is the least squares means of excreted mass in milligrams per grams wet weight of fish per hour (mg/g ww/hr) estimated at the grand mean mass of fish used in the trials. "Biomass" in grams (g) and "Day/Night" report the significance (* < 0.05) of the main effects of biomass and time of day on excretion rates, respectively. "Mass*Day/Night" reports the significance of the interaction effect between biomass and time of day.

Fish Species	Biomass (g)	Analyte	Time of Day (Day/ Night)	Excretion (mg/g ww/hr)	Biomass	Day/ Night	Mass · Day/Night
Eastern Mosquitofish	1.675	NH_4	Day	0.079 ± 0.002	*	*	*
			Night	0.036 ± 0.002			
		TDP	Day	0.024 ± 0.001	*	*	*
			Night	0.018 ± 0.001			
		TN	Day	0.584 ± 0.041	*	*	*
			Night	0.177 ± 0.038			
		TP	Day	0.188 ± 0.018	*	*	*
			Night	0.025 ± 0.016			
Bluefin Killifish	1.608	NH_4	Day	0.025 ± 0.001	*	*	*
			Night	0.020 ± 0.001			
		TDP	Day	0.012 ± 0.001	*		
			Night	0.012 ± 0.001			
		TN	Day	0.151 ± 0.015	*	*	
			Night	0.095 ± 0.015			
		TP	Day	0.028 ± 0.005	*	*	
			Night	0.016 ± 0.005			
Sailfin Molly	2.812	NH_4	Day	0.031 ± 0.001	*		
			Night	0.030 ± 0.001			
		TDP	Day	0.016 ± 0.001	*	*	
			Night	0.013 ± 0.001			
		TN	Day	0.381 ± 0.025	*	*	*
			Night	0.166 ± 0.025			
		TP	Day	0.093 ± 0.006	*	*	*
			Night	0.025 ± 0.006			
Largemouth Bass	264.1	NH_4	Day	2.325 ± 0.155	*		
			Night	2.603 ± 0.182			
		TDP	Day	0.137 ± 0.059	*		*
			Night	0.259 ± 0.069			
		TN	Day	4.295 ± 0.476	*		
			Night	4.570 ± 0.560			
		TP	Day	0.178 ± 0.068	*		*
			Night	0.321 ± 0.080			
Blue Tilapia	855.1	NH_4	Day	1.983 ± 0.148	*	*	
			Night	3.430 ± 0.144			
		TDP	Day	0.418 ± 0.123	*	*	
			Night	0.926 ± 0.120			
		TN	Day	6.581 ± 1.179	*	*	*
			Night	13.321 ± 1.151			
		TP	Day	0.607 ± 0.180	*	*	*
			Night	1.388 ± 0.176			
Sailfin Catfish	518.3	NH_4	Day	2.146 ± 0.192	*	*	*
			Night	3.018 ± 0.189			
		TDP	Day	0.141 ± 0.023	*		
			Night	0.182 ± 0.023			
		TN	Day	3.24 ± 0.318	*	*	*
			Night	4.90 ± 0.313			
		TP	Day	0.209 ± 0.038	*		
			Night	0.277 ± 0.038			

Table 4. A summary of experimental conditions. The column "Biomass" is the mass in grams of fish per liter (g/L) of water in each incubation chamber. "Chamber Temperature" and "STA Temperature" are the water temperatures of the water inside of the incubation chamber and the environmental water that the fish were collected from, respectively. Values in "Biomass" and "Chamber Temperature" columns are the mean and standard deviations derived from 20 incubations for each row.

Fish Species	Volume (L)	Time of Day (Day or Night)	Biomass (g/L)	Chamber Temperature (°C)	STA Temperature (°C)
Eastern Mosquitofish	0.26	Day	6.55 ± 4.02	22.06 ± 0.71	20.2
		Night	6.32 ± 5.22	15.72 ± 0.06	17.3
Bluefin Killifish	0.26	Day	6.86 ± 3.71	17.2 ± 1.23	16.1
		Night	5.5 ± 5.13	14.12 ± 0.15	15.3
Sailfin Molly	0.26	Day	9.74 ± 6.44	18.17 ± 0.37	18.2
		Night	11.89 ± 6.44	15.93 ± 0.39	16.6
Largemouth Bass	7.76	Day	29.21 ± 24.93	23.34 ± 1.24	17.9
		Night	62.82 ± 50.55	19.53 ± 0.39	22.7
Blue Tilapia	7.76	Day	106.5 ± 50.2	20.54 ± 1.86	18.2
		Night	113.7 ± 41.1	20.94 ± 0.64	22.1
Sailfin Catfish	7.76	Day	59.8 ± 18.3	22.02 ± 2.36	19.1
		Night	73.78 ± 21.47	22.24 ± 1.28	23.6

Table 5. Summary of the relationships between total biomass and total mass excreted. "Slope" and "Y Intercept" are the slope and intercept of the linear relationship between total mass of nutrients produced in an incubation and total biomass, and these values were used to calculate areal excretion estimates outlined in **Tables 7**, **8**, and **9**. The models were significant ($p < 0.05$) in all cases.

Species	Analyte	Time of Day (Day/Night)	Adjusted R^2	Slope	Y Intercept
Eastern Mosquitofish	NH_4	Day	0.96	0.0505	-0.0043
		Night	0.99	0.02	0.0019
	TDP	Day	0.96	0.0148	-0.0004
		Night	0.97	0.0115	-0.0006
	TN	Day	0.82	0.3625	-0.0189
		Night	0.85	0.1216	-0.0219
	TP	Day	0.67	0.1079	0.0054
		Night	0.87	0.017	-0.0025
Bluefin Killifish	NH_4	Day	0.81	0.0144	0.0004
		Night	0.97	0.015	-0.0035
	TDP	Day	0.82	0.0064	0.0014
		Night	0.84	0.0065	0.0011
	TN	Day	0.58	0.0815	0.0175
		Night	0.84	0.0489	0.0137
	TP	Day	0.48	0.011	0.0034
		Night	0.84	0.0086	0.0014
Sailfin Molly	NH_4	Day	0.9	0.0103	0.0013
		Night	0.94	0.0102	0.0009
	TDP	Day	0.93	0.005	0.0015
		Night	0.85	0.0044	0.0002
	TN	Day	0.7	0.1128	0.0551
		Night	0.89	0.0613	-0.0053
	TP	Day	0.75	0.0327	0.0011
		Night	0.7324	0.0096	-0.0008
Largemouth Bass	NH_4	Day	0.74	0.0059	0.6509
		Night	0.79	0.0046	0.4759
	TDP	Day	0.53	0.0004	0.0318
		Night	0.61	0.0013	-0.0449
	TN	Day	0.79	0.0128	0.7053
		Night	0.66	0.0116	0.213
	TP	Day	0.56	0.0005	0.0396
		Night	0.61	0.0016	-0.046
Blue Tilapia	NH_4	Day	0.76	0.0017	0.4144
		Night	0.70	0.0031	0.5930
	TDP	Day	0.60	0.0004	0.0321
		Night	0.39	0.0011	-0.0382
	TN	Day	0.69	0.0066	0.7617
		Night	0.58	0.0150	0.0391
	TP	Day	0.54	0.0006	0.0530
		Night	0.40	0.0014	-0.0600
Sailfin Catfish	NH_4	Day	0.55	0.0035	0.3781
		Night	0.80	0.0058	-0.0124
	TDP	Day	0.47	0.0003	0.0056
		Night	0.49	0.0004	-0.0107
	TN	Day	0.57	0.0050	0.5425
		Night	0.78	0.01	-0.0053
	TP	Day	0.49	0.0003	0.0307
		Night	0.44	0.0006	-0.0168

Table 6. Estimates of mean mass in grams per cell of nutrients excreted per day by fish in STA-2. (Note: #N/D indicates a negative estimate due to low biomass.)

Species	Nutrient	Cell 3	Cell 4	Cell 5	Cell 6	Total
	NH_4-N	1,821	6,679	4,880	2,565	15,945
	TDP	699	2,565	1,874	985	6,123
	TN	12,424	45,579	33,302	17,504	108,809
	TP	3,135	11,500	8,402	4,417	27,453
Bluefin Killifish	NH_4-N	2,153	2,566	952	1,899	7,569
	TDP	943	1,124	417	832	3,318
	TN	9,312	11,098	4,121	8,214	32,744
	TP	1,416	1,688	627	1,249	4,981
Sailfin Molly	NH_4-N	3,395	5,571	328	391	9,684
	TDP	1,548	2,541	149	178	4,417
	TN	28,060	46,048	2,708	3,234	80,051
	TP	6,657	10,924	642	767	18,990
Blue Tilapia	NH4-N	46	104	154	36	340
	TDP	6	15	23	4	49
	TN	161	425	651	118	1,355
	TP	9	24	36	7	76
Largemouth Bass	NH4-N	807	433	470	310	2,020
	TDP	136	72	78	51	337
	TN	1,867	992	1,079	705	4,643
	TP	168	89	97	63	416
Sailfin Catfish	NH4-N	170	0	10	5	186
	TDP	12	0	#N/D	#N/D	12
	TN	276	0	16	8	301
	TP	16	0	1	#N/D	17

Table 7. Estimates of mean mass in grams per cell of nutrients excreted per day by fish in STA-1E. (Note: #N/D indicates a negative estimate due to low biomass.)

Species	Nutrient	Cell 2	Cell 4N	Cell 4S	Cell 6	Total
Blue Tilapia	NH_4-N	0	0	0	0	0
	TDP	0	0	0	0	0
	TN	0	0	0	0	0
	TP	0	0	0	0	0
Largemouth Bass	NH_4-N	80	278	87	425	869
	TDP	11	45	12	70	139
	TN	166	629	184	973	1951
	TP	14	56	16	87	172
Sailfin Catfish	NH_4-N	8	14	29	261	312
	TDP	#N/D	1	2	19	21
	TN	12	23	46	424	505
	TP	#N/D	1	3	25	29

Table 8. Estimates of mean mass in grams per cell of nutrients excreted per day by fish in STA-1W. (Note: #N/D indicates a negative estimate due to low biomass.)

Species	Nutrient	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2	Total
Blue Tilapia	NH_4-N	0	0	0	0	0	0	0
	TDP	0	0	0	0	0	0	0
	TN	0	0	0	0	0	0	0
	TP	0	0	0	0	0	0	0
Largemouth Bass	NH_4-N	96	0	15	0	104	0	215
	TDP	14	0	#N/D	0	15	0	29
	TN	203	0	15	0	222	0	441
	TP	17	0	#N/D	0	19	0	37
Sailfin Catfish	NH_4-N	15	26	0	0	0	0	41
	TDP	1	2	0	0	0	0	3
	TN	24	41	0	0	0	0	65
	TP	1	2	0	0	0	0	3

DISCUSSION AND CONCLUSIONS

A brief literature review suggests that estimated excretion rates for the six species evaluated fall within a similar range of other freshwater fish species (Zimmer et al. 2006, Capps and Flecker 2013, Oliveira-Cunha et al. 2018). Though little work has considered the difference between diurnal and nocturnal excretion rates in freshwater fish, Oliveira-Cunha et al. (2018) found that this difference can vary greatly among species. We found that the rates of excretion, as well as the direction of difference between night and day differed among species, with large fish excreting more at night and small fish excreting more during the day in general. Though differences in diurnal and nocturnal excretion rates could be linked to differences in temperature between day and night, temperature was correlated to excretion in only a few cases.

These differences are likely explained by behaviors such as predator avoidance strategies. Smaller fish generally become less active at night (Helfman 1986), including most Everglades species (Obaza et al. 2011). We found small fish difficult to catch at night because they were hiding in thick mats of submerged vegetation. In contrast, during the day they were active in open water. Though it is unclear what caused differences in diurnal and nocturnal excretion, these differences were large enough to affect efforts to scale up estimated nutrient loads recycled by excretion and egestion from fish in the STAs.

Given the estimated biomass for fish in the STAs from Evans et al. (2019; **Table 1**), our estimates of excretion suggest that fish have a substantial effect on water column nutrient concentrations through excretion. We estimated that these six species excrete 228 kilograms (kg) of N and 52 kg of P per day in Cells 3, 4, 5, and 6 of STA-2 (combined). For N, this value is equivalent to 6% of the average annual N load from 2003 through 2016 entering all of STA-2 (1,305,880 kilograms per year [kg/y]; Chimney 2017). For P, this value is equivalent to 53% of the average P load from 2011 through 2019 entering all of STA-2 (36,247 kg/y; Hongying Zhao, South Florida Water Management District, personal communication). Note that these excretion values are not an additional source of nutrients, but rather recycled from the internal storage and external nutrient loads.

Our estimates of excretory contributions to STA-1E and STA-1W are highly speculative because small fish density estimates are not available from these regions. Small fish comprised 97 and 99% of excretory contributions of N and P reported for STA-2, respectively. If we assume that a similar proportion of small to large fish make up the biomass in other STAs, these six species excrete and estimated 82 kg of N and

6.7 kg of P in STA-1E, and 17 kg of N and 1.3 kg of P per day in STA-1W. For N, these values are equivalent to 9 and 1% of the average N load from 2007 through 2016 entering STA-1E (332,971 kg/y) and STA-1W (645,197 kg/y), respectively (Chimney 2017). For P, these values are equivalent to 12 and 1% of the average P load from 2008 through 2019 entering all of STA-1E (20,683 kg/y) and STA-1W (37,069 kg/y), respectively (Chapter 5B, Table 5B-1, of this volume). As noted above, unlike nutrients measured at the inflow to each STA, these are not new nutrients to the STAs. Further analysis is needed to understand the contributions of fish excretion to outflow concentrations. However, our calculations illustrate a significant role of fishes in recycling nutrients and possibly transforming them into bioavailable dissolved or small particulate forms that could elevate water column concentrations at the STA outflows.

Our estimates require several assumptions that need to be evaluated as part of their interpretation. First, differences in the slope of the relationship between biomass and excretion rate for day and night suggest that size in large fish is an important predictor in the mass of excreta produced during daytime and nighttime hours. Therefore, proper areal estimates of excretion would take the size distributions of fish into account, whereas the current estimates did not. Second, this experiment is representative of the winter months in South Florida, but environmental conditions associated with season probably affect fish metabolism and excretion. Thus, this experiment should be repeated in different seasons to scale areal estimations of excretion to an annual budget. Also, this work only addresses fish, but our sampling studies have demonstrated that macroinvertebrates, particularly grass shrimp (*Palaemonetes paludosus*) can be very dense in the STAs. Additional work is needed to incorporate data on macroinvertebrates in the P budget. Finally, the use of electrofishing surveys to estimate areal biomass requires calibration. Though correction factors for abundance/biomass estimates for electrofishing surveys exist for some native species (Chick et al. 2004, Schoenebeck et al. 2015), these factors were determined in different conditions than those found in the STAs. Notably, the STAs can have high conductivity (> 1200 microsiemens per centimeter [µS/cm]), which can affect the efficiency of the electroshocking equipment. Abundance of blue tilapia is known to be underestimated by electrofishing, which is evident from the zero catch rates in STA-1W and STA-1E (**Tables 7** and **8**); the areal estimates of excretion would be improved by correction factors derived from in situ calibration experiments.

The experiments reported in this year's South Florida Environmental Report took place during winter months that represent only a portion of the climate experienced by South Florida fishes. As fish are poikilotherms, seasonal variation in their excretion rates are expected and well documented in other systems. Furthermore, food resources vary seasonally, also affecting their excretory biology. Future efforts will expand the range of seasonal conditions studied to enhance the robustness of assessment of fish influences on STA nutrient budgets. This ongoing project provides a conservative estimate of the role of fishes in recycling N and P in the STAs. In last year's report, we documented that fishes are much more densely packed in the STAs than in Everglades wetlands and that those fish contain a significant mass of N and P in their tissues. This year we report progress in estimating the role of fishes in recycling N and P through excretion. In addition to high mass of these nutrients passing through fish digestive systems, the form of nutrients leaving fishes may be readily available for heterotrophic and autotrophic bacteria, algae, and vascular plants. The transformation of nutrients to bioavailable forms may be the most important implication of this ongoing work and will need to be examined in greater detail in future studies. We are currently analyzing new results on bioturbation by fishes that illustrate a second route by which fish are potentially affecting STA nutrient removal efficiency. We believe that our ongoing work and upcoming reports will provide helpful information for planning of STA management activities to achieve the mandated discharge limits in part through manipulation and control of fish populations.

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