

Appendix 5C-4: Effects of Abundant Faunal Species on Phosphorus Cycling in the Stormwater Treatment Areas

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SUMMARY

Fish and macroinvertebrate diversity were surveyed during three sampling periods in 2016 and 2017. The resulting quantification of species abundance and richness for the Everglades Stormwater Treatment Areas (STAs) allowed for comparisons of those patterns to other regions of the Everglades. The high abundance of aquatic animals in the STAs support the potential for fishes and macroinvertebrates there to have a greater impact on nutrient cycling than may be expected in other South Florida wetlands. Combining this survey data with vegetation survey data, small-bodied fish and macroinvertebrate areal biomass was estimated for STA-2 Cells 3, 4, 5, and 6. Moreover, large-bodied fish biomass was estimated for the submerged aquatic vegetation (SAV) treatment cells in STA-2, STA-1 East (STA-1E), and STA-1 West (STA-1W). Using these areal biomass estimates, it was possible to scale-up estimates of fish nutrient excretion rates. Furthermore, the mesocosm experiments illustrate the potential for bioturbation by fishes to increase water column nutrient concentration. The preliminary results indicate that because of the high density and biomass of fishes and macroinvertebrates in the STAs, animal excretion and bioturbation may be an important mechanism by which phosphorus (P) and nitrogen (N) are cycled within and among STA habitats. Aquatic animals have a large potential for translocation of P and N between the water column and benthos. Future research will focus on refining these estimates and incorporating the effects of aquatic fauna into nutrient budgets for the STAs.

INTRODUCTION

Fauna, including waterbirds, fish, and macroinvertebrates, can affect the cycling and reduction of P within the Everglades STAs through several pathways. First, animals function as a source of internal nutrient loading by directly mobilizing benthic or particulate nutrients through their feeding and excretion (Vanni et al. 2006). Second, they can have important indirect effects through modifications of the environment (e.g., bioturbation; Vanni et al. 2006) and by top-down consumptive effects involving predator limitation of food resources and cascading interactive effects (Kellog and Dorn 2012, Dorn 2013). Bioturbation elevates water column nutrients through the resuspension of benthic nutrients and seston. Top-down effects may affect nutrient cycles if they alter the abundance of key grazers or bioturbators, which alter the efficiency of animal-mediated nutrient cycles. Finally, aquatic animals can act as P sinks and vectors of P-transport (Vanni et al. 2006), especially by large, mobile animals such as birds, crocodilians, and fish.

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While the ecological literature points to a pivotal role of fauna in STA nutrient cycling, the direct and indirect roles of animals on nutrient cycles have not been studied or estimated in the STAs, and their effect on P transformations is currently unknown. The magnitudes of these processes within the STAs and ultimately the effect of fauna on water column total phosphorus (TP) concentrations in the STA outflow cells are determined from a series of four tasks: (1) estimate standing stock biomass (density) of fish and aquatic macroinvertebrates (STA-2), and aquatic faunal community compositional data (STA-1E and STA-1W); (2) determine stoichiometry (carbon [C], N, and P) of major fish species through tissue samples; (3) estimate mass-specific P consumption and excretion rates of the most abundant species; and (4) experimentally evaluate the potential of benthic aquatic species to enhance water column nutrient concentrations through bioturbation. Tasks 1 and 2 were the focus of last year's report and results from that work is summarized here to support its use in spatial scaling of results from Tasks 3 and 4 and establish a context for interpreting STA results through comparison to marshes in the Everglades. Biomass and excretion estimates are combined and scaled up to estimate areal (per hectare [ha]) P excretion by the entire aquatic faunal assemblage in STA-2 (i.e., rates of P released to the water column via excretion in micrograms phosphorus per hectare per hour [$\mu\text{g P/ha/h}$]). Excreted loads of P are compared to external loads of P and the nutrient demand of SAV through collaboration with vegetation scientists. Bioturbation estimates are used to evaluate the potential of animals to alter the efficiency of benthic sequestration of TP that may be included in future nutrient budgets and provide guidance for management actions aimed at improving P retention efficiency. This section summarizes the initial results of the biomass estimation, fish tissue stoichiometry, excretion incubations, and bioturbation experiments.

METHODS

QUANTIFYING AQUATIC FAUNAL BIOMASS

Fish and macroinvertebrate biomass and community composition were quantified for STA-2 outflow Cells 3, 4, 5, and 6 from June 2016 through March 2017. Large fish (≥ 8 centimeters [cm] standard length [SL]) abundance was quantified as average catch per unit effort (CPUE) based on replicated 5-minute electrofishing transects (Chick et al. 1999). Small fish (< 8 cm SL) and macroinvertebrates were quantified using 1-square meter (m^2) throw traps (Dorn et al. 2005, Jordan et al. 1997) in open water and SAV habitats to estimate density and species composition. All captured individuals were euthanized with MS222, preserved with formalin in the field, and processed in the lab in the weeks following the sampling.

A random sampling design stratified by vegetation type was used to incorporate spatial variation in animal biomass and associated habitat types within the STA outflow cells. Sampling effort (124 throw-trap samples and 16 electrofishing transects) was proportionally stratified based on the areal coverage of the four dominant habitats—*Ceratophyllum demersum*, *Najas* spp., *Chara* spp., and open water—found in the SAV outflow cells. To capture seasonal variation, throw-trap samples were collected in June and September 2016 as well as March 2017. Throw-trap collection yields data on density (number of individuals per 1-m^2) that are readily scalable. Electrofishing transect samples were collected in STA-2 during February, April, and October 2016, and March 2017. In addition, four electrofishing transect samples per outflow cell were collected in STA-1E (Cells 4S, 4N, and 6) and STA-1W (Cells 1B, 2B, 3, 4, 5B1, and 5B2). Electrofishing transects yield data on CPUE, which requires assumption of the areal coverage of the transect to be scaled-up spatially (see next paragraph). Funded by other sources, we collected throw trap and electrofishing data in three areas of the Everglades at approximately the same time as STA were collected. We report the Everglades data to establish a reference for interpretation of the STA data collected for this project. This comparison was the focus of our *2018 South Florida Environmental Report* (SFER) contribution and the details of collection of these data is reported there in Appendix 5C-3 of the 2018 SFER – Volume I (Villapando and King 2018). All Everglades study areas (Shark River Slough (SRS), Taylor Slough (TSL), Water Conservation Areas 3A and B (WCA 3) were oligotrophic or mesotrophic vegetated

marshes. Greater detail on the sampling methods and design is also reported in Appendix 5C-3 of the 2018 SFER – Volume I (Villapando and King 2018).

The areal biomass of fishes and macroinvertebrates was estimated by combining electrofishing and throw-trap survey data with the South Florida Water Management District's (SFWMD's or District's) November 2015 vegetation data. Mean throw-trap biomass estimates (grams per square meter [g/m^2]) for each of the four vegetation habitat types were scaled to the size of the STA cell using the areal coverage of the four habitats. Mean electrofishing-biomass estimates (grams per 5-minute transect [$\text{g}/5\text{-min transect}$]) were converted to areal estimates (g/m) by assuming the area sampled during transect was equal to the distance traversed during the transect multiplied by the width of the electrofisher anode array (2.5 meters [m]). Areal electrofishing biomass estimates were scaled to the size of the STA cell using the areal coverage of deep water (canal) and shallow water (marsh) habitats as estimated from aerial photographs. All chemistry analyses were conducted by the SFWMD Chemistry Laboratory and all statistical analyses were performed in R 3.4.2 (R Development Core Team, Vienna, Austria). Additional details on these results were reported in Appendix 5C-3 of the 2018 SFER – Volume I (Villapando and King 2018).

QUANTIFYING PHOSPHORUS AND NITROGEN SEQUESTRATION BY FISHES

To estimate the total mass of P and N sequestered in STA fishes, whole-body tissue stoichiometry (C:N:P) was determined for 10 abundant species in STA-2. Fish tissue stoichiometry samples were collected for bluefin killifish (*Lucania goodei*), eastern mosquitofish (*Gambusia holbrooki*), sailfin molly (*Poecilia latipinna*), largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), redear sunfish (*Lepomis microlophus*), Mayan cichlid (*Cichlasoma urophthalmus*), Florida gar (*Lepisosteus platyrhincus*), Seminole killifish (*Fundulus seminolis*), and blue tilapia (*Oreochromis aureus*). Two sets of samples of up to 10 replicates of each species representing the size range of individuals present in the STAs were collected and processed. The first set of samples was collected from August through November 2016. The second set of samples was collected from October through March 2017. Small fish ($\leq 5\text{-cm SL}$) tissue samples were processed by freeze drying whole, then grinding to a fine powder using a ball mill and mortar and pestle. Large-fish ($> 5\text{-cm SL}$) tissue samples were processed by first homogenizing the tissue using a meat grinder, freeze drying the homogenized sample, and then grinding to a fine powder using ball mill and mortar and pestle. Fish samples were analyzed for TP, total nitrogen (TN), total carbon (TC), and stoichiometric relationships based on molar ratios. Relationships of nutrient content and body size (length and mass) for the first and second sampling sets were compared for each of the 10 species via a Student's *t*-test. The quantities of C, N, and P in each STA cell were estimated by multiplying the total species-specific estimated dry mass by the mean nutrient content of each species.

ESTIMATING PHOSPHORUS AND NITROGEN EXCRETION BY FISHES

N and P excretion and egestion were measured for three large-bodied species captured in our electrofishing surveys and the three small-bodied species collected during our throw trap surveys. Large-bodied species included blue tilapia, vermiculated sailfin catfish (*Pterygoplichthys disjunctivus*), and largemouth bass. Small-bodied species included eastern mosquitofish, bluefin killifish, and sailfin molly. All species were selected for excretion study based on their high abundance except for vermiculated sailfin catfish, which was selected because of their potential impact on P budgets through bioturbation. TP, total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), TN, total dissolved nitrogen (TDN), and ammonium-nitrogen ($\text{NH}_4\text{-N}$) excretion rates were estimated via short-term incubations. Preliminary results for the three small-bodied species and vermiculated sailfin catfish are presented here. Further estimates for other species are underway.

From November 2017 through April 2018, daytime incubations were conducted for each of the four study species. Incubations followed methods similar to those employed by Torres and Vanni (2007), Whiles

et al. (2009), and Capps and Flecker (2013). Each incubation consisted of a 3.79-liter (L) polyethylene zip-top bag filled with 540 milliliters (mL) of STA water that was prefiltered through a 0.7-micromole (μM) glass fiber filter to remove phytoplankton and particulates. Immediately after filling with water, a multiple-individual sample of fish was placed into the bag and incubated for 60 minutes. Incubation biomasses varied by changing the number of individuals in each incubation. Individuals were selected from a representative sample of specimens collected in the sampling area; individual fish were too small to yield detectable levels of excretion in an experimental setting optimized to obtain field-relevant excretion estimates. Water samples were collected from each chamber immediately prior to adding the fish and at the conclusion of the incubation time to assess the change in nutrients due to fish. Additionally, fishless control bags were incubated in parallel, quantifying any non-fish related nutrient transformations. Water samples were processed and preserved according to standard methods and analyzed for TP/TN, TDP/TDN, SRP, and $\text{NH}_4\text{-N}$.

Nutrient excretion rates were estimated by regressing the excreta mass against the biomass of fish present in the incubation bags. For each of the three small-bodied fish species, 20 incubations have been completed. Following completion of the small-bodied fish excretion samples, the same methods were used to quantify excretion by vermiculated sailfin catfish (10 incubations) in 12-L incubation chambers. Vermiculated sailfin catfish samples were completed on a pilot basis.

Areal biomass estimates were combined with species-specific excretion rates to generate areal estimates of total assemblage excretion for each of the STA-2 cells. However, a lack of a significant positive relationship between fish biomass and excretion precluded estimating areal excretion estimates for some eastern mosquitofish, bluefin killifish, and vermiculated sailfin catfish N and P analytes. Biomass versus excretion regression equations were used to estimate the mass of $\text{NH}_4\text{-N}$ excreted per hour for bluefin killifish, sailfin molly, and vermiculated sailfin catfish. The mass of SRP excreted was estimated for eastern mosquitofish, bluefin killifish, and sailfin molly. The mass of TDN excreted was estimated for bluefin killifish and sailfin molly. The mass of TN excreted was estimated for bluefin killifish, sailfin molly, and vermiculated sailfin catfish. The mass of TDP excreted was estimated for bluefin killifish, sailfin molly, and vermiculated sailfin catfish. The mass of TP excreted was estimated for eastern mosquitofish, sailfin molly, and vermiculated sailfin catfish.

EVALUATING THE POTENTIAL FOR PHOSPHORUS AND NITROGEN RESUSPENSION BY FISHES

The effects of bioturbation on TP and TN concentration in the water column were assessed via in situ mesocosm experiments containing blue tilapia, Orinoco sailfin catfish (*Pterygoplichthys multiradiatus*), and largemouth bass. Orinoco sailfin catfish were used in the experiment rather than vermiculated sailfin catfish because of their greater abundance in STA-3/4, where the experiment was conducted. Mesocosm enclosures (3.2 x 3.2 m; 10.2 m²) were stocked with three densities of each target species including high density, low density, and fishless control treatments (**Table 1**). The high density treatment was approximately equal to the maximum density of the focal species observed during our electrofishing surveys in the STAs. The low density treatment was approximately equal to the average density of the focal species observed during our electrofishing surveys in the STAs. The fishless control treatments contained no individuals of the focal species. To ensure the fish did not escape, the bottoms of the enclosures were buried 20 to 30 cm into the substrate and tops of the enclosures were covered with bird netting. Enclosures were placed in the field at least five days prior to stocking with fish. Water samples were collected from each enclosure 7 and 14 days after the start of the experiment. To evaluate the effect of longer experimental trials, a third set of samples was collected after 28 days for the Orinoco sailfin catfish experimental trial. Water samples were collected by mid-water column grab sampling, acidified then analyzed for TN and TP. Water column TN and TP, nutrient contents, and stoichiometric ratios from the three treatment densities were compared via repeated measures analysis of variance (rmANOVA).

Table 1. Bioturbation experiment treatment densities (number of fish per enclosure). Each treatment was replicated three times (sample size [n] = 3). Mesocosms enclosed as surface area of 10.2 m².

Species	Control	Low Density	High Density
Orinoco sailfin catfish	0	1	2
blue tilapia	0	2	4
largemouth bass	0	2	3

RESULTS AND DISCUSSION

SMALL-BODIED FISH AND MACROINVERTEBRATE COMMUNITY COMPOSITION

In STA-2, in June and September 2016, and March 2017, 12,784 small-bodied fishes and 13,279 macroinvertebrates were collected from a total of 372 throw-trap samples (1-m²). The throw-trap samples consisted of 21 fish species and 33 macroinvertebrate taxa (**Table 2**; note some invertebrates could not be identified to species). Mean fish density (individuals per square meter [ind/m²]; $\pm$ standard error [SE]) was 42.4 ± 5.0 ind/m² in June, 35.5 ± 3.7 ind/m² in September, and 25.4 ± 3.6 ind/m² in March (**Figure 1**). Across all throw-trap samples, eastern mosquitofish (31% of total catch), sailfin molly (23% of total catch), bluefin killifish (19% of total catch), least killifish (*Heterandria formosa*; 12% of total catch), and Mayan cichlid (6% of total catch) were the most commonly captured fish species (**Figure 2**). Mean macroinvertebrate density was 38.6 ± 4.8 ind/m² in June, 20.3 ± 4.0 ind/m² in September, and 48.3 ± 6.5 ind/m² in March (**Figure 3**). Across all throw-trap samples, grass shrimp (*Palaemonetes paludosus*; 60% of total catch), four-spotted pennant (*Brachymesia gravida*; 9% of total catch), midge larva (Chironomidae; 7% of total catch), mesa rams-horn snail (*Planorbella scalaris* 6% of total catch), and marl pennant (*Macrodiplax balteata*; 5% of total catch) were the most commonly captured invertebrate species. Both fishes and macroinvertebrates were collected at higher density in the STAs than in any areas of the Everglades (**Figures 1 and 3**; discussed in more detail in the 2018 SFER).

Table 2. Taxa names and abbreviations for fishes and macroinvertebrates captured in throw-trap and electrofishing samples collected in STA-1E, STA-1W, and STA-2.

Common Name	Latin Binomial	Abbreviation	Common Name	Latin Binomial	Abbreviation
Fishes			Invertebrates		
African jewelfish	<i>Hemichromis letourneuxi</i>	HETLET	creeping water bug	<i>Pelocoris femoratus</i>	PELFEM
Amazon sailfin catfish	<i>Pterygoplichthys pardalis</i>	PTEPAR	apple snail	<i>Pomacea paludosa</i>	POMPAL
black crappie	<i>Pomoxis nigromaculatus</i>	POMNIG	Asian fingernail clam	<i>Corbicula fluminea</i>	CORFLU
blue tilapia	<i>Oreochromis aureus</i>	OREAUR	blue dasher	<i>Pachydiplax longipennis</i>	PACLON
bluefin killifish	<i>Lucania goodei</i>	LUCGOO	damselfly larva	Coenagrionidae	COENAG
bluegill	<i>Lepomis macrochirus</i>	LEPMAC	eastern amberwing	<i>Perithemis tenera</i>	PERTEN
bowfin	<i>Amia calva</i>	AMICAL	eastern pondhawk	<i>Erythemis simplicicollis</i>	ERYSIM
brook silverside	<i>Labidesthes sicculus</i>	LABSIC	everglades crayfish	<i>Procambarus alleni</i>	PROALL
brown bullhead	<i>Ameiurus nebulosus</i>	AMENEB	four-spotted pennant	<i>Brachymesia gravida</i>	BRAGRA
brown hoplo	<i>Hoplosternum littorale</i>	HOPLIT	freshwater mussel	<i>Villosa amygdala</i>	VILAMY
clown goby	<i>Microgobius gulosus</i>	MICGUL	garnet glider	<i>Tauriphila australis</i>	TAUAUS
eastern mosquitofish	<i>Gambusia holbrooki</i>	GAMHOL	giant water bug	<i>Belostoma spp.</i>	BELSPP
flagfish	<i>Jordanella floridae</i>	JORFLO	grass shrimp	<i>Palaemonetes paludosus</i>	PALPAL
Florida gar	<i>Lepisosteus platyrhincus</i>	LEPPLA	leeches	Hirudinea	HIRUDI
gizzard shad	<i>Dorosoma cepedianum</i>	DORCEP	marl pennant	<i>Macrodiplax balteata</i>	MACBAL
golden shiner	<i>Notemigonus crysoleucas</i>	NOTCRY	mayfly larva	<i>Ephemeroptera</i>	EPHEME
inland silverside	<i>Menidia beryllina</i>	MENBER	mesa rams-horn snail	<i>Planorbella scalaris</i>	PLASCA
lake chubsucker	<i>Erimyzon sucetta</i>	ERISUC	midge larva	Chironomidae	CHIRON
largemouth bass	<i>Micropterus salmoides</i>	MICSAL	mimic lymnaea	<i>Pseudosuccinea columella</i>	PSECOL
least killifish	<i>Heterandria formosa</i>	HETFOR	mysid shrimp	<i>Taphromysis louisianae</i>	TAPLOU
Mayan cichlid	<i>Cichlasoma urophthalmus</i>	CICURO	pennant dragonflies	<i>Celithemis spp.</i>	CELSPP
Nile tilapia	<i>Oreochromis niloticus</i>	ORENIL	predaceous diving beetle	<i>Cybister fimbriolatus</i>	CYBFIM
Orinoco sailfin catfish	<i>Pterygoplichthys multiradiatus</i>	PTMUL	red-rimmed melania	<i>Melanoides tuberculata</i>	MELTUB
redeer sunfish	<i>Lepomis microlophus</i>	LEPMIC	saddlebag gliders	<i>Tremea spp.</i>	TRASPP
sailfin molly	<i>Poecilia latipinna</i>	POELAT	skimmer dragonflies	<i>Libellula spp.</i>	LIBSPP
Seminole killifish	<i>Fundulus seminolis</i>	FUNSEM	slough crayfish	<i>Procambarus fallax</i>	PROFAL
spotted sunfish	<i>Lepomis punctatus</i>	LEPPUN	sponge	<i>Spongilla lacustris</i>	SPOLAC
spotted tilapia	<i>Tilapia mariae</i>	TILMAR	two-striped forceptail	<i>Aphylla williamsoni</i>	APHWIL
swamp darter	<i>Etheostoma fusiforme</i>	ETHFUS	unidentified aquatic beetle	Coleoptera	COLEOP
taillight shiner	<i>Notropis maculatus</i>	NOTMAC	unidentified dragonfly larva	Anisoptera	ANISOP
vermiculated sailfin catfish	<i>Pterygoplichthys disjunctivus</i>	PREDIS	unidentified fly larva	Diptera	DIPTER
walking catfish	<i>Clarias batrachus</i>	CLABAT	unidentified physid snails	<i>Haitia spp.</i>	HAISPP
warmouth	<i>Lepomis gulosus</i>	LEPGUL	water boatman	Corixidae	CORIXI
			water striders	Gerridae	GERRID

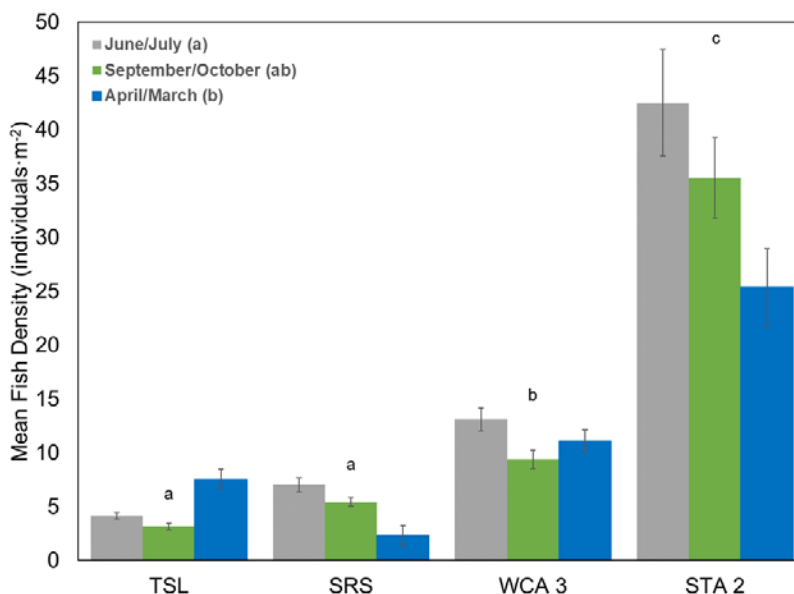


Figure 1. Mean fish density in individuals per square meter (individuals · m⁻²) estimated from throw-trap samples collected in summer 2016 (grey bars), fall 2016 (green bars), and spring 2017 (blue bars). STA-2 samples were collected in June 2016, September 2016, and March 2017 (n = 124 throws per sampling event). Water Conservation Area 3 (WCA 3) samples were collected July 2016, October 2016, and April 2017 (n = 150 throws). Shark River Slough (SRS) samples were collected July 2016, October 2016, and April 2017 (n = 126 throws). Taylor Slough (TSL) samples were collected June–July 2016, October 2016, and April (n = 175 throws). Letters above bars and in text box (sampling month) indicate significant mean separation ($\alpha = 0.05$). Error bars represent ± 1 SE.

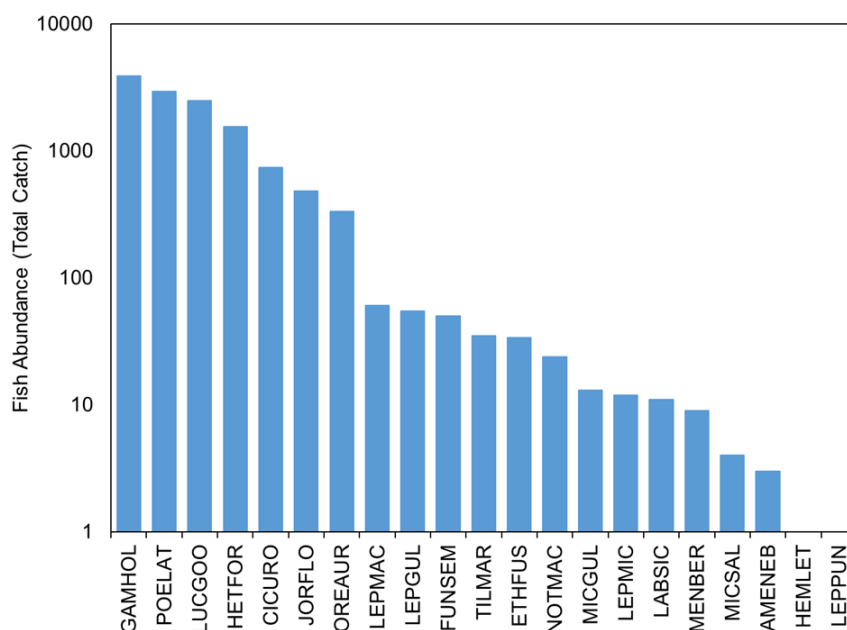


Figure 2. Relative abundance of fish species collected in STA-2 during throw-trap sampling. Relative abundance calculated by summing catches from all 372 throws collected during June 2016, September 2016, and March 2017. See **Table 2** for definitions of species abbreviations.

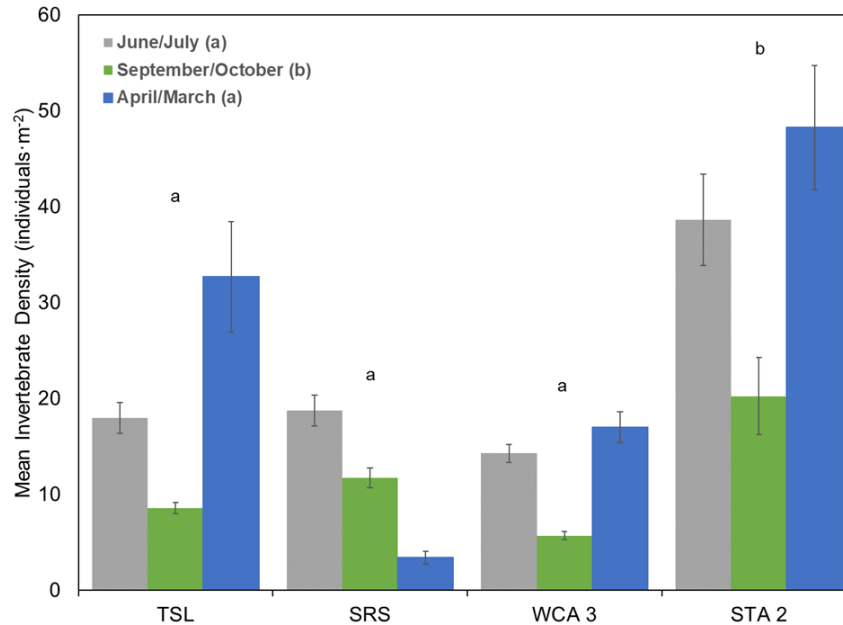


Figure 3. Mean macroinvertebrate density in individuals per square meter (individuals · m⁻²) estimated from throw-trap samples collected in summer 2016 (grey bars), fall 2016 (green bars), and spring 2017 (blue bars). STA-2 samples were collected in June 2016, September 2016, and March 2017 (n = 124 throws per sampling event). Water Conservation Area 3 (WCA 3) samples were collected July 2016, October 2016, and April 2017 (n = 150 throws). Shark River Slough (SRS) samples were collected July 2016, October 2016, and April 2017 (n = 126 throws). Taylor Slough (TSL) samples were collected June–July 2016, October 2016, and April (n = 175 throws). Letters above bars and in text box (sampling month) indicate significant mean separation among regions ($\alpha = 0.05$). Error bars represent ± 1 SE.

LARGE-BODIED FISH COMMUNITY COMPOSITION

Four electrofishing sampling events in STA-2, STA-1E, and STA-1W resulted in a total of 2,036 specimens from 221 electrofishing transects. Across all sampling events, 20 species were collected in STA-2, 21 species in STA-1E, and 19 species in STA-1W. Catch relative abundance was consistent among the sampling events, with largemouth bass, sunfish (*Lepomis* spp.), Seminole killifish and blue tilapia were among the most commonly collected species (**Table 3**). Mean CPUE in April was on average 1.8 times greater than in February and October (rmANOVA, probability [p] < 0.01). These differences appear to be heavily influenced by the disproportionately greater catches in STA-1W during the April sampling event (**Figure 4**) and relatively small catches in STA-2 and STA-1W during the October and March sampling events. Mean CPUE in March was similar to the other three sampling events. CPUE from the Everglades was also lower than in the STA study sites (**Figure 4**), similar to our findings for small fish, and are discussed in detail within Appendix 5C-3 of the 2018 SFER – Volume I (Villapando and King 2018).

Table 3. Total electrofishing catch (number of individuals) for STA-1E, STA-1W, and STA-2 during the four sampling periods. Catches are the sum total of all 5-minute transects completed within each STA.

Species	STA-2				STA-1E				STA-1W			
	February	April	October	March	February	April	October	March	February	April	October	March
brown bullhead	2	2	0	0	2	0	1	1	2	2	0	1
bowfin	11	3	2	0	5	9	2	1	1	9	3	1
Mayan cichlid	3	13	0	3	1	16	14	4	0	11	11	4
walking catfish	0	1	0	1	0	2	0	0	0	1	1	0
gizzard shad	0	0	0	0	3	6	1	7	0	1	1	7
lake chubsucker	1	0	1	0	2	3	4	3	1	0	0	3
Seminole killifish	15	8	5	1	32	54	19	7	65	23	0	7
brown hoplo	1	0	1	0	4	3	1	0	0	0	0	0
warmouth	2	8	1	4	2	21	3	0	0	11	0	0
bluegill	16	46	0	10	33	103	52	20	6	60	9	20
redecor sunfish	29	14	1	12	59	55	62	26	8	53	5	26
Florida gar	18	12	3	10	15	28	9	3	18	60	27	3
spotted sunfish	1	1	0	0	1	4	2	0	1	6	0	0
largemouth bass	44	52	7	11	36	43	15	9	11	21	2	9
golden shiner	2	0	2	0	14	10	0	0	2	10	6	0
blue tilapia	17	3	5	1	43	53	28	3	14	23	13	3
Nile tilapia	0	5	0	0	0	5	0	0	0	0	0	0
black crappie	0	0	0	0	0	2	0	0	0	0	1	0
vermiculated sailfin catfish	0	1	0	0	4	16	3	11	0	2	3	11
Orinoco sailfin catfish	1	1	0	0	3	0	0	3	2	0	0	3
Amazon sailfin catfish	0	1	0	0	1	5	0	0	0	0	0	0

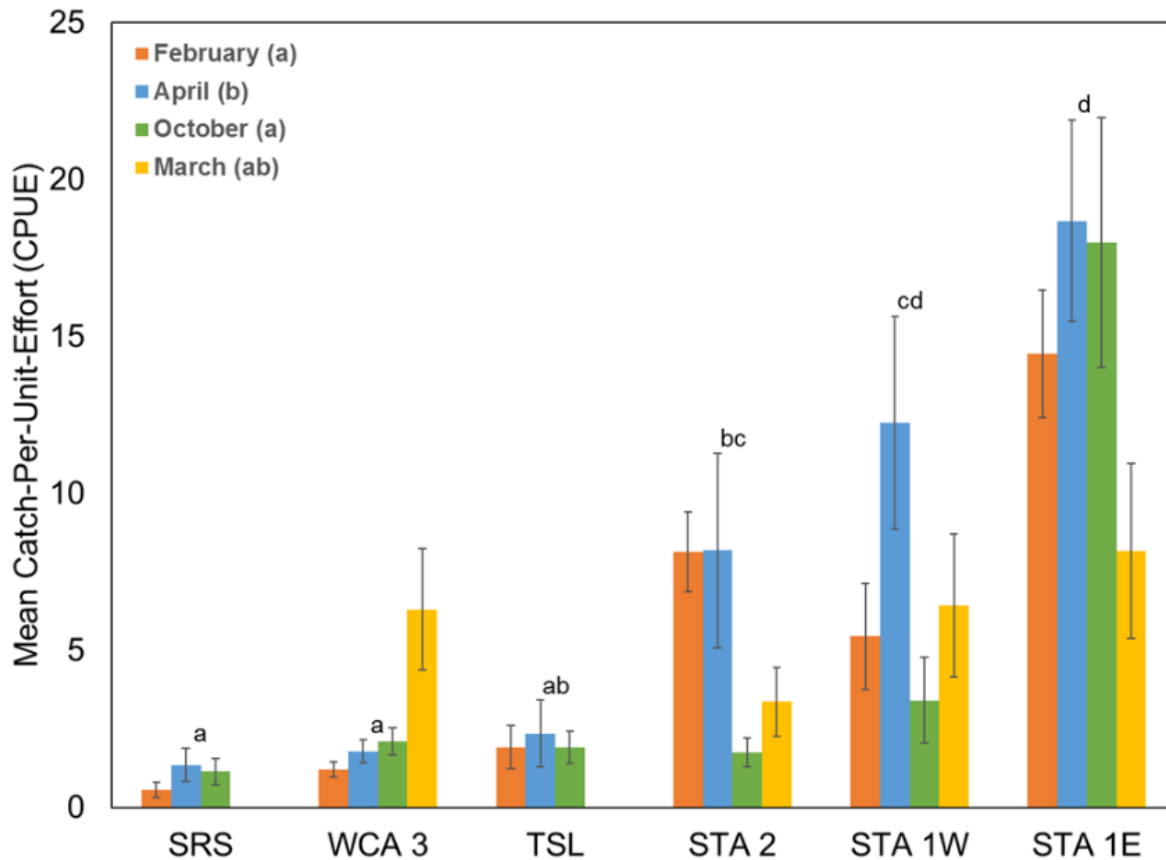


Figure 4. Mean electrofishing CPUE from 5-minute transect samples collected during January–February 2016, March–April 2016, September–October 2016, and March–April 2017. Shark River Slough (SRS) and Taylor Slough (TSL) were not sampled in March–April 2017 because of airboat inaccessibility resulting from shallow water conditions. Letters above bars and in text box (sampling month) indicate significant mean separation ($\alpha = 0.05$). Error bars represent ± 1 SE.

SMALL FISH AND MACROINVERTEBRATE BIOMASS

Species composition of fish and macroinvertebrates, as measured by biomass, differed among vegetation-defined habitats, supporting the need to stratify spatial up-scaling by relative coverage of habitat types. Two-way permutational multivariate analysis of variance (PERMANOVA), performed on biomass, indicated habitat differences in fish assemblage similarity ($p < 0.01$) with no difference among sampling months ($p > 0.05$). However, two-way PERMANOVA indicated differences in macroinvertebrate assemblage similarity between both habitats ($p < 0.01$) and sampling months ($p < 0.01$). Therefore, both habitat-specific and month-specific biomass estimates were calculated for all species. Total small-bodied fish biomass estimates for STA-2 (total of Cells 3, 4, 5, and 6) ranged from 89,185 kilograms (kg) in March 2017 to 147,715 kg in June 2016 (**Table 4**). Mean small-bodied fish biomass, averaged across months, was highest in Cell 4 and lowest in Cell 5 (**Table 4**). Total macroinvertebrate biomass estimates for STA-2 ranged from 32,159 kg in September 2016 to 74,365 kg in June 2016 (**Table 5**). Similar to fish, mean macroinvertebrate biomass, averaged across months, was highest in Cell 4 and lowest in Cell 5 (**Table 5**). Total large-bodied fish biomass estimates ranged from 5,437 kg in STA-1W for March 2017 to 108,003 kg in STA-2 for February 2016 (**Tables 6 through 9**). Mean large-bodied fish biomass, averaged across months, was highest in STA-2 and lowest in STA-1W (**Table 10**).

Table 4. Total estimated biomass in kg of small-bodied fishes in STA-2. Estimates scaled from 1-m² throw-trap samples using SFWMD vegetation data from November 2015. Throw-trap surveys were completed in June–July 2016, September–October 2016, and March 2017.

Species	Cell 3			Cell 4			Cell 5			Cell 6		
	June	September	March	June	September	March	June	September	March	June	September	March
brown bullhead	0	0	0	50	0	0	0	0	0	833	0	0
Mayan cichlid	8,225	3,010	585	1,789	7,067	0	3,724	5,133	2,819	11,913	9,100	9,171
swamp darter	99	0	57	339	0	0	64	0	14	206	0	70
Seminole killifish	3,155	659	115	128	2,483	325	38	37	27	3,731	560	391
eastern mosquitofish	3,622	844	2,258	10,483	11,599	2,582	6,639	9,295	2,086	2,309	3,875	3,288
African jewelfish	0	0	0	3	0	0	0	0	0	0	0	0
least killifish	141	32	135	2,126	1,741	3,044	605	324	289	1,247	1,411	778
flagfish	4,088	1,262	2,280	3,295	7,258	1,232	768	843	332	831	160	235
brook silverside	0	0	0	0	0	0	52	0	41	428	0	0
warmouth	0	0	0	28	0	0	333	0	0	1,353	166	181
bluegill	0	1,804	79	69	0	860	59	1,169	989	333	17	1,716
redear sunfish	0	3,252	0	180	0	0	0	242	0	886	332	1,540
spotted sunfish	0	0	0	2,021	0	0	0	0	0	0	192	0
bluefin killifish	5,108	5,817	7,345	9,414	6,424	5,937	3,977	1,977	2,131	6,882	2,060	7,173
inland silverside	560	0	105	0	0	0	0	0	0	0	0	0
clown goby	0	58	496	0	0	0	0	17	80	55	0	0
taillight shiner	378	12	0	0	0	0	28	0	0	21	0	1,203
blue tilapia	1,225	10	256	1,054	0	119	25	526	211	738	6	10
sailfin molly	21,444	4,006	15,965	16,870	44,157	6,938	1,016	2,063	918	664	1,328	2,780
spotted tilapia	0	0	0	0	0	0	427	937	0	1,637	1,529	0
Total	48,044	20,766	29,675	47,849	80,729	21,036	17,755	22,562	9,936	34,067	20,738	28,538
Cell Mean		32,828			49,871			16,751			27,781	
June Total	147,715											
September Total	144,795											
March Total	89,185											

Table 5. Total estimated biomass in kg of macroinvertebrates in STA-2. Estimates scaled from 1-m² throw-trap samples using SFWMD vegetation data from November 2015. Throw-trap surveys were completed in June–July 2016, September–October 2016, and March 2017.

Species	Cell 3			Cell 4			Cell 5			Cell 6		
	June	September	March	June	September	March	June	September	March	June	September	March
unidentified dragonfly larvae	0	22	0	0	102	18	0	13	76	0	0	30
two-striped forceptail	0	0	70	0	0	0	0	43	43	0	0	41
giant water bug	0	0	0	0	0	0	0	105	0	34	135	0
four-spotted pennant	620	186	887	374	1,921	1,318	117	296	797	155	361	1,602
pennant dragonflies	0	6	43	17	13	144	28	7	55	0	0	241
midge larva	36	123	317	28	10	356	18	2	58	7	3	57
damsselfy larva	17	3	103	24	20	205	4	14	97	10	10	143
aquatic beetles	13	0	0	0	185	0	0	3	0	0	0	0
Asian fingernail clam	920	0	920	965	0	0	3,940	0	563	546	0	0
waterboatman	24	9	47	12	24	321	3	16	46	2	0	43
predaceous diving beetle	29	0	0	0	0	0	0	0	0	0	0	0
unidentified fly larva	0	0	0	0	0	0	0	0	0	0	18	0
mayfly larva	0	0	1	0	1	5	0	0	18	1	1	8
eastern pondhawk	0	0	0	0	0	0	0	7	0	0	10	0
water striders	0	0	0	0	0	0	0	3	0	0	0	2
physid snails	8	0	2	0	0	2	0	0	0	1	18	0
leeches	57	23	23	36	0	88	0	0	0	0	0	0
skimmer dragonflies	0	0	0	0	9	0	0	0	0	0	0	0
marl pennant	0	50	305	0	2,422	954	0	189	810	0	459	4,715
red-rimmed melania	343	0	86	0	0	0	0	0	34	0	0	76
blue dasher	0	0	124	20	95	95	19	95	25	0	29	129
grass shrimp	5,843	1,310	8,600	16,906	14,962	10,093	4,095	1,389	624	2,757	811	7,058
creeping waterbug	18	3	0	0	28	40	2	34	3	2	11	12

Table 5. Continued.

Species	Cell 3			Cell 4			Cell 5			Cell 6		
	June	September	March	June	September	March	June	September	March	June	September	March
eastern amberwing	0	0	0	0	0	0	25	0	0	0	0	116
mesa rams-horn	4,270	775	625	5,042	874	629	25	16	32	12	0	155
Everglades crayfish	0	0	0	0	0	0	0	0	0	0	0	0
slough crayfish	1,075	538	2,133	10,223	2,350	1,189	3,223	428	705	3,063	1,531	4,405
mimic lymnaea	0	0	8	0	0	13	0	0	0	0	12	0
apple snail	0	0	0	948	0	0	0	0	0	0	0	858
Sponge	0	0	0	0	0	0	562	0	4	474	0	22
mysid shrimp	7	0	0	10	0	0	0	0	0	12	0	9
garnet glider	0	0	0	0	0	0	0	28	0	0	0	0
saddlebag glider	1,307	0	0	3,479	0	0	538	0	0	287	0	0
freshwater mussel	1,046	0	0	686	0	0	0	0	0	0	0	0
Total	15,634	3,048	14,295	38,771	23,017	15,468	12,599	2,684	3,989	7,362	3,410	19,722
Cell Mean	10,992			25,752			6,424			10,161		
June Total	74,365											
September Total	32,159											
March Total	53,475											

Table 6. Total estimated biomass in kg of large-bodied fishes during February 2016. Estimates scaled from 5-minute electrofishing transect surveys using estimates of the proportion of deep water and shallow water habitat in each STA cell.

Species	STA-2				STA-1E				STA-1W					
	Cell 3	Cell 4	Cell 5	Cell 6	Cell 2	Cell 4N	Cell 4S	Cell 6	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2
brown bullhead	0	0	0	0	0	488	0	0	0	0	0	337	887	0
bowfin	11,323	0	8,325	0	0	825	1,685	0	0	0	0	33	0	0
Mayan cichlid	191	3,084	0	0	0	0	0	32	0	0	0	0	0	0
walking catfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gizzard shad	0	0	0	0	21	3	0	0	0	0	0	0	0	0
lake chubsucker	0	0	198	0	0	166	33	0	0	0	0	0	0	144
Seminole killifish	15	194	199	39	0	200	190	29	0	59	1	0	233	272
brown hoplo	0	0	0	0	34	0	0	0	0	0	0	0	0	0
warmouth	0	390	0	0	0	99	0	0	0	0	0	0	0	0
bluegill	0	2,517	0	0	0	1,592	225	0	0	0	5	0	1	61
redear sunfish	0	1,319	8,990	362	643	2,917	336	365	34	0	35	0	0	549
Florida gar	878	10,463	9,579	0	1,402	1,785	99	6,382	386	332	1,518	0	2,382	4,810
spotted sunfish	0	0	131	0	0	0	61	0	0	0	5	0	0	0
largemouth bass	18,914	8,360	9,129	7,569	532	2,121	595	3,301	662	0	17	0	725	0
golden shiner	0	0	0	0	0	122	0	221	0	0	6	0	1	0
blue tilapia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nile tilapia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
black crappie	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vermiculated sailfin catfish	0	0	0	0	0	0	216	757	0	0	0	0	0	0
Orinoco sailfin catfish	5,833	0	0	0	33	90	0	963	97	192	0	0	0	0
Amazon sailfin catfish	0	0	0	0	0	0	0	536	0	0	0	0	0	0
STA Total		108,003				29,100						13,783		

Table 7. Total estimated biomass in kg of large-bodied fishes during April 2016. Estimates scaled from 5-minute electrofishing transect surveys using estimates of the proportion of deep water and shallow water habitat in each STA cell.

Species	STA-2				STA-1E				STA-1W					
	Cell 3	Cell 4	Cell 5	Cell 6	Cell 2	Cell 4N	Cell 4S	Cell 6	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2
brown bullhead	480	0	2,706	0	0	0	0	0	0	616	473	0	0	0
bowfin	435	0	2,518	0	1,436	1,147	0	5,679	0	351	99	419	0	508
Mayan cichlid	0	0	1,163	237	0	0	94	872	0	0	0	142	0	288
walking catfish	0	0	57	0	0	0	132	0	0	108	0	0	0	0
gizzard shad	0	0	0	0	13	0	179	377	0	0	0	0	0	0
lake chubsucker	0	0	0	0	0	111	0	269	0	0	0	0	0	0
Seminole killifish	0	0	15	259	22	367	143	313	0	16	6	1	204	377
brown hoplo	0	0	0	0	259	0	0	0	0	0	0	0	0	0
warmouth	105	0	1,197	0	0	82	287	373	136	33	77	5	0	0
bluegill	505	0	2,035	164	35	764	312	1,414	54	3	48	8	525	578
redeer sunfish	0	0	147	392	7	1,188	380	109	59	83	24	15	1,558	948
Florida gar	11,823	0	13,114	5,173	1,341	1,024	4,513	9,453	6,250	3,557	9,627	1,299	9,736	22,988
spotted sunfish	0	0	31	0	0	0	0	0	0	7	2	6	0	28
largemouth bass	3,382	0	4,579	1,631	84	1,348	1,467	713	372	38	38	11	3,220	0
golden shiner	0	0	0	0	0	0	0	0	13	19	13	5	0	24
blue tilapia	280	0	3,198	0	1,858	6,005	2,245	8,607	171	480	39	0	558	816
Nile tilapia	0	0	6,841	5,744	0	0	0	0	0	0	0	0	0	0
black crappie	0	0	0	0	18	0	0	121	0	0	0	0	0	0
vermiculated sailfin catfish	0	0	0	46	182	98	250	4,424	0	156	0	0	0	0
Orinoco sailfin catfish	0	0	114	0	0	0	0	0	0	0	0	0	0	0
Amazon sailfin catfish	0	0	114	0	0	856	554	915	0	0	0	0	0	0
STA Total	68,485				62,440				67,234					

Table 8. Total estimated biomass in kg of large-bodied fishes during October 2016. Estimates scaled from 5-minute electrofishing transect surveys using estimates of the proportion of deep water and shallow water habitat in each STA cell.

Species	STA-2				STA-1E				STA-1W					
	Cell 3	Cell 4	Cell 5	Cell 6	Cell 2	Cell 4N	Cell 4S	Cell 6	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2
brown bullhead	0	0	0	0	0	211	0	0	0	0	0	0	0	0
bowfin	0	1,524	0	0	6,583	0	0	0	0	0	126	0	0	550
Mayan cichlid	0	0	0	0	342	109	0	1,579	0	98	21	484	161	167
walking catfish	0	0	0	0	0	0	0	0	0	430	0	0	0	0
gizzard shad	0	0	0	0	0	14	0	0	0	0	0	0	39	0
lake chubsucker	185	0	0	0	0	1,474	0	0	0	0	0	0	0	0
Seminole killifish	0	0	0	0	448	0	162	0	0	0	0	0	0	0
brown hoplo	0	1,736	0	0	0	77	0	0	0	0	0	0	0	0
warmouth	285	0	0	0	167	286	254	0	0	0	0	0	0	0
bluegill	0	0	0	0	2,192	694	1,555	60	44	165	30	0	128	0
redecor sunfish	208	0	0	0	4,635	1,296	3,972	113	0	0	58	0	16	0
Florida gar	7,848	0	0	0	2,346	4,386	7,623	1,115	353	910	529	2,290	464	747
spotted sunfish	0	0	0	0	0	0	474	0	0	0	0	0	0	0
largemouth bass	3,731	972	0	331	0	33	4,611	2,435	0	0	0	0	0	164
golden shiner	64	12	0	0	0	0	0	0	0	0	0	0	0	11
blue tilapia	4,637	5,348	0	630	0	14,433	6,862	2,855	0	0	0	6,296	6	1,403
Nile tilapia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
black crappie	0	0	0	0	0	0	0	0	66	0	0	0	0	0
vermiculated sailfin catfish	0	0	0	0	0	0	2,366	465	411	0	0	0	0	501
Orinoco sailfin catfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amazon sailfin catfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STA Total	27,513				76,228				16,671					

Table 9. Total estimated biomass in kg of large-bodied fishes during March 2017. Estimates scaled from 5-minute electrofishing transect surveys using estimates of the proportion of deep water and shallow water habitat in each STA cell.

Species	STA 2				STA-1E				STA-1W					
	Cell 3	Cell 4	Cell 5	Cell 6	Cell 2	Cell 4N	Cell 4S	Cell 6	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2
brown bullhead	0	0	0	0	0	28	0	0	0	0	0	0	0	0
bowfin	0	0	0	0	0	0	0	0	0	908	0	0	17	0
Mayan cichlid	0	0	0	528	0	0	0	107	595	189	0	0	53	230
walking catfish	0	0	2,147	0	0	0	0	0	0	0	0	0	0	107
gizzard shad	0	0	0	0	0	0	0	143	0	0	0	0	14	0
lake chubsucker	0	0	0	0	0	239	117	0	0	0	0	0	0	0
Seminole killifish	0	37	0	0	0	160	0	30	0	0	2	0	0	0
brown hoplo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
warmouth	0	860	0	94	0	0	0	0	0	0	6	0	0	0
bluegill	2,332	403	79	0	0	44	153	356	31	0	0	0	5	24
redeer sunfish	7,927	754	215	0	0	191	255	548	531	126	142	0	20	126
Florida gar	6,520	7,736	13,499	5,424	0	0	3,050	0	52	744	25	640	133	145
spotted sunfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0
largemouth bass	2,303	2,130	112	0	0	110	0	0	176	0	12	0	0	32
golden shiner	0	0	0	0	0	0	0	0	0	38	0	0	0	0
blue tilapia	0	0	0	0	0	112	0	0	0	0	0	0	71	113
Nile tilapia	0	0	0	0	0	0	0	0	0	132	0	0	0	0
black crappie	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vermiculated sailfin catfish	0	0	0	0	0	1,163	0	1,263	0	0	0	0	0	0
Orinoco sailfin catfish	0	0	0	0	0	752	0	173	0	0	0	0	0	0
Amazon sailfin catfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STA Total	53,097				8,994				5,437					

Table 10. Mean estimated biomass in kg of large-bodied fishes averaged across the February 2016, April 2016, October 2016, and March 2017 sampling events. Estimates scaled from 5-minute electrofishing transect surveys using estimates of the proportion of deep water and shallow water habitat in each STA cell.

Species	STA-2				STA-1E				STA-1W					
	Cell 3	Cell 4	Cell 5	Cell 6	Cell 2	Cell 4N	Cell 4S	Cell 6	Cell 3	Cell 4	Cell 1B	Cell 2B	Cell 5B1	Cell 5B2
brown bullhead	120	0	676	0	0	182	0	0	0	154	118	84	296	0
bowfin	2,939	381	2,711	0	718	2,139	421	1,420	0	315	56	113	4	265
Mayan cichlid	48	771	291	191	0	113	23	648	149	72	5	36	215	187
walking catfish	0	0	551	0	0	0	33	0	0	135	0	0	0	27
gizzard shad	0	0	0	0	17	4	45	130	0	0	0	0	13	0
lake chubsucker	0	46	50	0	0	129	406	67	0	0	0	0	0	36
Seminole killifish	87	58	54	74	11	294	83	134	0	19	2	0	145	213
brown hoplo	0	0	434	0	147	19	0	0	0	0	0	0	0	0
warmouth	26	384	299	23	0	87	143	157	34	8	21	1	0	0
bluegill	709	730	528	41	17	1,170	324	847	32	1	62	2	179	231
redecor sunfish	2,034	518	2,338	188	325	2,233	567	1,277	156	52	65	4	525	451
Florida gar	4,805	6,512	9,048	2,649	1,372	1,342	2,959	6,143	1,760	1,386	2,925	543	4,858	9,441
spotted sunfish	0	0	40	0	0	0	15	119	0	2	2	2	0	7
largemouth bass	6,369	3,368	3,665	2,383	308	903	530	2,750	302	10	16	3	1,301	49
golden shiner	0	19	0	0	0	31	0	55	3	14	5	1	0	9
blue tilapia	70	1,159	2,136	158	929	1,836	3,863	4,581	43	120	10	0	2,303	583
Nile tilapia	0	0	1,710	1,436	0	0	0	0	0	33	0	0	0	0
black crappie	0	0	0	0	9	0	0	30	16	0	0	0	0	0
vermiculated sailfin catfish	0	0	0	12	91	315	214	2,221	103	39	0	0	0	125
Orinoco sailfin catfish	1,458	0	28	0	16	210	0	284	24	48	0	0	0	0
Amazon sailfin catfish	0	0	28	0	0	214	139	363	0	0	0	0	0	0
Total	18,666	13,947	24,589	7,156	3,960	11,219	9,767	21,224	2,623	2,406	3,287	788	9,839	11,624
STA Mean	16,089				11,543				5,095					

PHOSPHORUS AND NITROGEN SEQUESTRATION BY FISHES

Fish tissue stoichiometry measurements were determined for 168 samples collected from the first sampling event and 200 from the second sampling event (**Table 11**). Stoichiometry ratios of these samples were similar to those reported by Hagerthey et al. (2014) for nutrient enriched sites in Water Conservation Area (WCA) 2A (**Figure 5**). Percent P (mean $\pm$ standard deviation [SD]) in body tissue ranged from $2.0 \pm 0.8\%$ (blue tilapia) to $3.7 \pm 1.2\%$ (Mayan cichlid) (**Table 12**). Generally, P content varied more within species (among individuals) than among species. Intraspecific differences in P content among individuals ranged from 1.1% (bluefin killifish) to 5.1% (Mayan cichlid). Percent N (mean $\pm$ SD) in body tissue ranged from $9.3 \pm 0.7\%$ (blue tilapia) to $11.6 \pm 0.8\%$ (largemouth bass). Differences in N content were similar both within and among species and ranged from 0.8% to 2.5%. Percent C (mean $\pm$ SD) in body tissue ranged from $37.4 \pm 4.5\%$ (Mayan cichlid) to $47.7 \pm 3.6\%$ (blue tilapia). Differences in C content were slightly greater within species than among species and ranged from 7.9% to 18.0%. Stoichiometric ratios (C:P, C:N, and N:P) varied, but were highest for blue tilapia compared to the other species. Mean C:N ratios did not differ between the two sampling periods for all species except largemouth bass ($p = 0.04$; **Figure 5**). Mean C:P ratios did not differ between the two sampling periods for all species except bluefin killifish ($p < 0.01$), largemouth bass ($p < 0.001$), and sailfin molly ($p < 0.001$). Mean N:P ratios did not differ between the two sampling periods for species except Mayan cichlid ($p = 0.04$), bluefin killifish ($p < 0.001$), largemouth bass ($p < 0.001$), blue tilapia ($p = 0.04$), and sailfin molly ($p < 0.001$). Mean nutrient contents differed between the two sampling periods for many of the species (**Figure 6**). However, magnitude of the differences between the means for the two sampling periods was small with a mean difference of 0.5% for N (range = 0.04–1.1%), 0.4% for P (range = 0.2–0.9%), and 2.5% for C (range = 0.2–5.7%).

Total areal estimates of the mass of P and N stored within the tissue of the STA fishes were similar for the first and second sampling periods. The similarity of the estimates suggests that approximately 1,065 kg of P and 3,733 kg of N are stored within the tissue of the 10 fish species in STA-2 (total of Cells 3, 4, 5, and 6; **Table 13**). Averaged across both stoichiometry samples, all STA cells, and vegetation habitat types, overall fish mean N is 0.13g/m^2 and P is 0.04g/m^2 . Average N and P storage per kg of fish tissue are equivalent to the specific nutrient contents in **Table 12** (i.e. $3.1\% = 0.031\text{kg P}$). These results suggest that fish biomass may comprise a nontrivial sink for N and P within the STAs (Noe et al. 2013).

Table 11. Number of fish tissue stoichiometry processed for each of the 10 STA-2 species. 2016 sample specimens were collected from August 23, 2016, through November 14, 2016. 2017 sample specimens were collected from March 20, 2017, through October 25, 2017.

Species	2016 Number of Samples	2017 Number of Samples
Mayan cichlid	25	20
Seminole killifish	13	20
eastern mosquitofish	16	20
Florida gar	13	20
bluegill	12	20
redecor sunfish	18	20
bluefin killifish	17	20
largemouth bass	19	20
blue tilapia	18	20
sailfin molly	17	20
Total	168	200

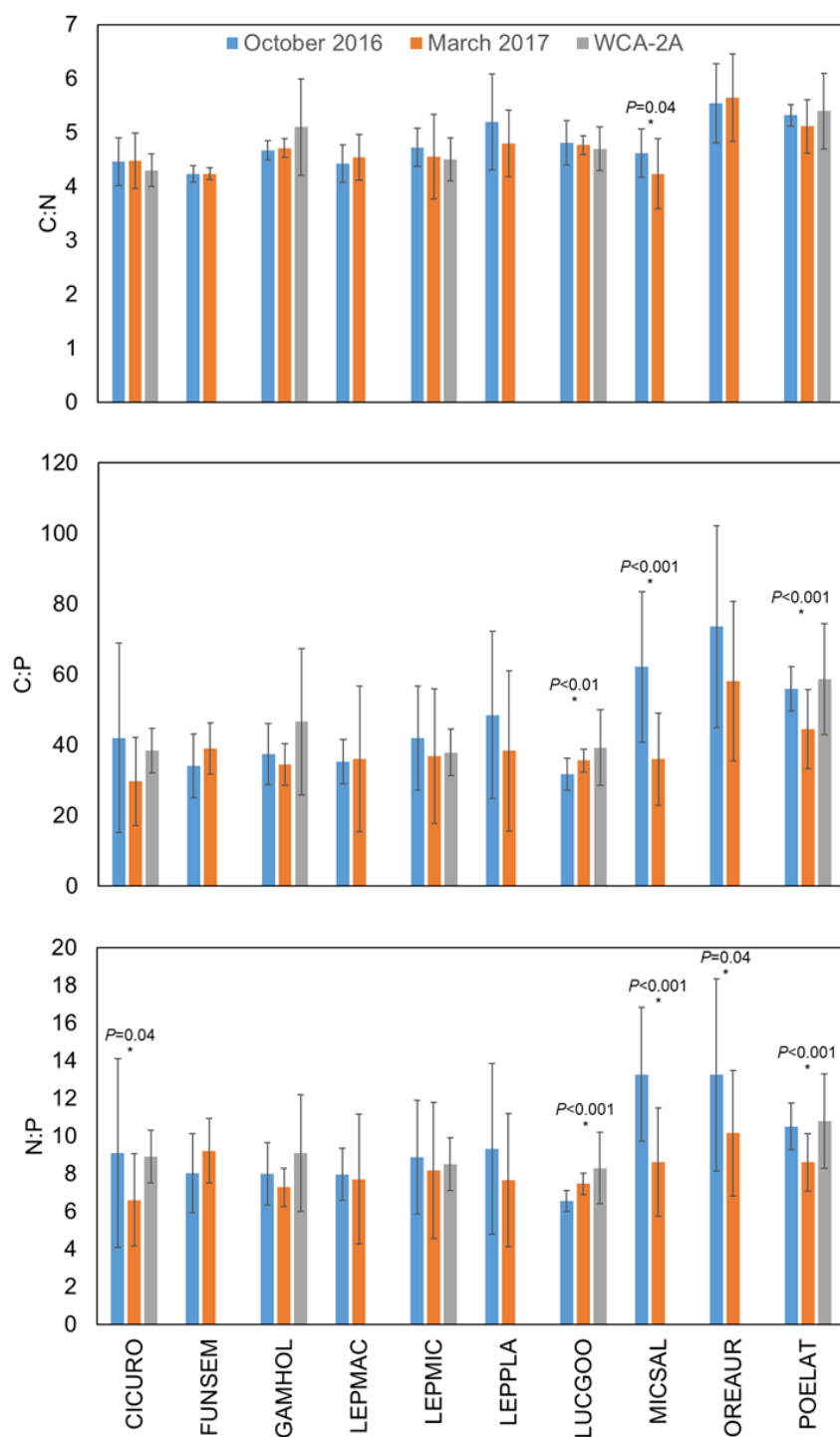


Figure 5. Mean stoichiometric ratios for fish tissue collected from STA-2 (this study: October 2016 sample = blue and March 2017 sample = orange) and WCA-2A (Hagerthey et al. 2014; grey). p-values correspond to a t-test between the two STA sampling periods. Error bars represent ± 1 SE. See Table 2 for species abbreviations.

Table 12. C, N, and P contents and stoichiometric ratios (mean $\pm$ SD) for fishes captured in STA-2. Fish were collected during two sampling periods (October 2016 and March 2017).

	C%			N%			P%			C:N			C:P			N:P		
October 2016																		
Mayan cichlid	40.4	±	4.3	10.6	±	0.5	3.1	±	1.1	4.5	±	0.4	42.0	±	26.9	9.1	±	5.0
Seminole killifish	39.3	±	2.5	10.8	±	0.7	3.1	±	0.6	4.2	±	0.2	34.0	±	9.0	8.0	±	2.1
eastern mosquitofish	40.2	±	1.9	10.0	±	0.3	2.9	±	0.5	4.7	±	0.2	37.4	±	8.6	8.0	±	1.7
Bluegill	40.3	±	1.5	10.7	±	0.8	3.0	±	0.5	4.4	±	0.3	35.2	±	6.3	8.0	±	1.4
redear sunfish	42.0	±	2.7	10.4	±	0.9	2.8	±	0.7	4.7	±	0.4	41.9	±	14.8	8.9	±	3.0
Florida gar	45.0	±	3.9	10.2	±	0.9	2.9	±	1.2	5.2	±	0.9	48.6	±	23.7	9.3	±	4.5
bluefin killifish	39.2	±	2.2	9.5	±	0.4	3.2	±	0.3	4.8	±	0.4	31.7	±	4.5	6.6	±	0.6
largemouth bass	45.6	±	3.2	11.6	±	0.8	2.1	±	0.7	4.6	±	0.5	62.2	±	21.3	13.3	±	3.6
blue tilapia	47.7	±	3.6	10.1	±	0.8	2.0	±	0.8	5.5	±	0.7	73.6	±	28.6	13.3	±	5.1
sailfin molly	44.0	±	1.0	9.6	±	0.3	2.1	±	0.2	5.3	±	0.2	55.9	±	6.3	10.5	±	1.2
March 2017																		
Mayan cichlid	37.4	±	4.5	9.8	±	1.1	3.7	±	1.2	4.5	±	0.5	29.7	±	12.5	6.6	±	2.5
Seminole killifish	39.5	±	2.2	10.9	±	0.7	2.7	±	0.4	4.2	±	0.1	39.0	±	7.3	9.2	±	1.7
eastern mosquitofish	40.4	±	1.7	10.0	±	0.2	3.1	±	0.4	4.7	±	0.2	34.4	±	5.9	7.3	±	1.0
Bluegill	39.1	±	4.1	10.1	±	0.6	3.3	±	1.0	4.5	±	0.4	36.0	±	20.6	7.7	±	3.4
redear sunfish	39.9	±	3.2	9.8	±	1.2	3.1	±	1.0	4.5	±	0.8	36.8	±	19.1	8.2	±	3.6
Florida gar	39.3	±	4.8	9.6	±	0.4	3.2	±	1.1	4.8	±	0.6	38.4	±	22.8	7.7	±	3.5
bluefin killifish	41.6	±	1.8	10.2	±	0.5	3.0	±	0.2	4.8	±	0.2	35.6	±	3.3	7.5	±	0.6
largemouth bass	39.9	±	2.8	10.5	±	0.9	3.0	±	0.8	4.2	±	0.7	36.0	±	13.1	8.6	±	2.9
blue tilapia	44.6	±	4.5	9.3	±	0.7	2.2	±	0.7	5.6	±	0.8	58.1	±	22.6	10.2	±	3.3
sailfin molly	42.4	±	2.7	9.7	±	0.4	2.6	±	0.5	5.1	±	0.5	44.5	±	11.2	8.6	±	1.5

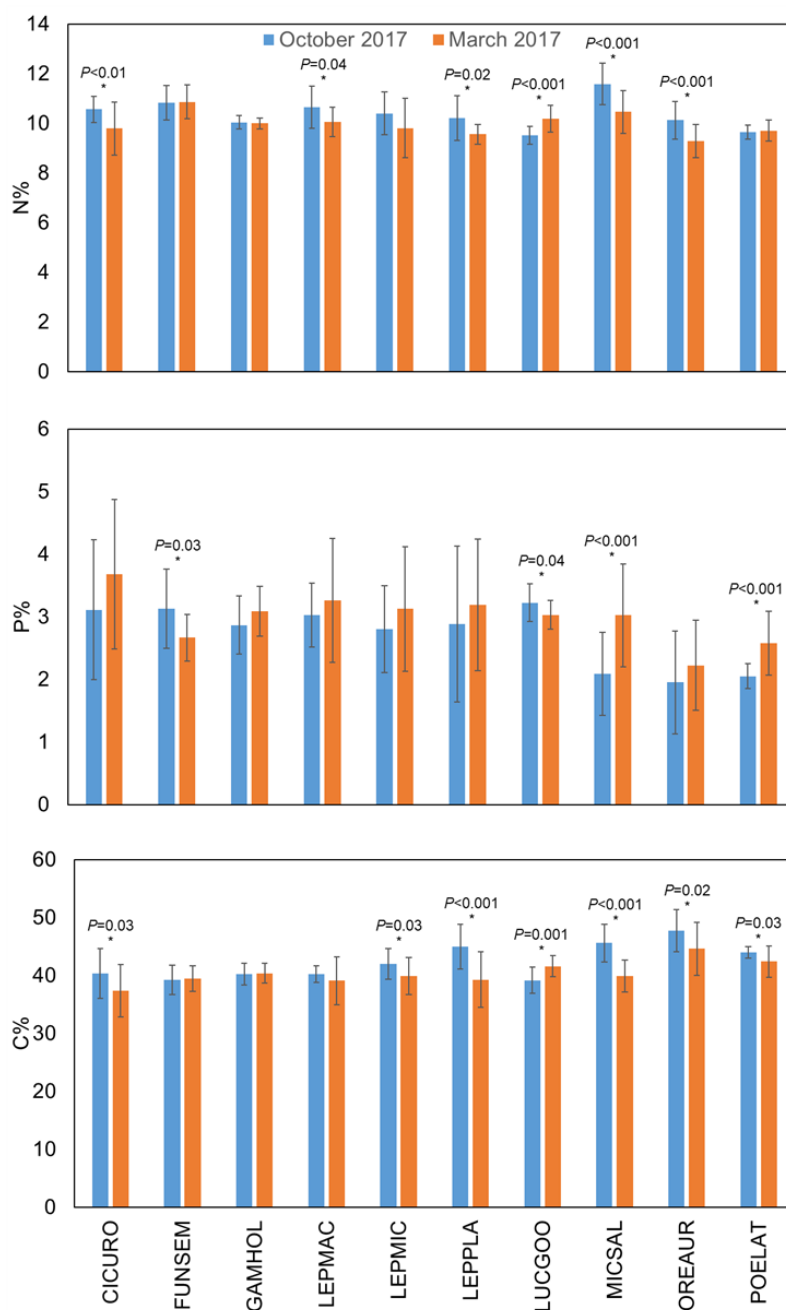


Figure 6. Mean nutrient content for fish tissue collected from STA-2 in October 2016 (blue) and March 2017 (orange). p-values correspond to a t-test between the two STA sampling periods. Error bars represent ± 1 SE. See **Table 2** for species abbreviations.

Table 13. Estimated mass in kg of P and N stored in STA-2 fish tissue. The bottom panel reports the mean P and N when data from October 2016 and March 2017 were combined.

Species	Cell 3		Cell 4		Cell 5		Cell 6	
	P	N	P	N	P	N	P	N
October 2016								
Mayan cichlid	31.2	105.9	29.1	98.9	32.7	111.1	80.2	272.3
Seminole killifish	8.9	30.7	6.6	22.9	0.2	0.8	10.6	36.6
eastern mosquitofish	11.9	41.5	43.5	152.2	31.8	111.2	16.7	58.4
bluegill	8.5	30.1	6.6	23.4	8.1	28.5	4.7	16.4
redeer sunfish	21.7	80.5	4.0	14.9	16.8	62.5	7.7	28.6
Florida gar	45.3	160.3	61.4	217.3	85.3	301.9	25.0	88.4
bluefin killifish	34.3	101.4	40.9	120.8	15.2	44.9	30.3	89.4
largemouth bass	29.1	161.3	15.4	85.3	16.7	92.8	10.9	60.3
blue tilapia	2.9	15.2	8.0	41.7	12.4	64.3	2.1	11.0
sailfin molly	57.1	286.6	93.8	470.3	5.5	27.7	6.6	33.0
Cell Total	250.9	1013.4	309.4	1247.7	224.8	845.5	194.6	694.5
STA Total P	979.7							
STA Total N	3801.2							
March 2017								
Mayan cichlid	36.8	98.2	34.4	91.7	38.7	103.0	94.7	252.5
Seminole killifish	7.6	30.8	5.6	23.0	0.2	0.8	9.0	36.7
eastern mosquitofish	12.8	41.3	46.8	151.6	34.2	110.8	18.0	58.2
bluegill	9.2	28.4	7.2	22.1	8.7	26.9	5.0	15.5
redeer sunfish	24.2	75.9	4.5	14.1	18.8	58.9	8.6	27.0
Florida gar	50.0	150.0	67.8	203.2	94.2	282.4	27.6	82.7
bluefin killifish	32.3	108.5	38.5	129.3	14.3	48.0	28.5	95.7
largemouth bass	42.1	145.8	22.3	77.1	24.2	83.9	15.8	54.5
blue tilapia	3.4	14.0	9.2	38.2	14.1	59.0	2.4	10.1
sailfin molly	76.6	288.5	125.7	473.4	7.4	27.8	8.8	33.2
Cell Total	294.9	981.3	361.9	1223.7	254.8	801.4	218.4	666.1
STA Total P	1129.9							
STA Total N	3672.5							
Both Samples Combined								
Mayan cichlid	33.8	102.4	31.5	95.6	35.4	107.4	86.8	263.3
Seminole killifish	8.1	30.8	6.0	23.0	0.2	0.8	9.6	36.7
eastern mosquitofish	12.4	41.4	45.3	151.8	33.1	110.9	17.4	58.3
bluegill	9.0	29.0	7.0	22.6	8.5	27.5	4.9	15.8
redeer sunfish	23.0	78.1	4.3	14.5	17.8	60.6	8.2	27.8
Florida gar	48.2	154.0	65.3	208.8	90.7	290.1	26.6	84.9
bluefin killifish	33.2	105.2	39.6	125.4	14.7	46.5	29.3	92.8
largemouth bass	35.8	153.3	18.9	81.1	20.6	88.2	13.4	57.4
blue tilapia	3.2	14.6	8.6	39.9	13.3	61.5	2.3	10.5
sailfin molly	69.9	287.7	114.7	472.1	6.7	27.8	8.1	33.2
Cell Total	276.3	996.5	341.2	1,234.7	241.1	821.4	206.4	680.6
STA Total P	1,065.1							
STA Total N	3,733.1							

PHOSPHORUS AND NITROGEN EXCRETION BY FISHES

In addition to preliminary incubation trials for methods development, 301 incubation-chamber experiments were conducted. Nutrient concentrations in equipment blanks were below detection for all samples and analytes. Except for a few incubations, effect sizes for fishless controls were below detection limits for all analytes. Likewise, with a few exceptions, effect sizes were greater than detection limits for all N and P analytes across all species. Positive relationships between fish biomass and nutrient excretion rate for some or all the analytes were found for eastern mosquitofish, bluefin killifish, sailfin molly, and vermiculated sailfin catfish (**Figures 7 through 10**). These preliminary results suggest that the methods sufficiently increased effect sizes above detection limits for all analytes. However, the lack of significant positive relationships between fish biomass and excretion rates for some nutrients and species suggests further modification to the methods is necessary to increase the amount of heterogeneity among the samples and improve excretion estimate precision.

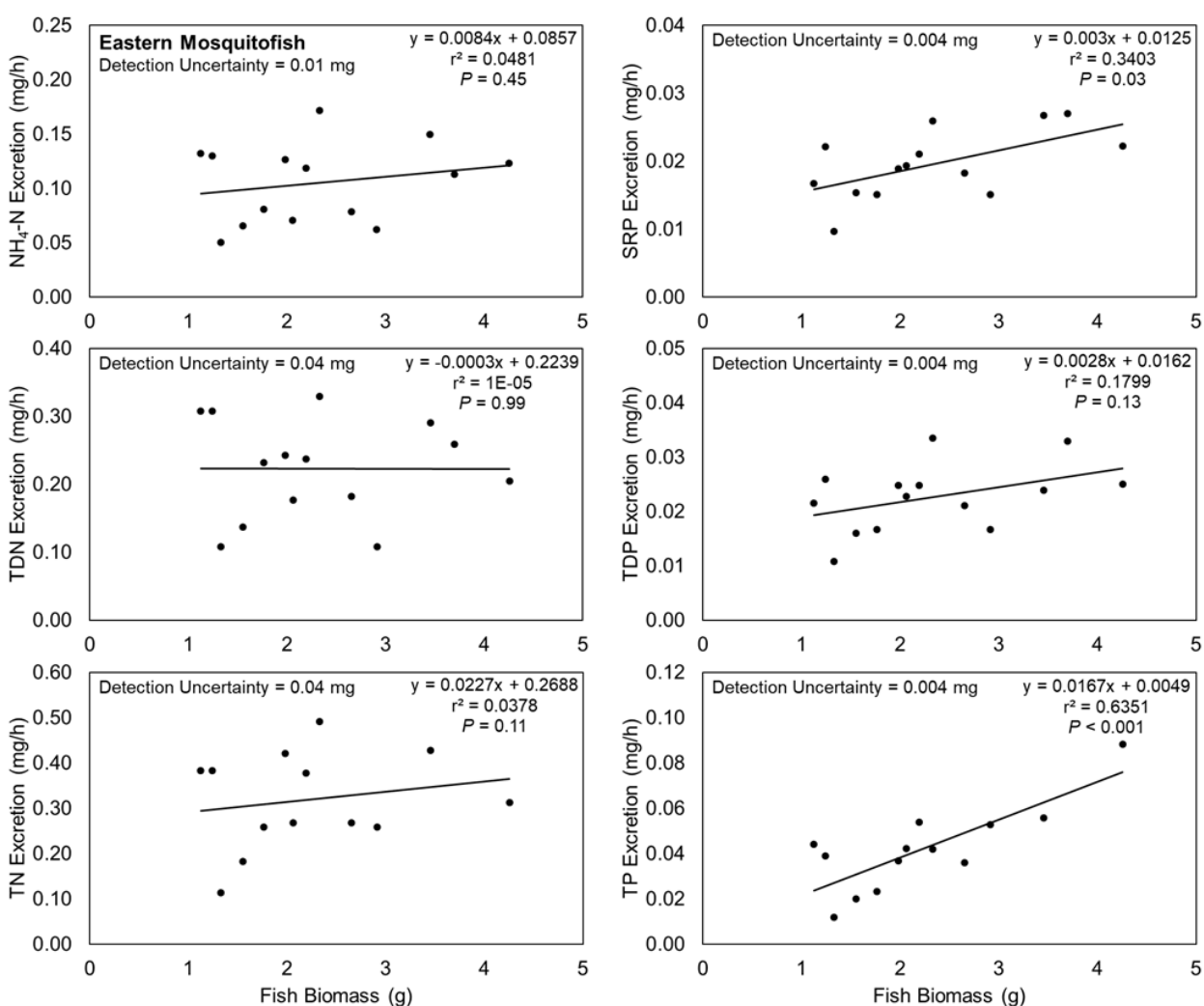


Figure 7. Ammonium nitrogen (NH₄-N), total dissolved nitrogen (TDN), total nitrogen (TN), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) excretion after 60 minutes by eastern mosquitofish (biomass) in incubation chambers.

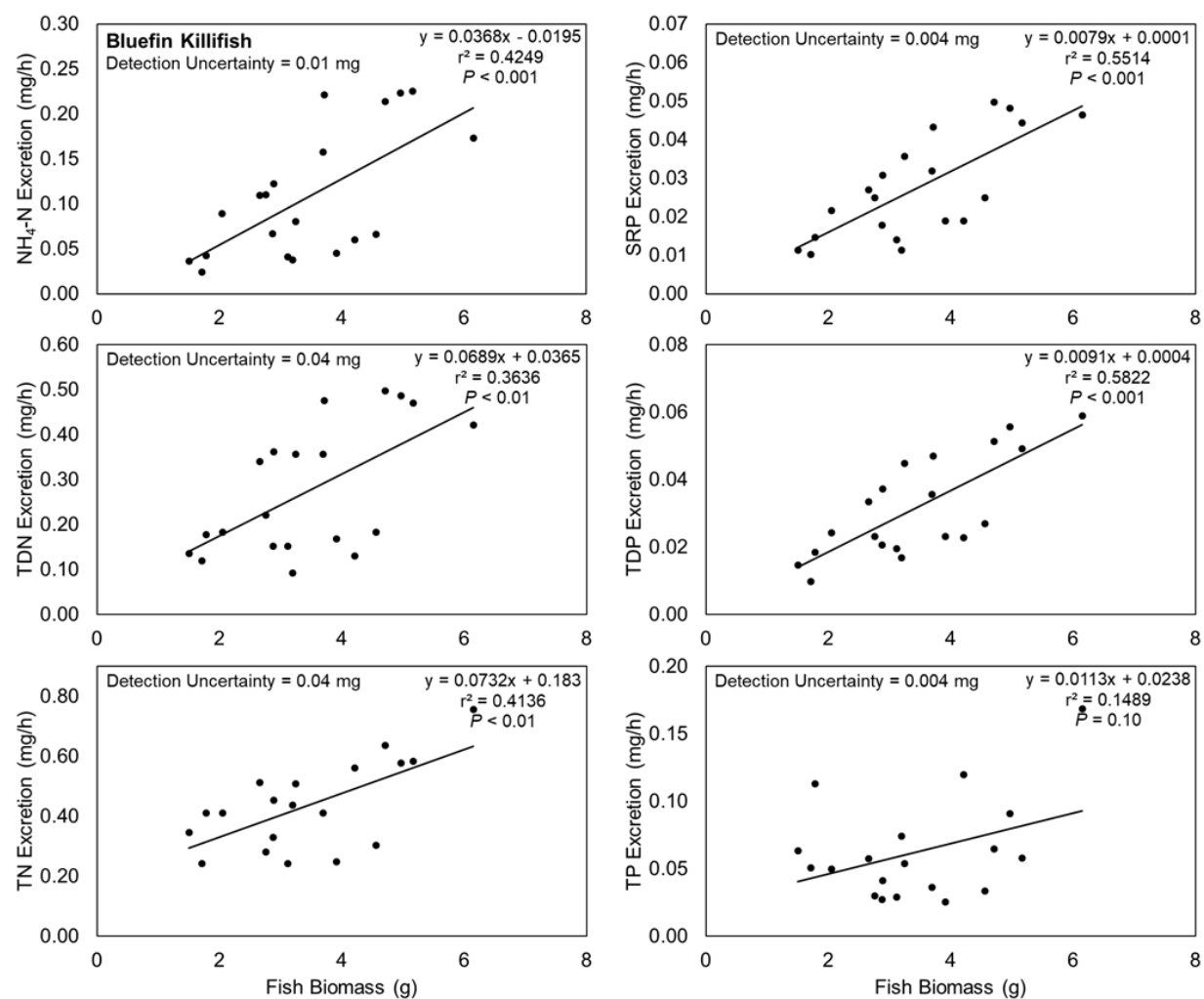


Figure 8. Ammonium nitrogen (NH₄-N), total dissolved nitrogen (TDN), total nitrogen (TN), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) excreted after 60 minutes by bluefin killifish (biomass) in incubation chambers.

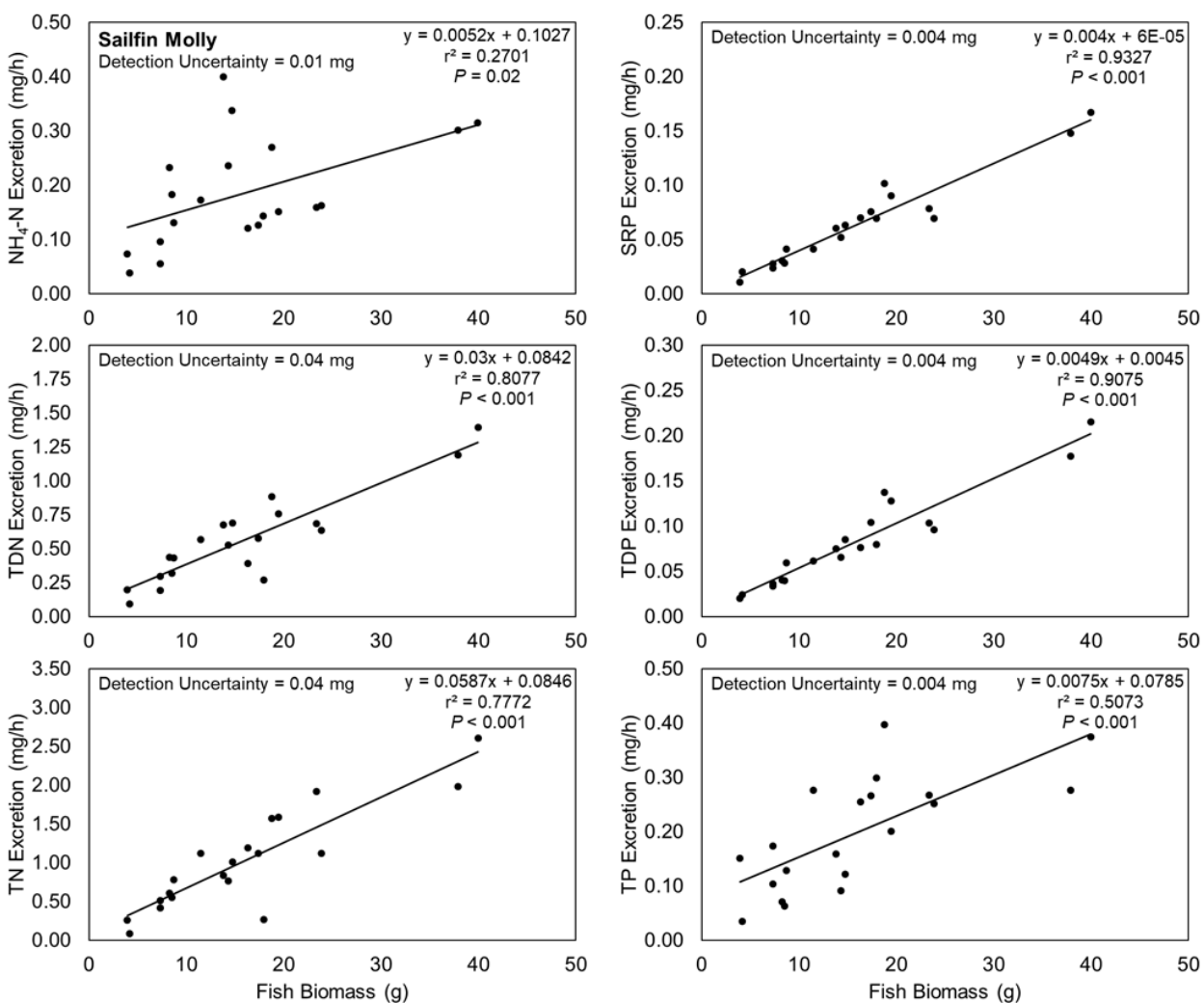


Figure 9. Ammonium nitrogen (NH₄-N), total dissolved nitrogen (TDN), total nitrogen (TN), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) excretion after 60 minutes by sailfin mollies (biomass) in incubation chambers.

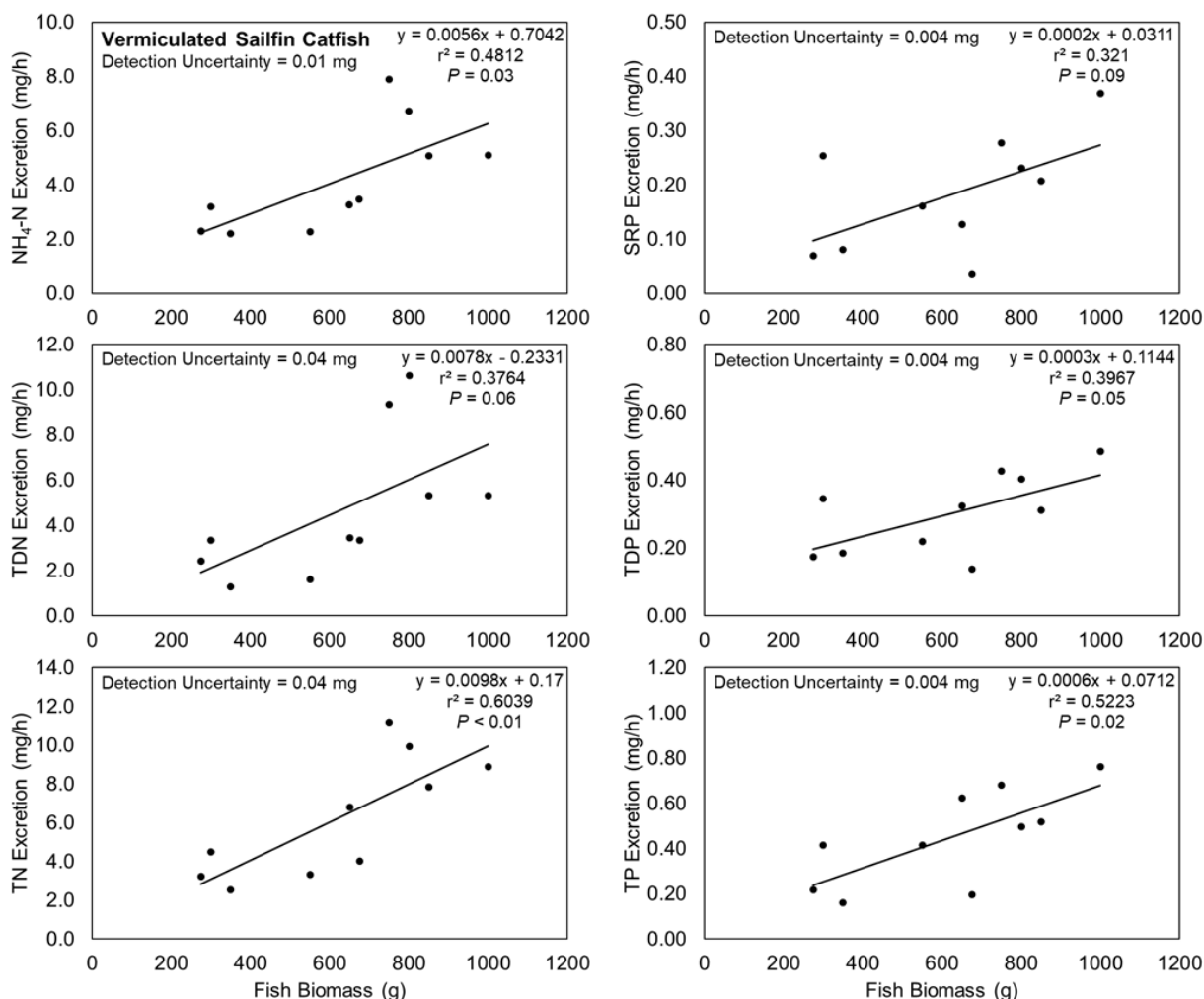


Figure 10. Ammonium nitrogen (NH₄-N), total dissolved nitrogen (TDN), total nitrogen (TN), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) excreted over 60 minutes by vermiculated sailfin catfish (biomass) in incubation chambers

Total areal estimates of N and P excretion rates (milligrams per hectare [mg/ha]) could only be estimated for the species that illustrated a significant positive relationship between incubation chamber fish biomass and excretion quantity. Therefore, sailfin molly were the only species for which areal estimates could be calculated for all six analytes. For the other species, areal estimates could be estimated for five for bluefin killifish, four for vermiculated sailfin catfish, and two for eastern mosquitofish. No estimates could be made for any largemouth bass and blue tilapia analytes. Estimates varied among the species with small-bodied fishes illustrating higher mass-adjusted excretions rates than vermiculated sailfin catfish (**Table 14**). Overall, the results indicate the potential for excretion by STA fishes to contribute to nutrient cycling in the STAs. Future incubations will include additional eastern mosquitofish, bluefin killifish, sailfin molly, and vermiculated sailfin catfish trials, as well as largemouth bass and blue tilapia incubations. The work to date indicates that future incubations will benefit from reducing water volume in the incubation chambers and quantifying excretion over a larger range of biomass for each study species.

Table 14. Estimated mean mass in grams (g) of nutrients excreted per hour by select fishes in STA-2 cells. Dashes indicate no relationship between fish biomass and excretion in the incubation chambers.

Parameter	Cell 3	Cell 4	Cell 5	Cell 6
Eastern Mosquitofish				
NH ₄ -N	-	-	-	-
SRP	6,830	25,022	18,286	9,618
TDN	-	-	-	-
TDP	-	-	-	-
TN	-	-	-	-
TP	37,486	137,500	100,465	52,811
Bluefin Killifish				
NH ₄ -N	223,946	266,919	99,086	197,536
SRP	48,011	57,223	21,245	42,350
TDN	419,333	499,785	185,577	369,891
TDP	55,258	65,861	24,452	48,742
TN	446,127	531,692	197,515	393,542
TP	-	-	-	-
Sailfin Molly				
NH ₄ -N	72,193	118,409	7,059	8,411
SRP	55,278	90,717	5,334	6,371
TDN	414,921	680,870	40,112	47,893
TDP	68,255	112,010	6,590	7,870
TN	810,593	1,330,205	78,291	93,494
TP	104,048	170,703	10,111	12,061
Vermiculated Sailfin Catfish				
NH ₄ -N	8,811	704	1,021	769
SRP	-	-	-	-
TDN	-	-	-	-
TDP	554	114	132	118
TN	14,427	170	726	284
TP	960	0	106	78

PHOSPHORUS AND NITROGEN RESUSPENSION BY FISHES

Preliminary results from the bioturbation experiments indicated density-related effects of blue tilapia on water column nutrients. Mean water column TN was significantly greater in the high density blue tilapia enclosures than in the control treatment enclosures (rmANOVA, $p = 0.01$). Mean water column TN was approximately 1.5 times greater in the high density treatment enclosures than in the control treatment enclosures (**Figure 11**). Mean water column TN in the low density treatment enclosures was intermediate and not different from either the control treatment enclosures or the high density treatment enclosures. Mean water column TP was approximately 2 times greater in the high density blue tilapia enclosures relative to both the control treatment and low density treatment enclosures (rmANOVA, $p = 0.02$; **Figure 12**). No significant differences in water column TN or TP were observed among the largemouth bass or Orinoco sailfin catfish treatments. These results suggest that fish species, like blue tilapia, have the potential to

increase water column TN and TP via excretion and bioturbation, while other species, like largemouth bass and Orinoco sailfin catfish, may have minimal effects on water column nutrient concentrations.

Our results provide proof of concept that bioturbation by at least one of the study species may contribute a considerable amount of P to the STA P budget. Finding blue tilapia to increase water column P through their presence in enclosures when stocked at field densities supports our impression that this species may function similarly to European carp that have been introduced into U.S. waters. This and related species have been demonstrated to change the trophic state of aquatic systems through their behavior and suspension of benthic organic matter. We hypothesize that Orinoco catfish may ultimately be shown to have similar impacts because of their extensive burrowing activities. Largemouth bass are primarily associated with benthic habits during spawning, which makes them a reference species for the integrity of our experiments; we do not expect strong bioturbation impacts from this species unless nesting is observed. More replication of this experimental design is needed before conclusions can be drawn. Future experimental trials will benefit from longer experimental trials and use of more stocking density levels, including treatments with higher fish stocking densities to increase our ability to detect a relationship between suspended P and fish biomass.

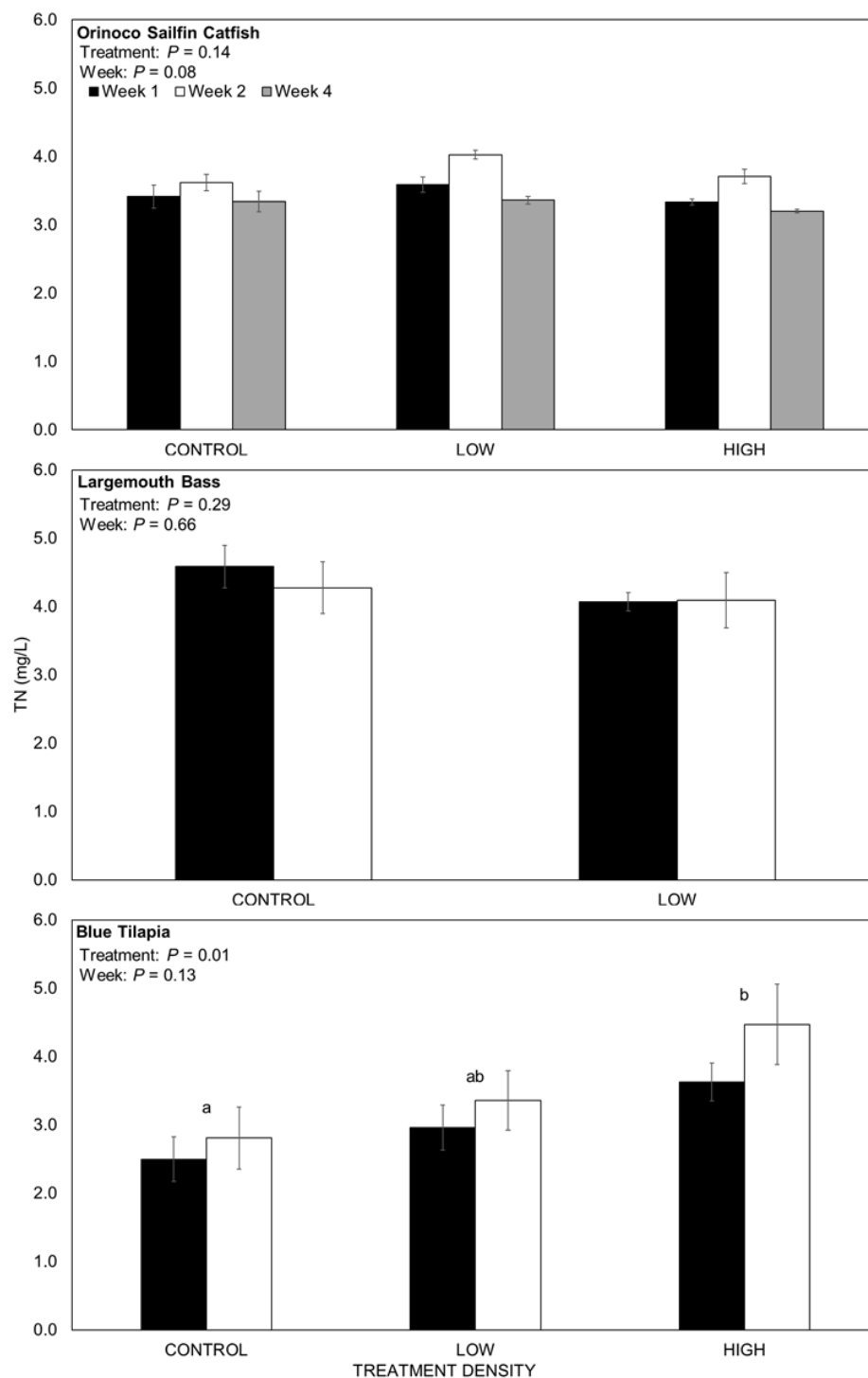


Figure 11. Mean water column TN in milligrams per liter (mg/L) from each of the three bioturbation trials and enclosure density treatments (see **Table 1** for species densities). p-values correspond to rMANOVA. Letters above bars indicate significant mean separation among density treatments ($\alpha = 0.05$). Error bars represent ± 1 SE.

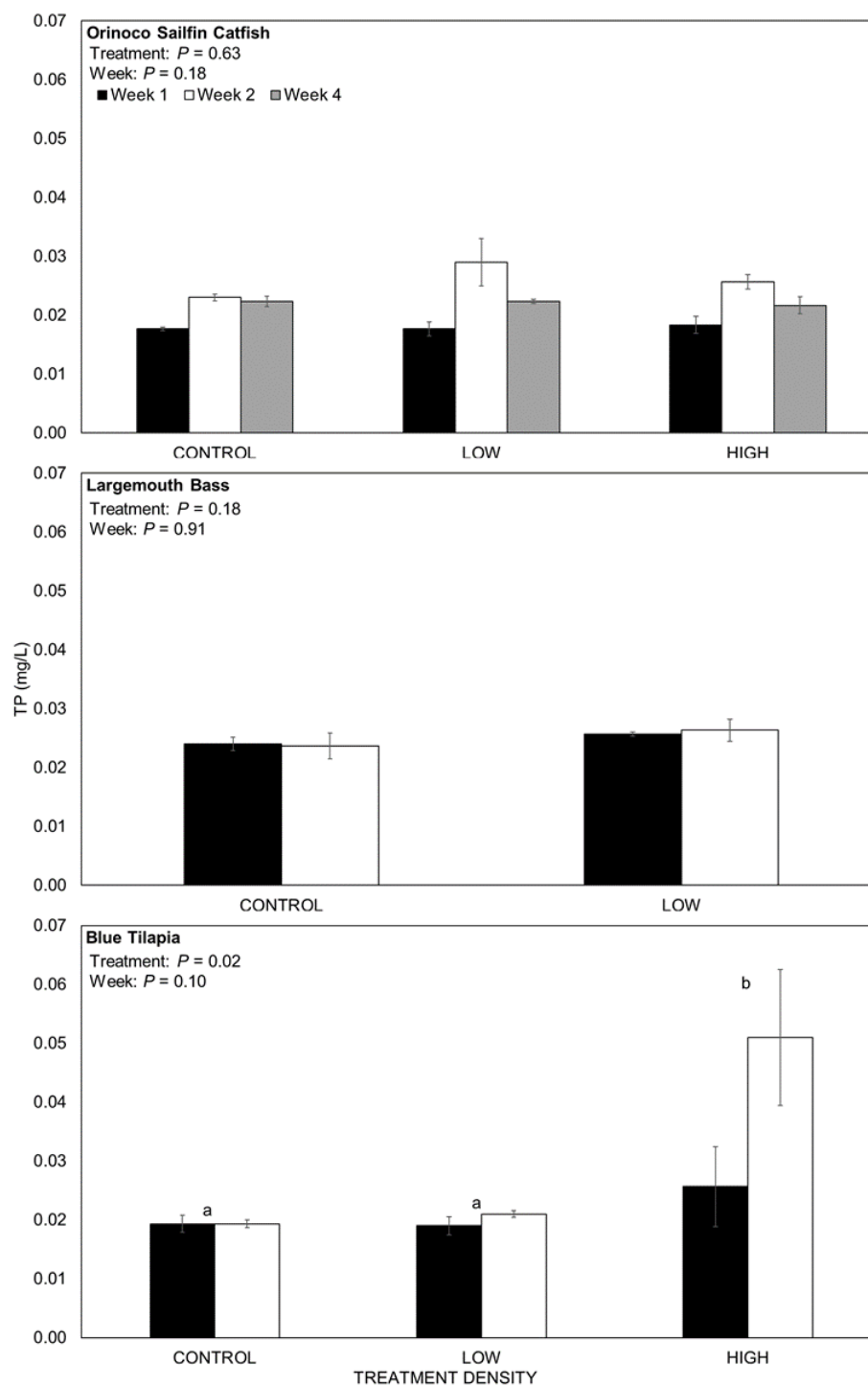


Figure 12. Mean water column TP in milligrams per liter (mg/L) from each of the three bioturbation trials and enclosure density treatments (see **Table 1** for species densities). p-values correspond to rmANOVA. Letters above bars indicate significant mean separation among density treatments ($\alpha = 0.05$). Error bars represent ± 1 SE.

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