



FY16 Evaluation of Factors Influencing Methylmercury Accumulation in South Florida Marshes

Annual Report

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AGRICULTURAL AREA ENVIRONMENTAL PROTECTION DISTRICT

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Introduction

This report covers two areas under DB Environmental's (DBE) contract with Florida Department of Agriculture and Consumer Services. Task 1 is a comparison of DBE's mercury (Hg) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) data collected on water, soil, plants, detritus, invertebrates, and cyprinodontoid fish (including *Gambusia holbrooki* [eastern mosquitofish]) in 2013, 2014, and 2015 with other previously collected published and unpublished Everglades databases. Other water quality parameters that have been shown to affect Hg methylation rates and concentrations, such as sulfate, sulfide, dissolved (diss) organic carbon (DOC) concentration and aromaticity, and iron, are included in the comparisons.

Task 2 describes the methods and results of DBE's field investigations in WCA 2A, WCA 3A and the Everglades National Park (ENP) during the contract period. Much of the effort in this investigation focused on biogeochemical parameters that influence Hg cycling in the south Florida marshes, as well as on characterizing the relationships between the trophic position of *Gambusia* and the Hg concentrations in its diet and tissues among six sampling sites, with varying histories of high or low sulfate and methylmercury (MeHg) concentrations. Findings from this effort, and recommendations for future follow-up investigations, are provided at the end of the Task 2 section.

Summary of Previous DB Environmental Research Findings

DB Environmental (DBE) completed Year Three of a multi-year research program, funded by the Everglades Agricultural Area Environmental Protection District (EAAEPD), Florida Department of Agriculture and Consumer Services (FDACS), Florida Department of Environmental Regulation (FDEP) and the South Florida Water Management District (SFWMD). DBE's first year's research effort focused on in situ and ex situ studies in WCA-3A, near a site (3A-15) that was considered a Hg "hot spot" with respect to fish tissue total Hg (THg) levels. Our initial research emphasis was on clarifying the role of sulfate in Hg methylation at that location through complementary field and laboratory experiments. From a laboratory soil slurry experiment, we found a positive relationship between sulfate levels and methylmercury (MeHg) production, in agreement with some early Everglades research. However, we also found that the availability of inorganic Hg contributed to MeHg accumulation much more strongly than did availability of sulfate, and that the relative stimulation of net MeHg production by sulfate was greatest at ultra-low sulfate levels (< 1.5 mg/L), calling into question the "Goldilocks" paradigm of peak MeHg concentrations associated with moderate sulfate levels. Further, three years of sulfate additions (3 – 48 mg/L) failed to affect MeHg levels within in situ mesocosms at the same site. Also discovered in DBE's first year, Bae et al. (2014) determined that syntrophic taxa (i.e., sulfate-reducing bacteria (SRB) which utilize sulfate facultatively, rather than obligatorily) dominate the methylating

consortium in central WCA-3A, raising the possibility that Hg methylation could proceed in the absence of sulfate.

DBE's effort in our second research year expanded the focus from Year 1 to include in situ and ex situ studies in WCA-2A, WCA-3A and Everglades National Park. We maintained an emphasis on clarifying the role of sulfate in Hg methylation, but we also examined other biogeochemical, physicochemical and ecological drivers known or suspected to influence MeHg bioaccumulation. In Year 2, research yielded two principal advancements in understanding. First, in a laboratory incubation, soil slurries from central Shark River Slough in ENP responded to sulfate and Hg additions similarly to WCA-3A soils, reinforcing those results, and indicating ubiquity of the relatively greater influence of inorganic Hg than sulfate on MeHg accumulation. Second, we found that dietary exposure to MeHg was different for mosquitofish collected from different vegetation communities in WCA-2A (ridge and slough vs. monotypic cattail), corresponded to mosquitofish tissue THg concentrations (as expected), and was not related to expected chemical constituents, including sulfate and aqueous MeHg. This suggested that ecological factors related to habitat structure are significant determinants of fish THg levels across the Everglades.

In the third year, we analyzed our data from Years 1 and 2 within the context of external datasets, including REMAP and the Everglades Mercury Hotspot Study. In particular, in the REMAP dataset, we found mosquitofish collected from cattail habitats to be markedly lower in THg than mosquitofish collected from ridge and slough habitats. Therefore, DBE designed the field research program for the third year to investigate habitat structure influences on Hg bioaccumulation, while continuing to generate data to address early fundamental questions, like the role of sulfate in Hg methylation. Analysis of stable isotopes and gut and tissue Hg concentrations for a variety of organisms suggested that food chain lengths are longer in phosphorus (P)-enriched cattail communities than in P-limited ridge and slough areas, but that these differences did not account for differences in mosquitofish Hg levels. However, our data offered compelling evidence that the Hg levels at all trophic positions are principally controlled by MeHg concentrations in the primary consumers at each site. Therefore, the biogeochemical or ecological controls on the entry of MeHg into the food web are likely to account for spatial differences (e.g., "hot spots") in Hg concentrations in mosquitofish and other organisms.

Objectives for Year Four

DBE's Year Four research effort seeks to advance our understanding of the findings from the first three years by two complementary approaches, building on the successes of Year Three. Field investigations were performed to elucidate the ecological drivers of Everglades Hg cycling. Additionally, we continued our analyses of DBE data against existing data from other studies to ensure maximum utilization and interpretation of all newly generated field data.

Task 1: Data Analysis to Compare Ongoing DBE Findings with Results of Related Investigations

Introduction and rationale

In our first three project years, DBE has produced interesting findings that have generated new insights into Hg dynamics in the Everglades. These observations have suggested the need for novel re-analysis of existing datasets and prior investigations. Such analyses are prudent to increase understanding of Hg cycling at minimal expense (compared to conducting new experiments) and should provide guidance to work plans of this and future years.

Much of Everglades Hg research attributes the current enrichment of Hg in the Everglades environment and biota to increases in the Hg and/or sulfate loading to the system in the last several decades. Systemic monitoring of sulfate and, especially, Hg in the Everglades began sometime after the earliest indications of elevated Hg levels in biota, so the precise timelines of changes to sulfate loading, Hg loading and biological Hg enrichment are not well known. In this section, we collate and evaluate three disparate databases:

1. DB Environmental's own database assembled between 2011 and 2015 at an eight-station subset from within WCA-2A, WCA-3A, and ENP (Figure 1);
2. The Everglades Mercury Hot Spot (EMHS) database, where several of the stations sampled between 2010 and 2013 were near the same locations sampled by DB Environmental.
3. The R-EMAP data set collected in 1995-96, 1999, and 2005 during wet (high water) and dry (low water) periods during each of those three sampling intervals. To make valid comparisons between DB Environmental's and EMHS databases with that of R-EMAP, we selected data from only locations in the R-EMAP data set that were within a 5-km radius of the six sampling sites contained in DB Environmental's data sets.

Data availability and usage

We examined R-EMAP and Everglades Emerging Hot Spot (EMHS) data collected earlier than DB Environmental's data set. Data were collected at randomized locations throughout the Everglades by EPA in 1995-96, 1999, 2005, and 2013-14, under usually wet and dry seasons (Scheidt and Kalla 2007). We did not include the 2013-14 R-EMAP data in this report since the data are still considered "Preliminary". Since the station locations sampled each year in R-EMAP changed due to the probability-based statistical approach (Scheidt and Kalla 2007), we arbitrarily defined any R-EMAP station that was within a radius of 5 km of one of DB Environmental's fixed station locations as qualifying to be included in the comparisons.

Over a total of seven trips between 2011 and 2013, all seven trips collected surface water (SW) and porewater (PW) samples, while six trips sampled *Gambusia holbrooki*, four trips retrieved

soil, and 4-6 trips harvested periphyton in the EMHS study. There were two locations (U3 and 3A-15) in the EMHS study that approximated those of DB Environmental's, although 3A-15 is approximately 9.5 km from DB-15. The EMHS database is unpublished, but can be requested from the South Florida Water Management District, which funded the research.

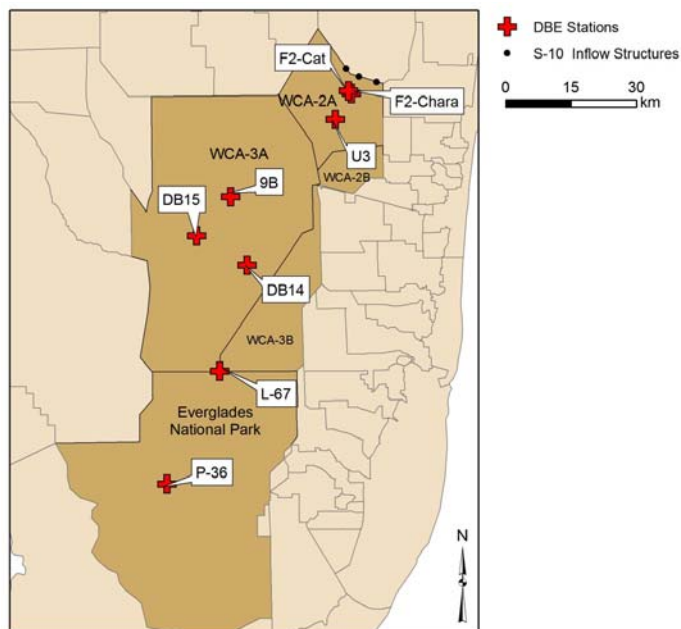


Figure 1. Map showing the locations of the eight sites where samples were collected by DB Environmental in 2011 to 2015.

Results

Generally, SW sulfate concentrations among five key marsh stations (F2-Cat, U3, DB-15, DB-14, and P-36) did not exhibit a distinct temporal trend (Figure 2), whereas the surface water (SW) TMeHg (unfiltered) concentrations decreased over the period of record at DB-15 and DB-14 (Figure 3), responding to the decrease in atmospheric Hg loading in the two decades from the mid-1990s (Pollman 2012; Stober et al. 2001). Why the SW at U3 and P-36 did not conform to the trend observed at DB-15 and DB-14 is interesting, and likely due to different biogeochemical factors (DOC, trophic interaction, community biotype) and/or lower Hg(II) atmospheric loadings.

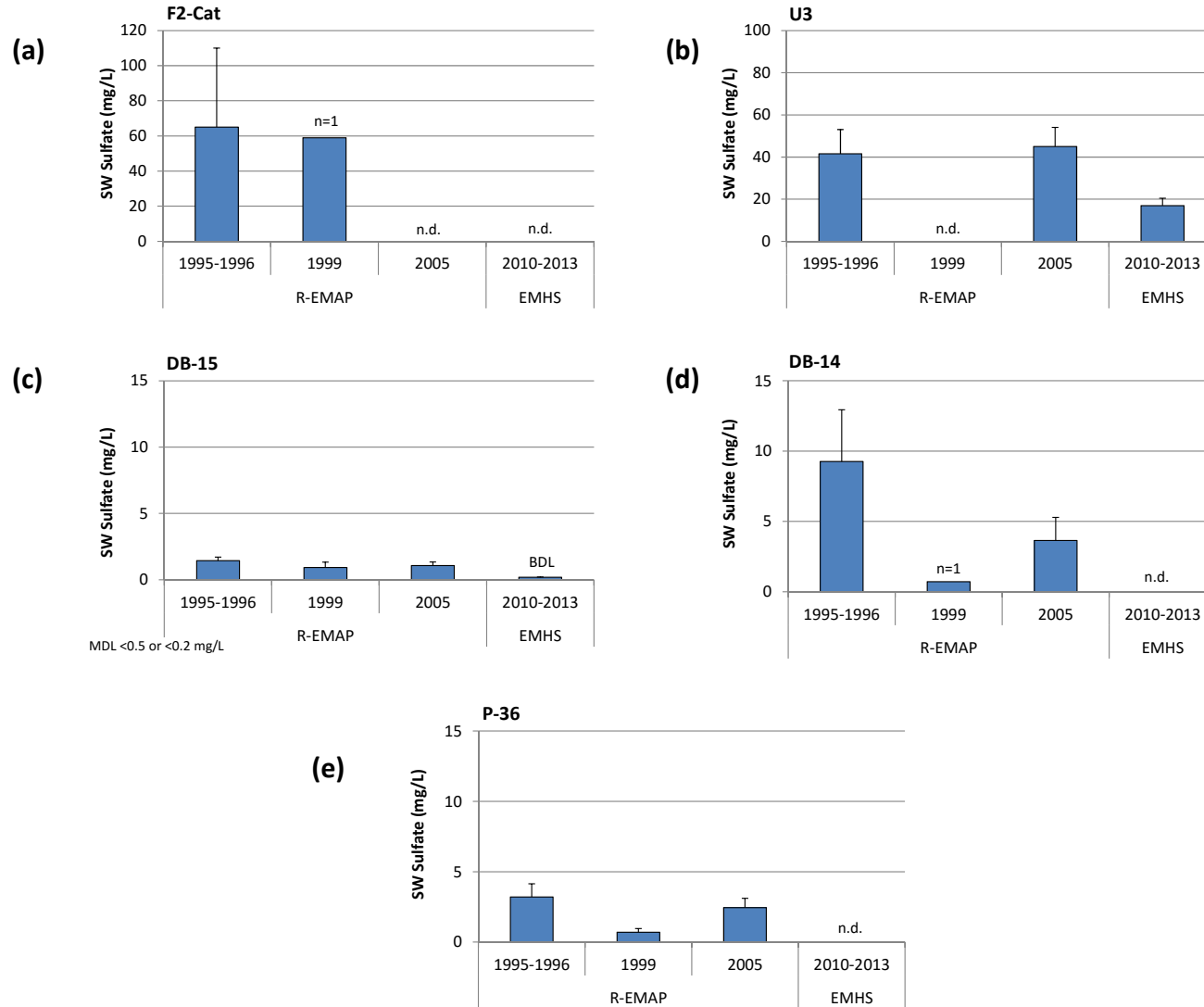


Figure 2. The surface water (SW) sulfate concentration changes over nearly two decades at five stations throughout the Water Conservation Areas (WCA) 2A (F2-Cat) and 3A (DB-15 and DB-14) and the Everglades National Park (ENP) (P-36).

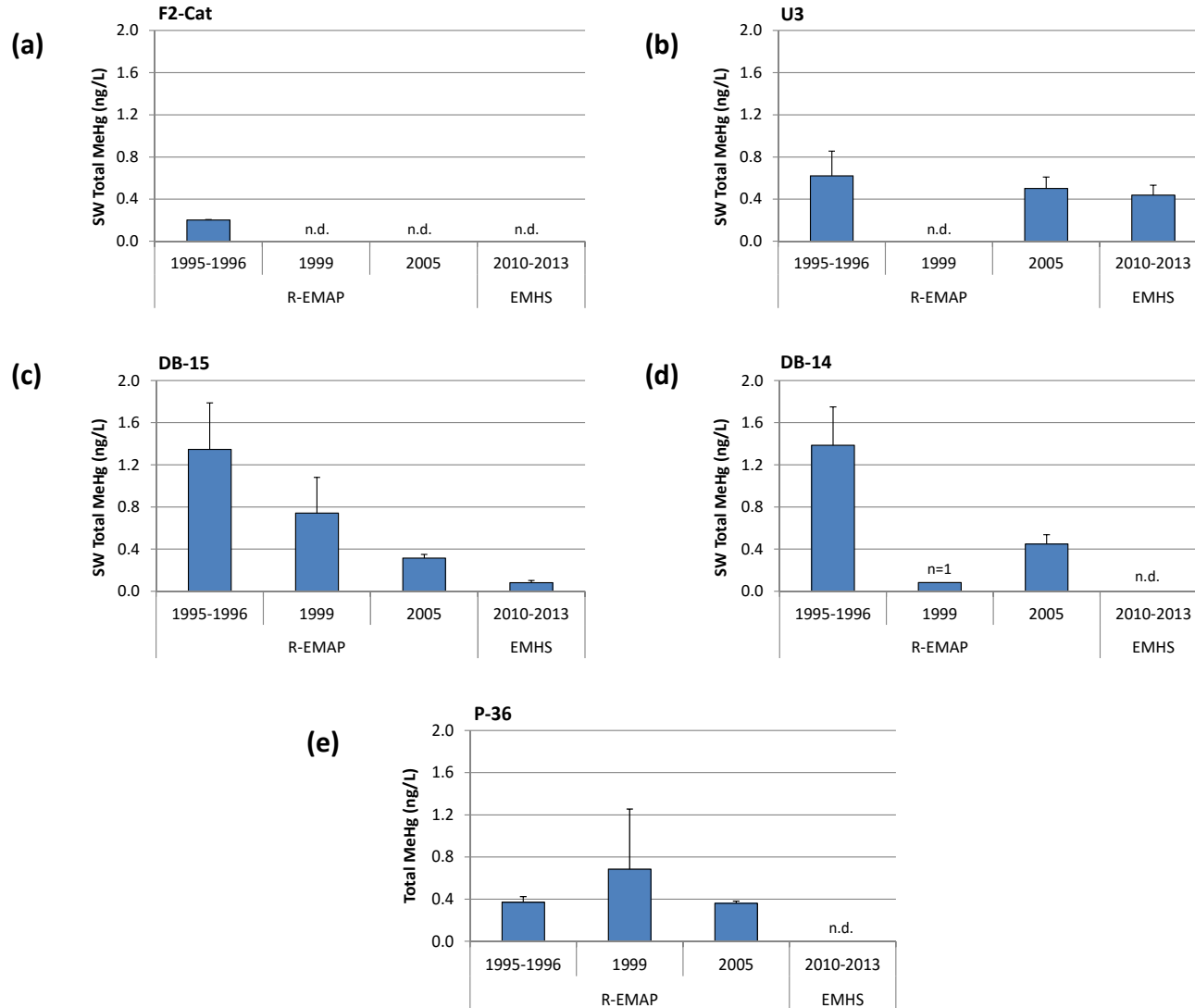


Figure 3. The surface water (SW) total MeHg (unfiltered) concentration changes over nearly two decades at five stations throughout the Water Conservation Areas (WCA) 2A (F2-Cat) and 3A (DB-15 and DB-14) and the Everglades National Park (ENP) (P-36).

Periphyton was not present at some sites during the R-EMAP surveys, but for DB-15 and DB-14 the periphyton MeHg content decreased, and increased at P-36, with time (Figure 4). We could not include periphyton MeHg reported the EMHS data set because the MeHg concentrations were not reported on a dry weight, but instead on a wet weight, basis.

The temporal variations for periphyton at DB-15, DB-14, and P-36 (Figure 4) were mimicked by THg in *G. holbrooki* at those locations (Figure 5), suggesting that the bioconcentration factors (BCFs) for periphyton and biomagnification factors (BMFs) for *G. holbrooki* were influenced by the same environmental conditions or Hg loading unique to each site. The increasing, rather than decreasing, trend at P-36 is intriguing, and deserves further study. Note the multiple-times lower THg concentration in *G. holbrooki* from F2-Cat, a high sulfate site, than for fish from DB-15, DB-14, and P-36, which are low sulfate sites (Figure 2).

The combined R-EMAP and EMHS data sets did not display meaningful relationships between SW DOC, specific UV absorbance at 254 nm (SUVA₂₅₄), and sulfate, and either SW total (unfiltered) Hg or SW total (unfiltered) MeHg concentrations (Figure 6). This is contrary to the significant relationships reported for DOC and SUVA₂₅₄, with SW diss MeHg concentrations in DB Environmental's database (Figure 7), and likely is due to the unfiltered nature of the SW THg and SW MeHg in the R-EMAP and EMHS samples. Particulate THg and MeHg present in the sample effectively masked any relationships between the dissolved Hg species and DOC.

When SW diss MeHg concentration is plotted versus periphyton MeHg concentration, there is no correlation between the two variables (Figure 8a). On the other hand, positive correlations emerge when SW total MeHg (instead of SW diss MeHg) is the independent variable, as shown in the R-EMAP (Figure 8b) and EMHS (Figure 8c) data sets. The direct correlation between SW total MeHg concentration, which includes particles, and periphyton MeHg, may be an artifact due to the autocorrelation: some of the particles measured in the total MeHg aqueous sample may have originated from the nearby periphyton mats.

Periphyton MeHg concentrations were not related to SW sulfate or SW DOC in any of the three datasets, separately or in combination (Figure 9).

The correlation between detrital MeHg and both SW DOC and SW sulfate concentrations using only DB Environmental's 2011-15 data set provided an R² of 0.11 and 0.39, respectively. Adding R-EMAP data to DB Environmental's 2011-15 data set reduced the strength of the correlations between these parameters (R²=0.07 for both correlations; Figure 10a and b). Although the SW MeHg concentrations were unfiltered in the R-EMAP data set, there was still an inverse correlation between detritus and SW total MeHg (Figure 10c).

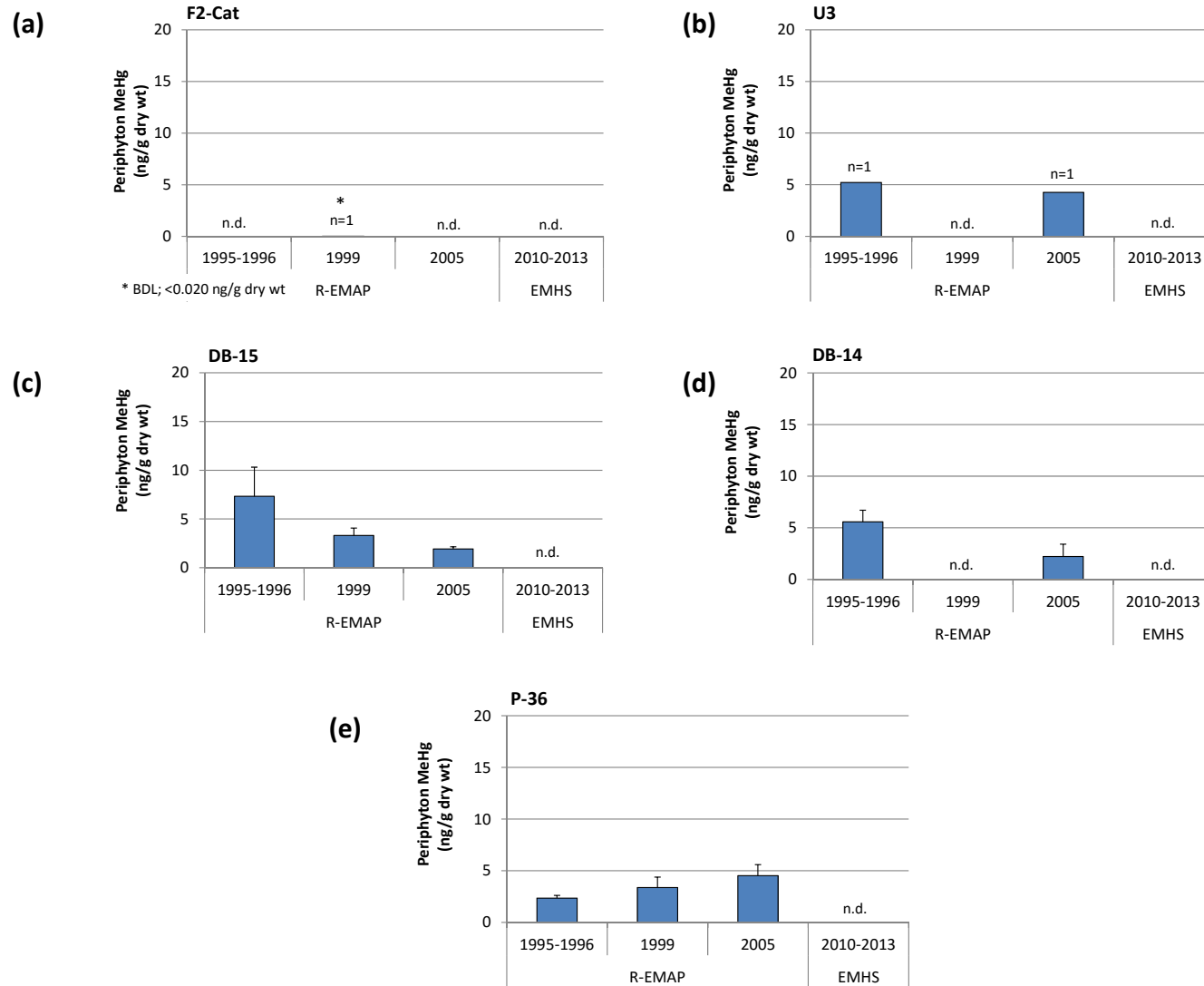


Figure 4. The methylmercury (MeHg) concentration changes in the periphyton over nearly two decades at five stations throughout the Water Conservation Areas (WCA) 2A (F2-Cat) and 3A (DB-15 and DB-14) and the Everglades National Park (ENP) (P-36).

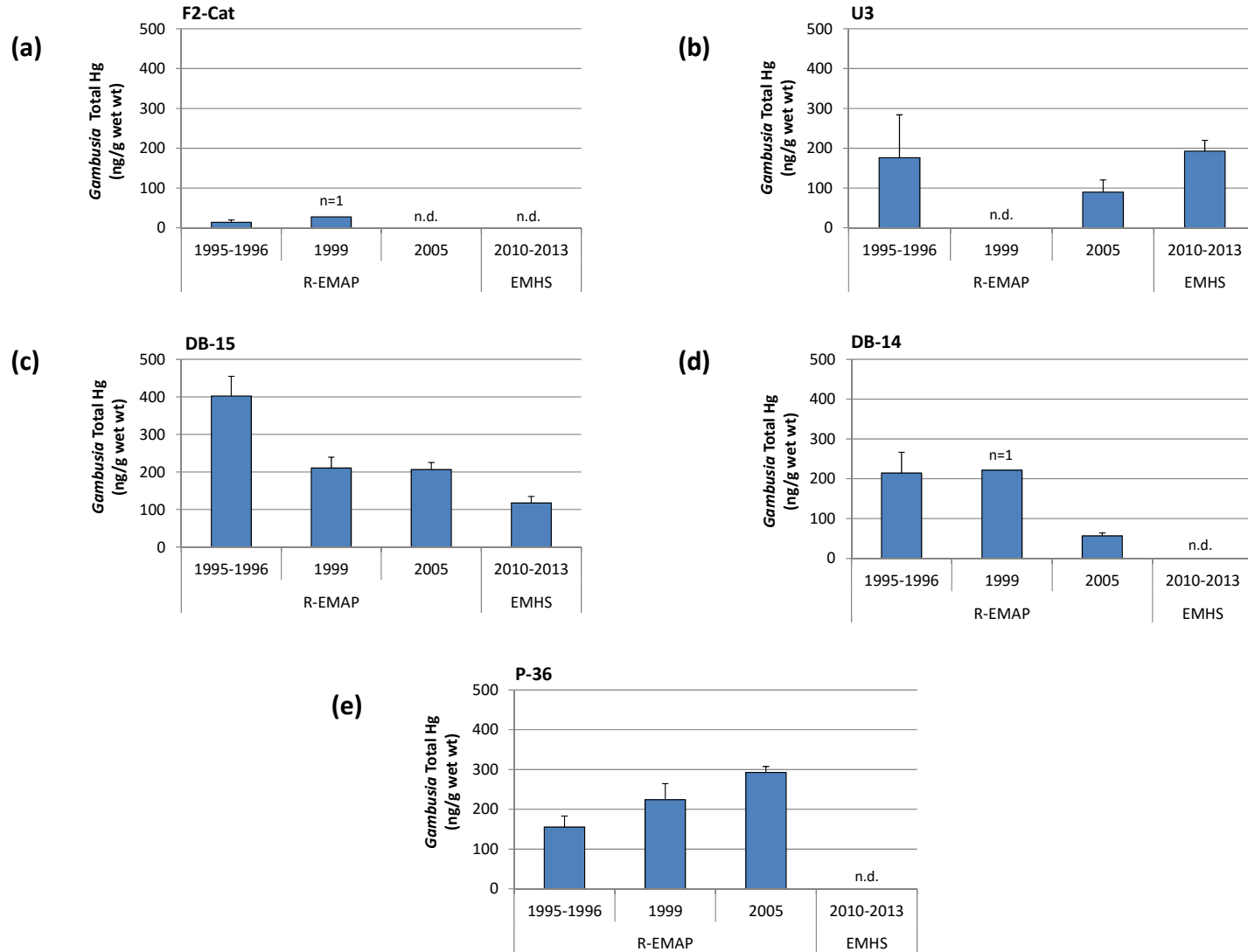


Figure 5. The total mercury (THg) concentration changes in the periphyton over nearly two decades at five stations throughout the Water Conservation Areas (WCA) 2A (F2-Cat) and 3A (DB-15 and DB-14) and the Everglades National Park (ENP) (P-36).

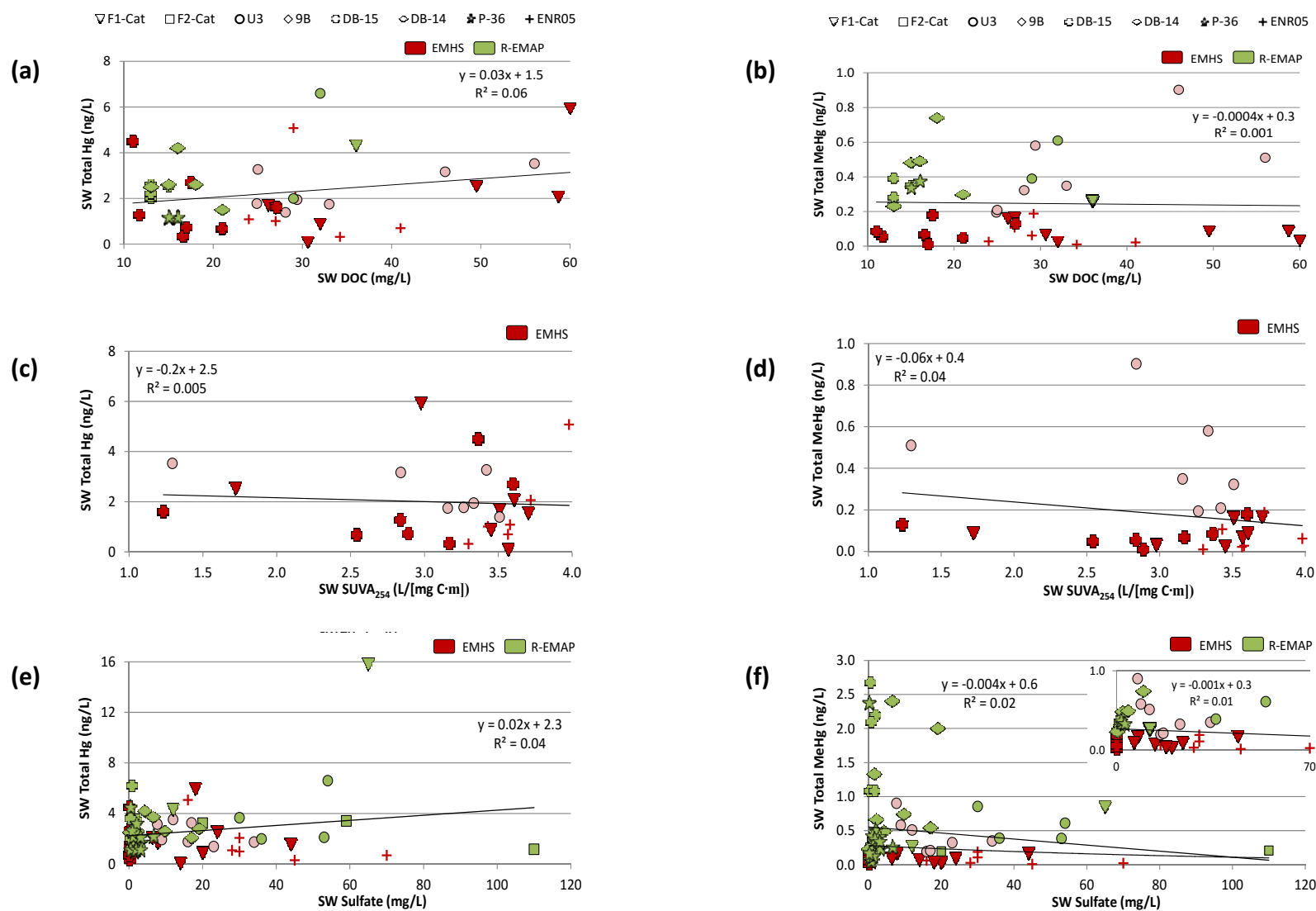


Figure 6. The distributions between surface water (SW) dissolved organic carbon (DOC) concentrations, specific UV absorbance at 254 nm (SUVA₂₅₄), or sulfate concentrations, and SW total (unfiltered) mercury (Hg) concentrations (panels a, c, and e) and SW total (unfiltered) methylmercury (MeHg) concentrations (panels b, d, and f) from R-EMAP and Everglades Mercury Hot Spots (ENHS) data sets.

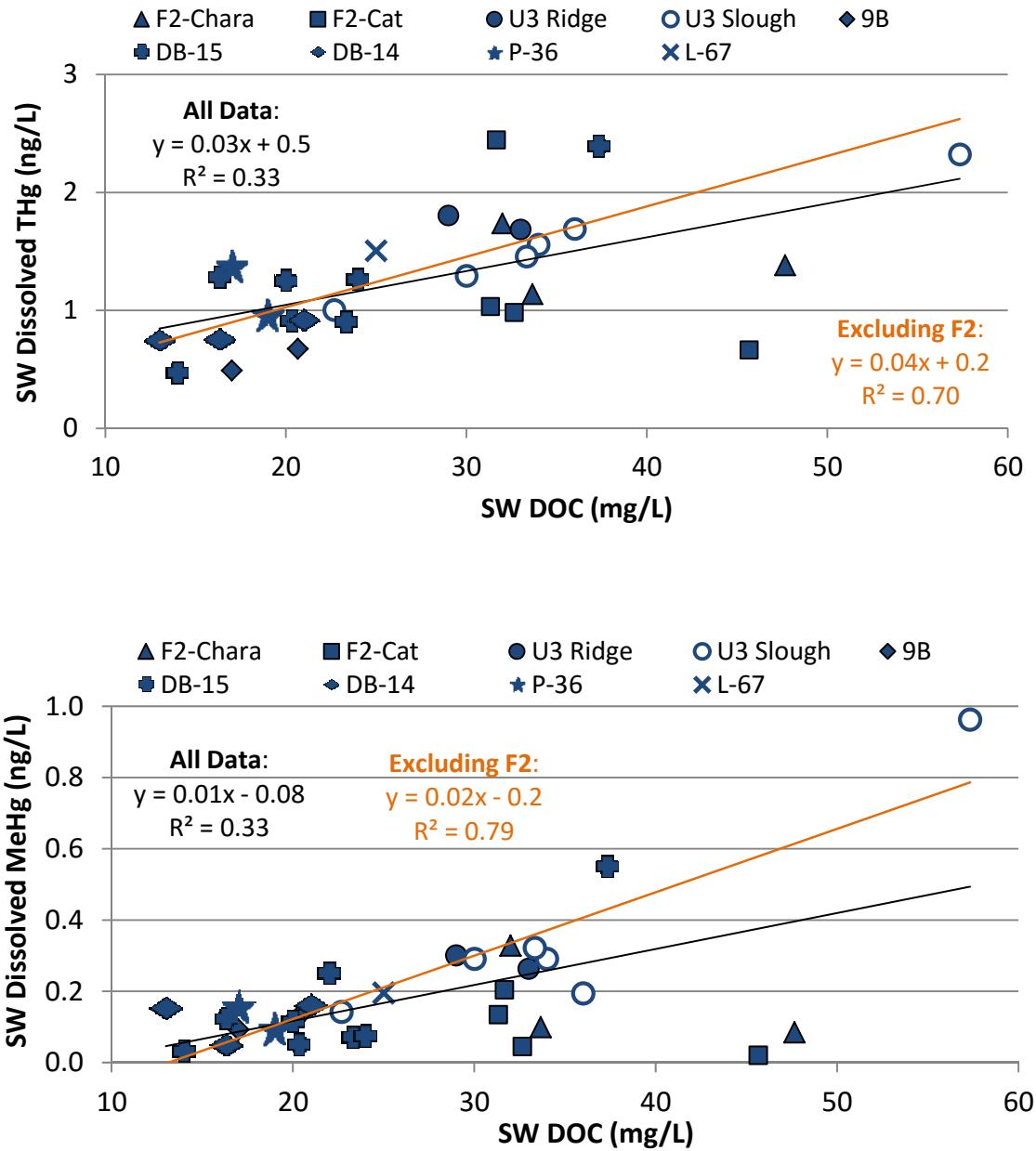


Figure 7. The relationship between the mean (N=1-3) surface water (SW) dissolved total mercury (THg) or dissolved methylmercury (MeHg) concentrations and dissolved organic carbon (DOC) concentrations.

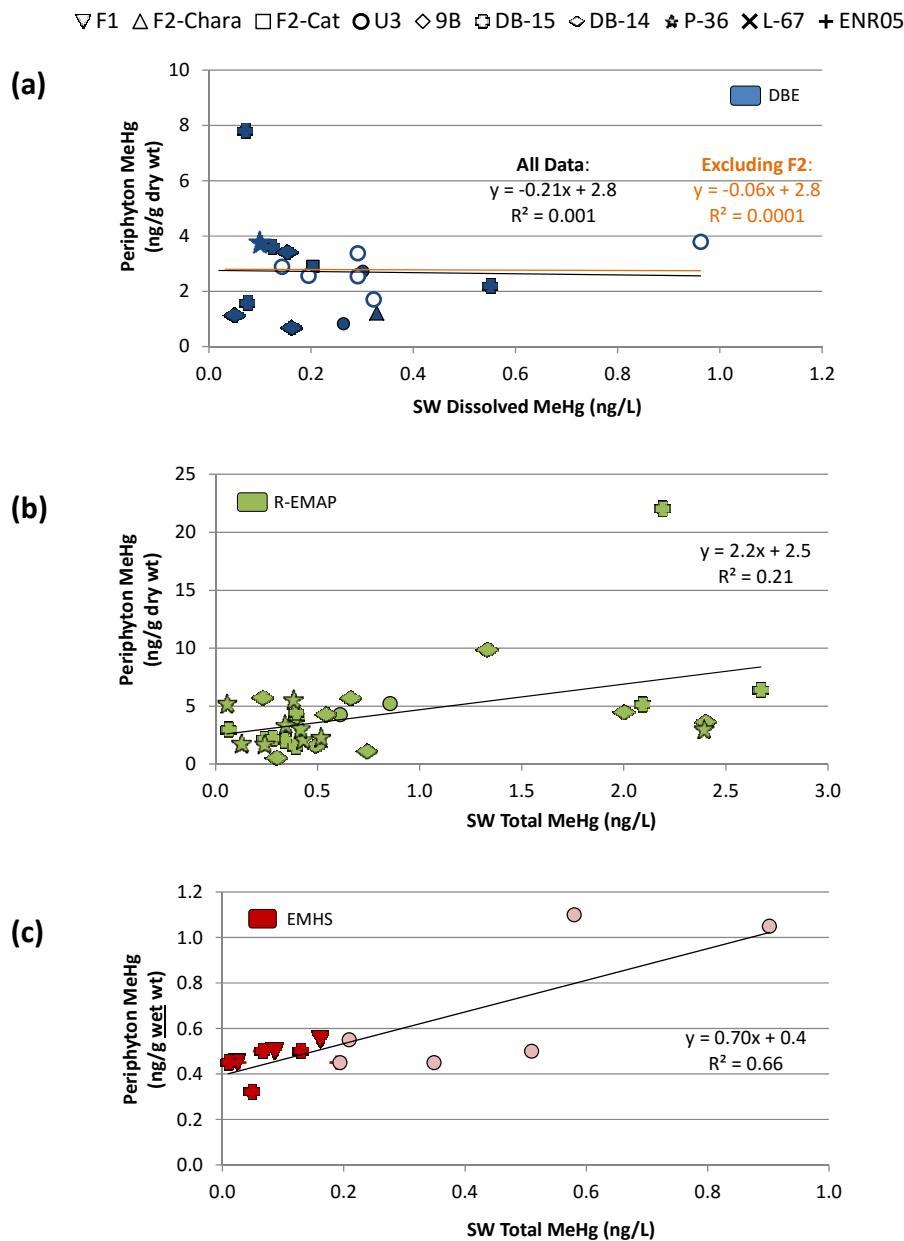


Figure 8. The relationship between periphyton methylmercury (MeHg) content and the (a) surface waters (SW) dissolved MeHg and (b and c) SW total (unfiltered) MeHg concentrations from DB Environmental 2011-15, R-EMAP and Everglades Mercury Hot Spots (ENHS) data sets. Note that the periphyton MeHg concentration for the EMHS (panel c) is on a wet weight basis. U3: closed circle= ridge, open circle=slough; shaded circle= unspecified habitat type. Note vertical and horizontal scales are different in each panel.

▽ F1 △ F2-Chara □ F2-Cat ○ U3 ◇ 9B ◻ DB-15 ◇ DB-14 ★ P-36 ✕ L-67 + ENR05

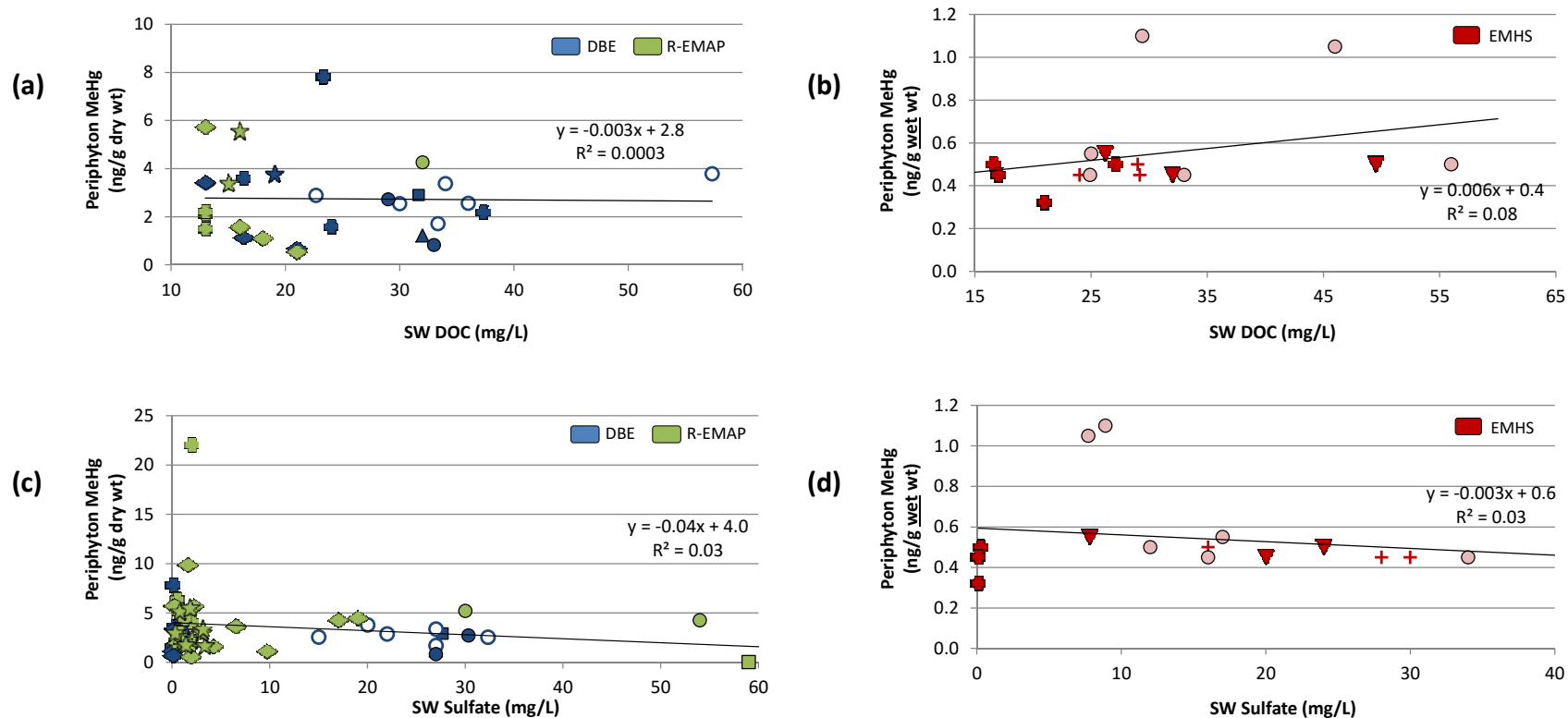


Figure 9. The correspondence between periphyton methylmercury (MeHg) and surface water (SW) dissolved organic carbon (DOC) and SW sulfate concentrations in (a and c) DB Environmental 2011-2015 and R-EMAP data sets and (b and d) EMHS data set. Note that the periphyton MeHg concentration for the EMHS (panels b and d) is on a wet weight basis. U3: closed circle= ridge, open circle=slough; shaded circle= unspecified habitat type.

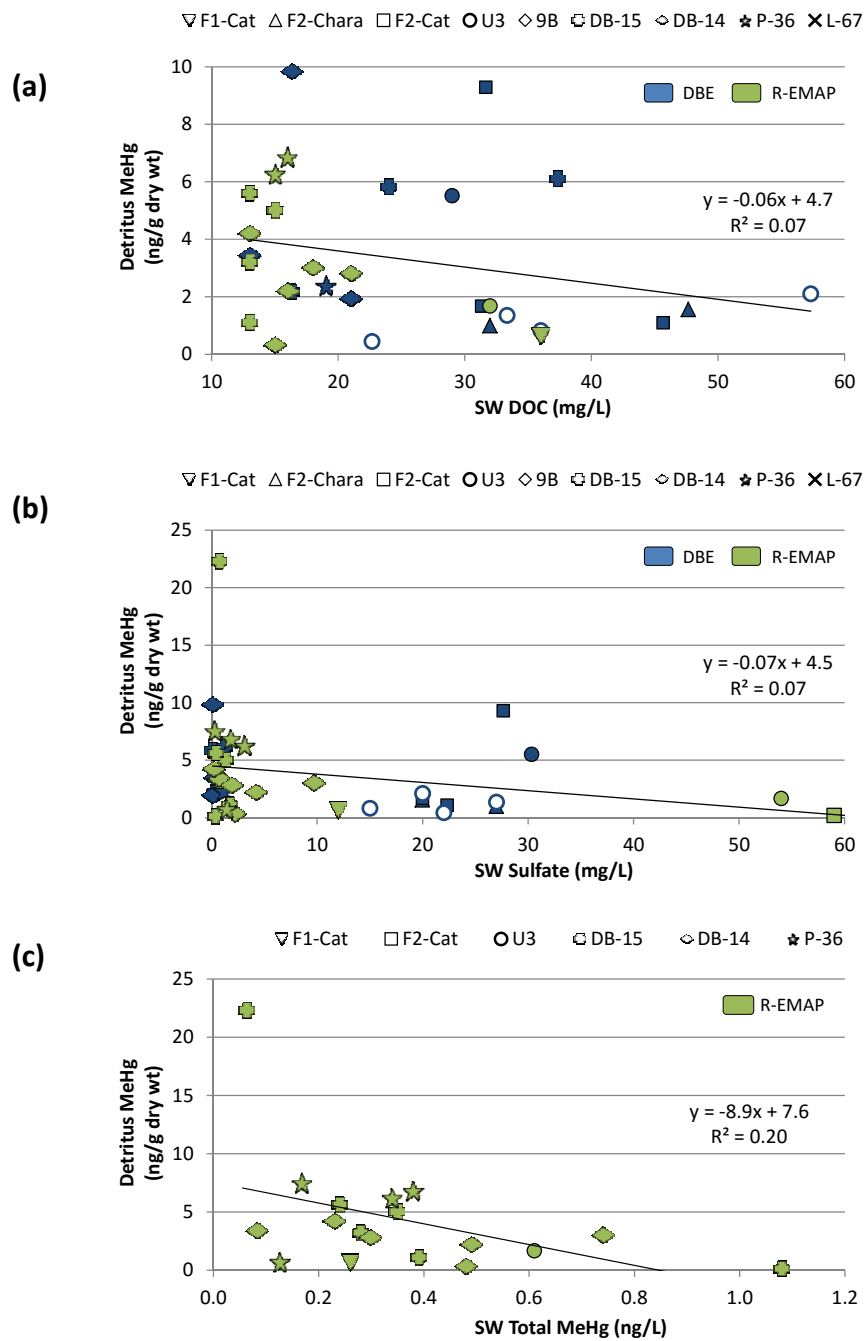


Figure 10. The correlation between detritus methylmercury (MeHg) and surface water (SW) (a) dissolved organic carbon (DOC), (b) SW sulfate, and (c) SW total MeHg concentrations in the DB Environmental 2011-15 and R-EMAP data sets. U3: closed circle= ridge, open circle=slough; shaded circle= unspecified habitat type.

Whereas there was no discernible relationship between SW diss (filtered) MeHg and *G. holbrooki* THg for DBE's data (Figure 11a), there was a noticeable positive association between SW total (unfiltered) MeHg and *G. holbrooki* THg in the EMHS and R-EMAP data sets (Figure 11b). This difference was likely because the total MeHg in the EMHS and R-EMAP data included particles, which are like to be consumed by the fish or their prey, whereas the MeHg dissolved in the water is not assimilated by the fish via gill uptake. For the combined DBE, EMHS, and R-EMAP data sets, SW DOC concentration was inversely related to *G. holbrooki* THg (Figure 11c), likely due to the linkage between increased partitioning of diss MeHg by higher DOC concentrations (Tsui and Finlay 2011; Liu et al. 2008), leaving less water column MeHg available to be adsorbed or taken up by the food sources (detritus, periphyton) of *G. holbrooki* or their prey.

There was also an inverse relationship, both linearly ($r^2 = 0.18$) and exponentially ($r^2 = 0.31$), between sulfate and *G. holbrooki* THg for the combined DB Environmental, EMHS, and R-EMAP data sets (Figure 11d). We did not discern a unimodal response as reported by Pollman (2012). Instead, the highest *G. holbrooki* THg concentrations occurred at extremely low (< 1.0 mg/L) sulfate concentrations, which have been considered to be too low to support sulfate reduction by sulfate-reducing bacteria (SRB) (Corrales et al. 2011; Gilmour et al. 2007; Orem et al., 2011). These data support the findings by Bae et al. (2014) that SRB can still contribute to net methylation even in the absence of sulfate by "switching" metabolic pathways from one of sulfate reduction when sulfate is high to a syntrophic relationship with methanogens at a lower sulfate concentration.

Interestingly, of all the chemical (DOC, sulfate) and biological variables (e.g., periphyton MeHg) considered here, perhaps the strongest correlate with *G. holbrooki* THg concentrations was MeHg in detritus (Figure 12). This suggests that MeHg enters the food web, and transfers up to *G. holbrooki* via a detrital pathway. The origin and fate of MeHg in detritus may be critical controls on the spatial patterns in Hg bioaccumulation in the Everglades.

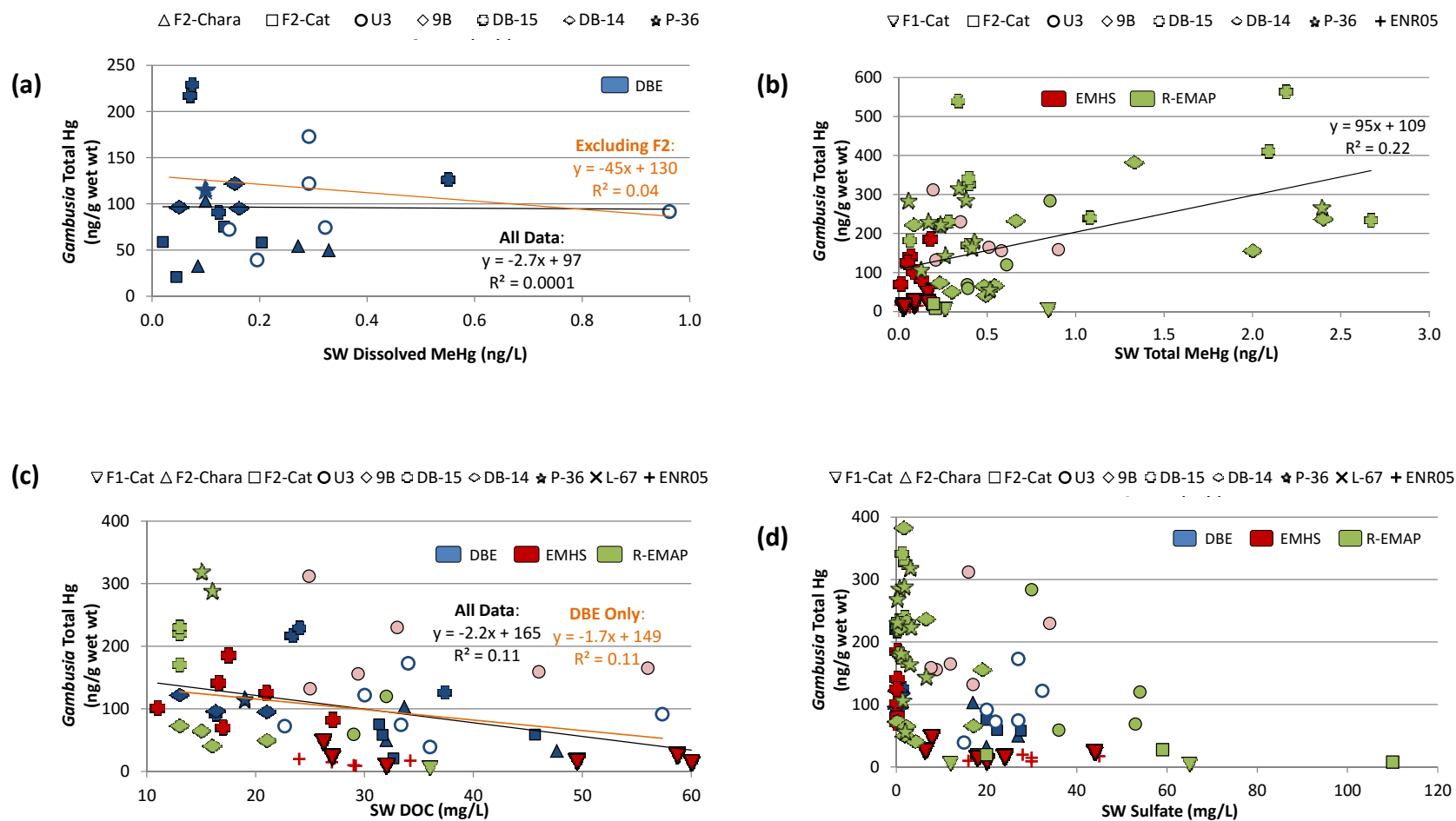


Figure 11. The relationships between *Gambusia holbrooki* total mercury (Hg) and surface water (SW) methylmercury (MeHg) concentrations in DB Environmental's (DBE) data set (panel a), SW total (unfiltered) MeHg in EMHS and R-EMAP data sets (panel b), SW dissolved organic carbon (DOC) concentrations in DBE, EMHS, and R-EMAP data sets (panel C), and SW sulfate concentrations in DBE, EMHS, and R-EMAP data sets (panel d). U3: closed circle= ridge; open circle=slough; shaded circle= unspecified habitat type.

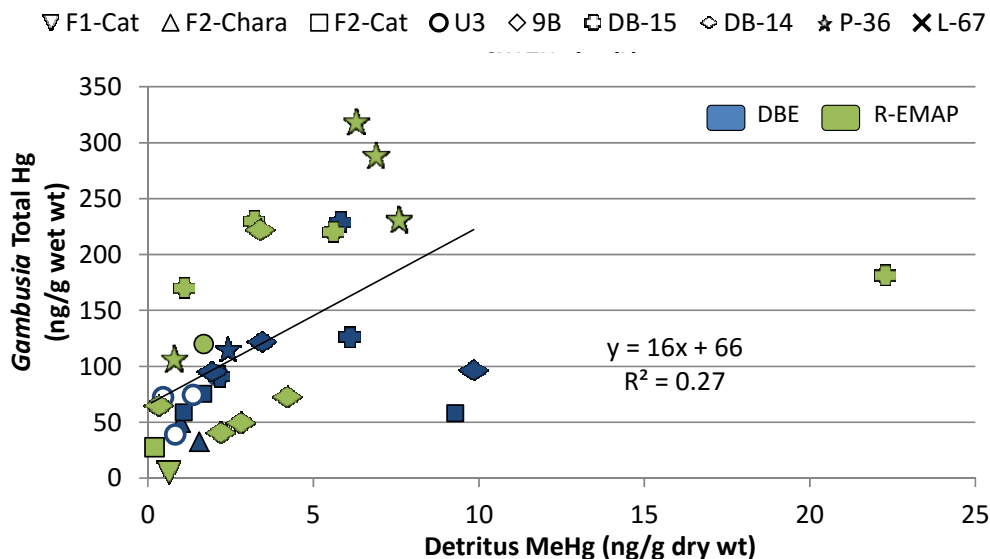


Figure 12. The correspondence between MeHg concentration in detritus and THg concentration in *Gambusia holbrooki* for DB Environmental 2011-15 and R-EMAP data sets. Outlier excluded from regression analysis.

Conclusions

Among the biogeochemical factors collected by DBE, EMHS and R-EMAP, DOC concentration was directly correlated to aqueous MeHg concentration, but inversely correlated to total Hg and MeHg in *Utricularia*, *Gambusia*, and detritus (no relationship was found for periphyton). Through the strong binding capacities of dissolved organic matter (DOM), which DOC is a surrogate, Hg and MeHg become less available for uptake by basal food sources (plants and detritus). This effect cascades up the food web to *Gambusia*. There were few relationships between fish Hg and other biogeochemical variables. Detritus may be an important vector of MeHg into the food web.

Task 2: Biogeochemical and Ecological Controls on Bioaccumulation of MeHg by Everglades Biota

Background and Rationale

The identification of gross community effects on Hg bioaccumulation and the specific relationships between trophic position and Hg accumulation in Everglades biota within DB Environmental and other data sets in Year Three lead to the Year Four focus on the ecological contributors to Hg cycling.

MeHg concentrations have been found to increase along the Everglades food chain (Liu et al. 2008). Bioaccumulation of MeHg, particularly in mosquitofish (*Gambusia holbrooki*), is poorly correlated to water column MeHg concentrations across the greater Everglades, and may be mediated in part by periphyton MeHg concentrations (Scheidt and Kalla 2007; Liu et al. 2008) and food web structure (Loftus 2000). Results from the SFWMD Everglades Mercury Hot Spot monitoring study (EMHS) (Figure 13) and data from DB Environmental research Years Two, Three, and Four (Figure 14) have shown widely varying concentrations of Hg in fish tissue among sampling sites. Differences in tissue Hg content cannot be adequately explained by variability in commonly cited factors such as sulfate concentration. For example, *Gambusia* Hg content is substantially greater at station WCA2A-U3 than at northern WCA2A (e.g., F1) (Figure 13), yet these sites are both highly sulfate-impacted (Figure 13) with high levels of porewater sulfide.

Spatially, mosquitofish THg levels vary substantially over narrow ranges of sulfate concentration (Pollman 2012.). Further, at fixed locations within the marsh, mosquitofish THg can vary considerably over short time periods, especially where median concentrations are relatively high (Figure 14). Our findings from Year Three indicated that spatial patterns in Hg contamination were consistent across food chain strata. That is, the MeHg concentration in primary consumers poised the MeHg concentrations in a variety of higher consumers at each monitoring site. Therefore, the controls on the entry of MeHg into the base of the food web may explain spatial distributions of Hg enrichment in *Gambusia* (and other biota), so are of chief interest this research Year.

Given the aforementioned interest in MeHg transfer among the lower trophic strata (primary producers and primary consumers), particular attention was devoted to the character of detritus and primary producers, so those materials received special consideration.

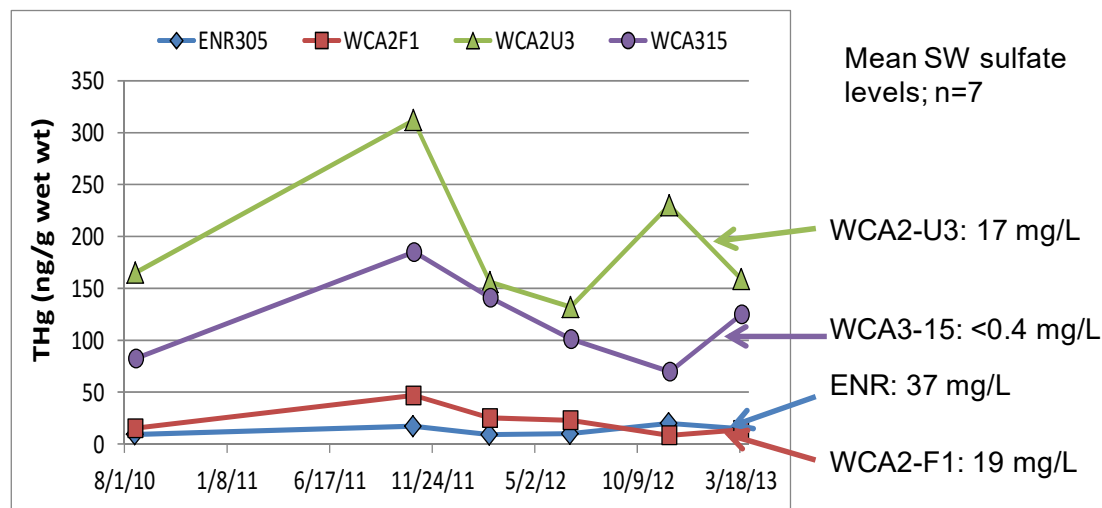


Figure 13. Concentration of fish tissue (*Gambusia*) Hg and surface water sulfate concentrations measured at four sites within the Everglades during the Everglades Mercury Hot Spot monitoring study.

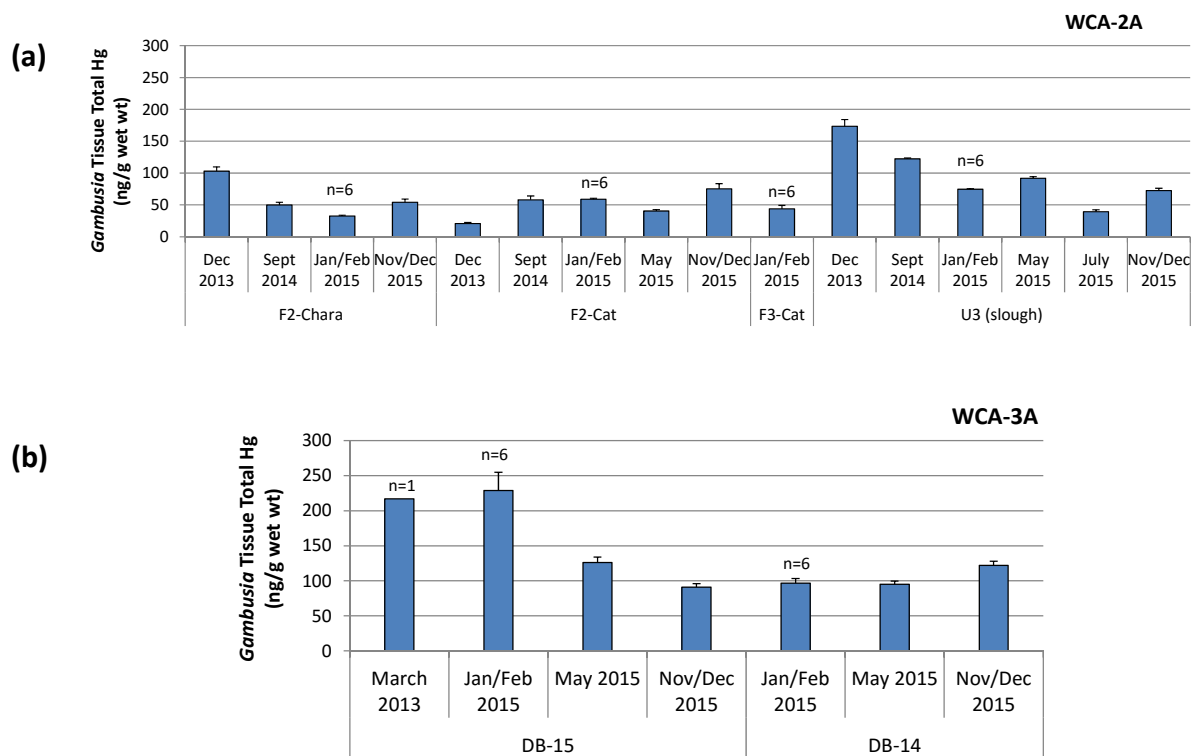


Figure 14. Mean (+1S.E.) historical *Gambusia* tissue total mercury (THg) concentrations reported by DB Environmental for sites within the (A) Water Conservation Areas (WCA) -2A and (B) -3A. n=3.

Spatial Differences in Water Quality and Biota in Year 4

Six marsh locations (a subset of DBE's historical monitoring sites) spanning a range of sulfate concentrations, plant community types and known fish Hg levels within the Everglades Protection Area (EvPA) and ENP were sampled this year (Figure 15). Two locations (DB-14 and DB-15 within WCA-3A) were sampled November 30, 2015, followed by three locations (U3, F2-Cat, and F2-Chara) in WCA-2A the next day (December 1, 2015), and finally P-36 in ENP on December 2, 2015.

Sampled matrices focused on biological ecosystem components that include surface water, porewater, detritus, primary producers, invertebrates and cyprinodontoid fish. Analyses, especially of biological materials, included THg, MeHg, and stable isotopes ^{13}C and ^{15}N , in addition to routine biogeochemical analytes such as nutrients, sulfur species and organic C. All field and laboratory methodologies have been described in the 2015 FDACS Annual Report (DBE 2015).

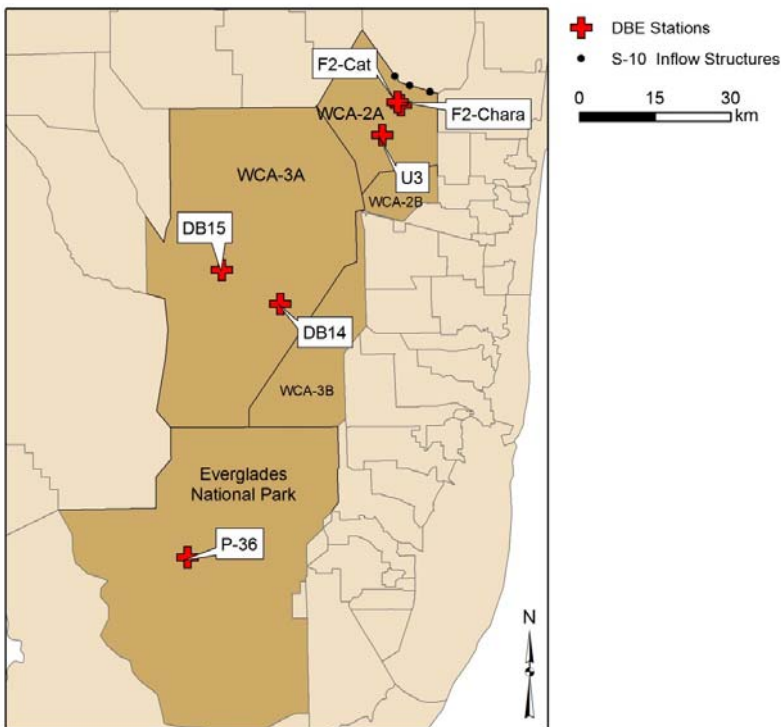


Figure 15. Map showing the locations of the six sites where samples were collected in Nov. 30 – Dec. 2, 2015.

Surface and Pore Waters

The F2-Chara plot contained the SW concentrations for both dissolved (diss) THg and diss MeHg than the remaining five stations, which varied within a narrow range of 0.747-1.28 ng/L for diss THg and 0.099-0.153 ng/L for diss MeHg (Figure 16a and b). The diss MeHg:THg ratio, a measure of the methylation efficiency, was highest at DB-14 with a value of 0.21 ng/L; the diss MeHg:THg ratios at the other locations ranged between 0.10 and 0.15 (Figure 16c).

The SW DOC concentrations were highest for the sites within WCA-2A, whereas concentration within WCA-3A and ENP varied between 13 and 19 mg/L (Figure 17a). The $SUVA_{254}$ is a measure of the aromaticity of the dissolved organic matter (DOM), and was highest at F2-Cat and lowest at P-36 in the ENP (Figure 17b). Surface water from the two WCA-3A stations, DB-15 and DB-14, had $SUVA_{254}$ values 3.3 and 3.4 L/[mg C m], respectively. The spectral slope between 275 and 295 nm ($S_{275-295}$) is an alternative means of measuring the aromatic content of DOM, where low values represent more aromaticity. Thus F2-Cat and P-36 had the lowest and highest $S_{275-295}$, respectively, consistent with the $SUVA_{254}$ data where higher values indicate more aromatic compounds. However, the aromaticity was higher at DB-14 than DB-15 (Figure 17c), unlike the equal $SUVA_{254}$ values (Figure 17b), demonstrating that while both $SUVA_{254}$ and $S_{275-295}$ are considered to be a measure of aromaticity, they differentially measure groups of aromatic compounds in DOM.

In addition to optical techniques that rely on absorbance of light passing through DOM at certain wavelengths, there are also optical techniques for measuring the nature of DOM by fluorescence. The DOM molecules that emit fluorescence at 460 nm wavelength (λ) after being excited by light energy at λ of 360 nm is defined as the fluorescent DOM, or FDOM. Its unit of measurement is quinine sulfate equivalents (QSE) in $\mu\text{g/L}$. As with SUVA_{254} , higher values of QSE equate to higher molecular weight, or humic, compounds. Surface water from F2-Cat exhibited the highest, and DB-14 the lowest, FDOM value in the five-station group (Figure 17d). The second fluorescence measurement is called the relative fluorescence efficiency (RFE), which is the FDOM divided by the absorbance at 340 nm. Thus the RFE normalizes the FDOM to the DOC concentration. Among the five stations, the RFE was highest at U3 and P-36, and lowest at F2-Cat; the RFE for DB-15 and DB-14 were intermediate and approximately the same (Figure 17e). The relationship between SUVA_{254} and RFE was inversely correlated to a high degree ($r = -0.94$). Since both parameters are normalized to the C concentration, and higher SUVA_{254} values indicate higher aromaticity, then apparently lower RFE values correspond to lower aromatic content of the DOM.

The SW pH values ranged from 7.4 at F2-Cat and 7.8 at DB-15 (Figure 18a); PW pH values were approximately 1 pH unit lower (Figure 18b). The oxidation-reduction potential (ORP) was negative for soils in WCA-2A, and positive at WCA-3A and ENP soils (Figure 18c).

Sulfate and sulfide concentrations have been cited as important factors in regulating the extent of net MeHg production in the Everglades (Benoit et al. 2003; Gilmour et al. 1998; Gilmour et al. 2007; Skyllberg 2008). For the locations included in our data set, both SW and PW sulfate and sulfide concentrations varied widely, depending on whether the stations were located within or outside WCA-2A (Figure 19a-d). Sulfate and sulfide concentrations in the SW and PW were highest at the two WCA-2A stations, and lowest at the WCA-3A and ENP stations. The origin of the sulfate is the agricultural drainage water that has been released into the northern area of WCA-2A through the S-10 structures. The reduced conditions in the soils (Figure 18c), coupled with the high sulfate concentrations, resulted in the elevated sulfide concentration in the PW in WCA-2A (Figure 19d).

Dissolved iron (Fe) concentrations in SW and PW were considerably lower at the WCA-2A sampling stations, compared to the sites outside WCA-2A (Figure 20a and b). There was also considerable disparity in PW diss Fe concentration between DB-15 (3.73 mg/L) and DB-14 (0.27 mg/L) within WCA-3A (Figure 20b). Although not documented as of yet in the Everglades, bacteria capable of Fe reduction have been shown to methylate Hg in other environments (Fleming et al. 2006; Kerin et al. 2006; Skyllberg 2008). Fe-reducing bacteria in soil at WCA-3A and ENP may partially (or wholly) explain why SW MeHg concentrations for the two sites in WCA-3A and P-36 in ENP, where sulfate concentrations are negligible (Figure 19a and c), are comparable to the SW MeHg concentrations at the sulfate-enriched sites in WCA-2A (Figure 19b).

Dissolved calcium (Ca) concentrations among the sites were comparable, with SW concentrations ranging from 52-55 mg/L except for the 47 mg/L at DB-14 (Figure 20c). SW total P (TP) concentrations were $\leq 4 \mu\text{g/L}$ except for the elevated level ($16 \mu\text{g/L}$) at F2-Cat (Figure 20d), the station in WCA-2A closest to the S-10 inflow structures.

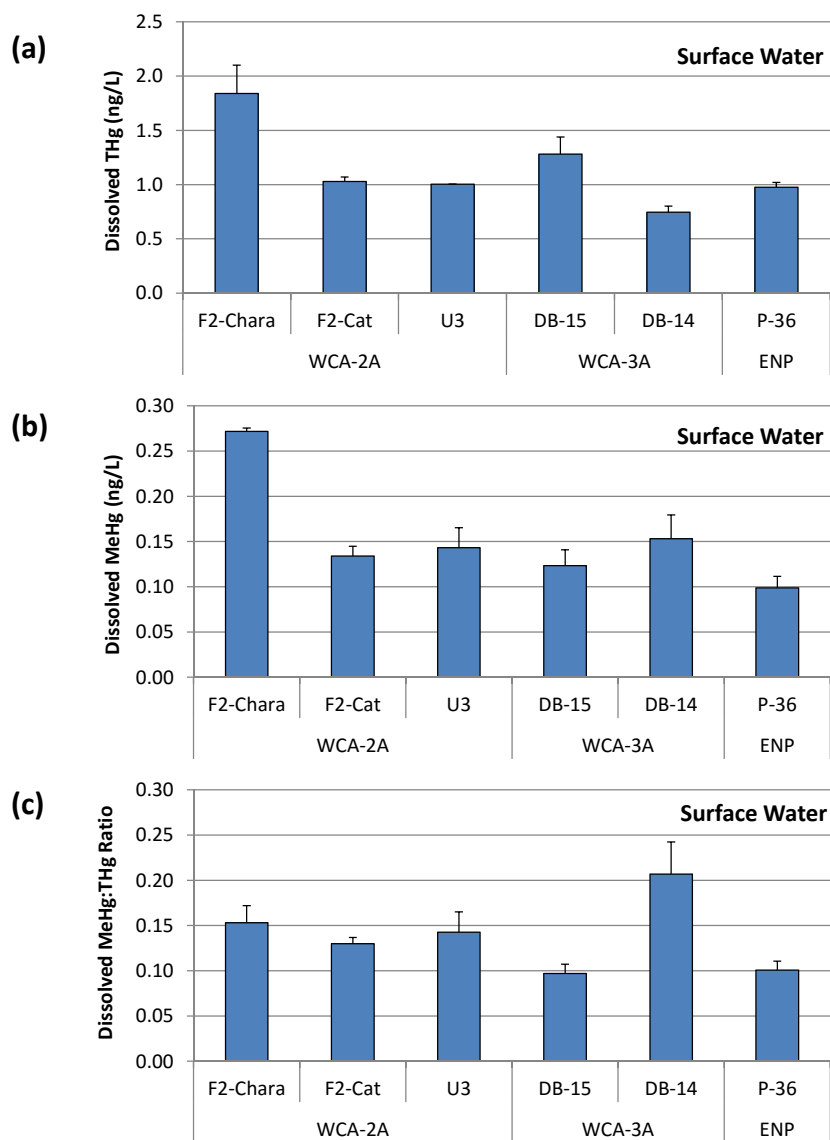


Figure 16. The mean (+1 S.E.) dissolved total mercury (THg, panel a), dissolved methylmercury (MeHg, panel b), and the ratio of dissolved MeHg:dissolved THg (panel c) among six sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3.

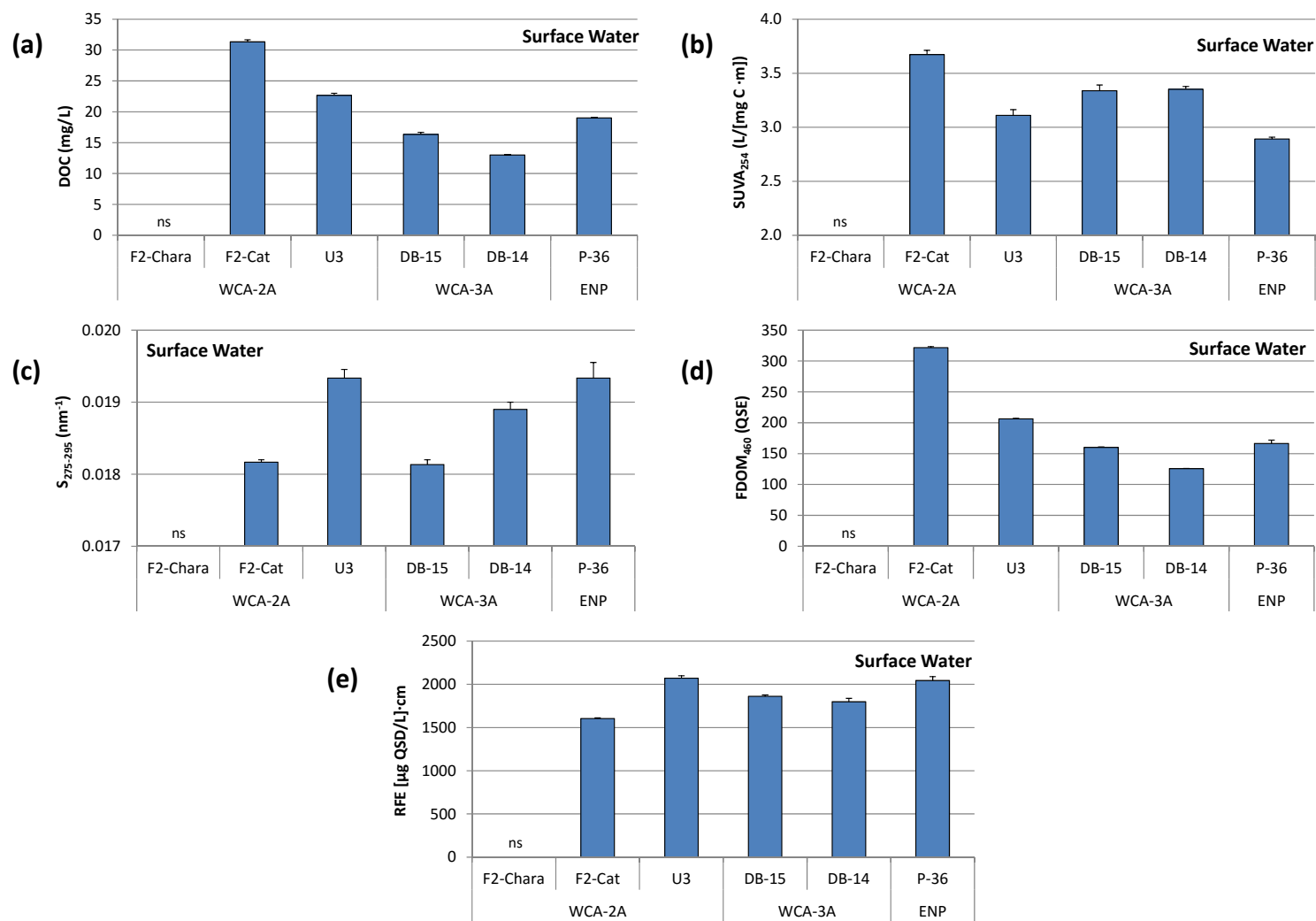


Figure 17. The mean (+1 S.E.) dissolved organic carbon concentration (DOC, panel a), specific UV absorbance at 254 nm (SUVA₂₅₄, panel b), spectral slope between 275 nm and 295 nm wavelengths (S₂₇₅₋₂₉₅, panel c), fluorescent dissolved organic matter at wavelengths of 360 nm for excitation and 460 nm for emission (FDOM₄₆₀, panel d), and the relative fluorescent efficiency (RFE, panel 3e) among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns = not sampled.

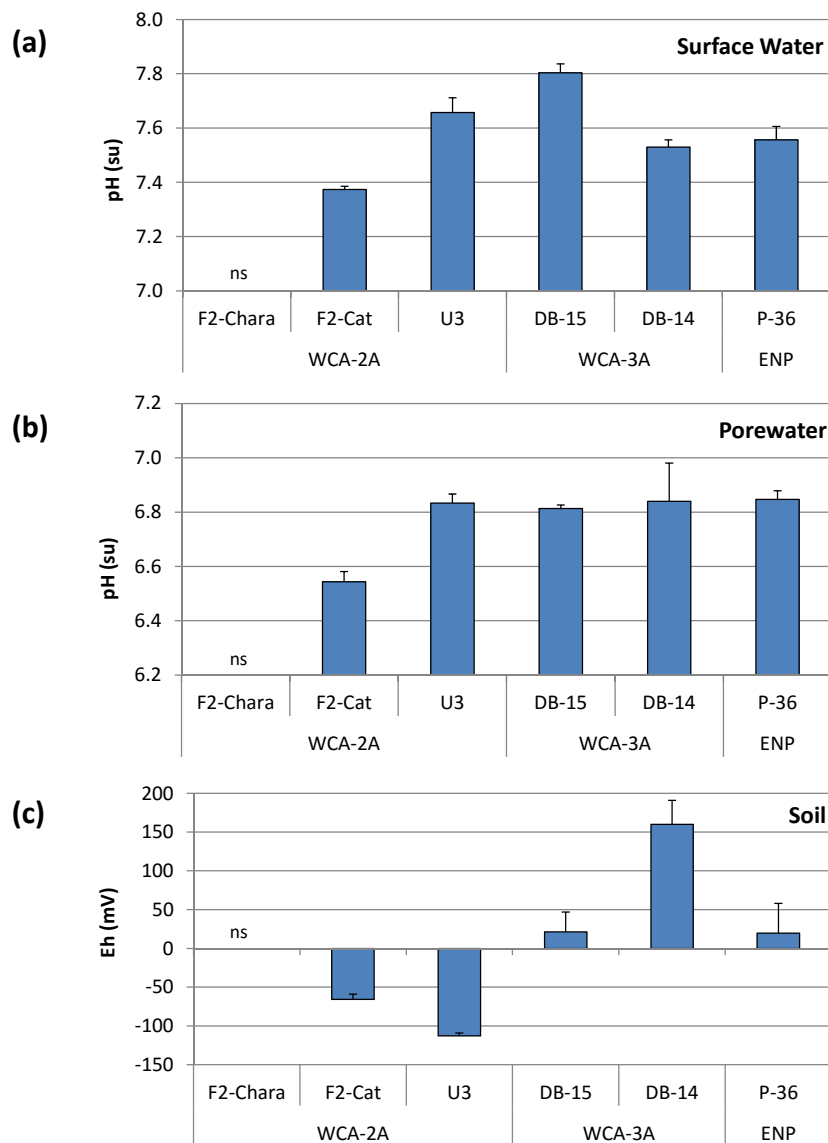


Figure 18. The mean (+1 S.E.) surface (panel a) and pore water (6-9 cm depth) (panel b) pH values, and soil oxidation-reduction potentials (E_h , panel c) among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns = not sampled.

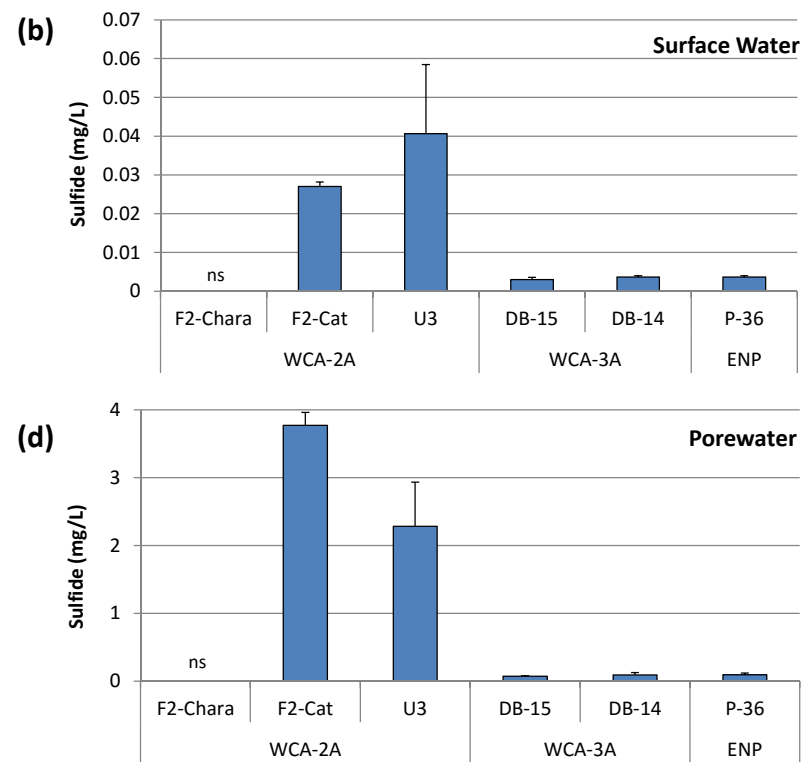
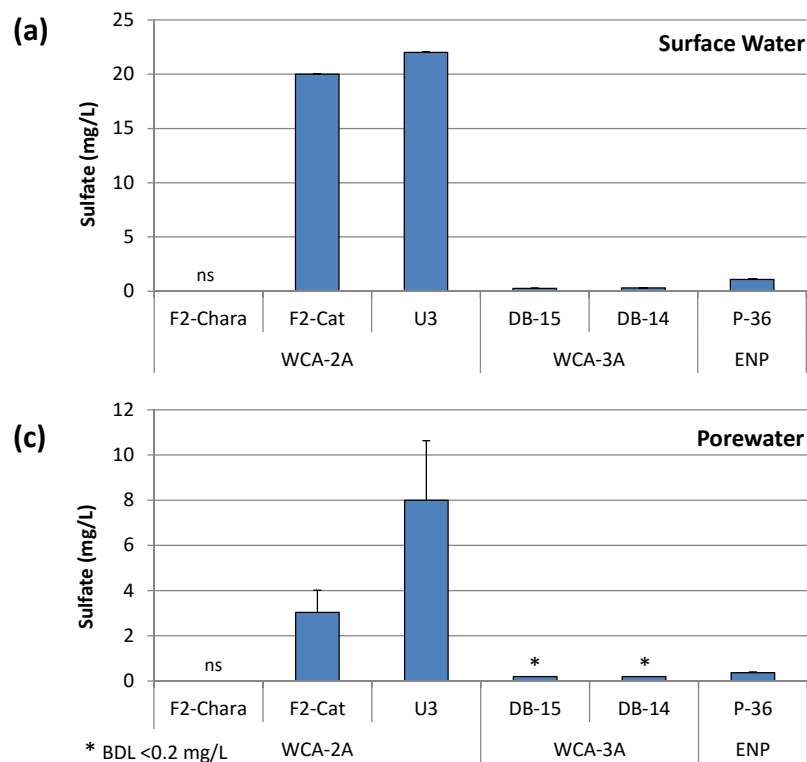


Figure 19. The mean (+1 S.E.) dissolved surface water sulfate (panel a) and sulfide (panel b), and the dissolved porewater sulfate (panel c) and sulfide (panel d), concentrations among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns = not sampled.

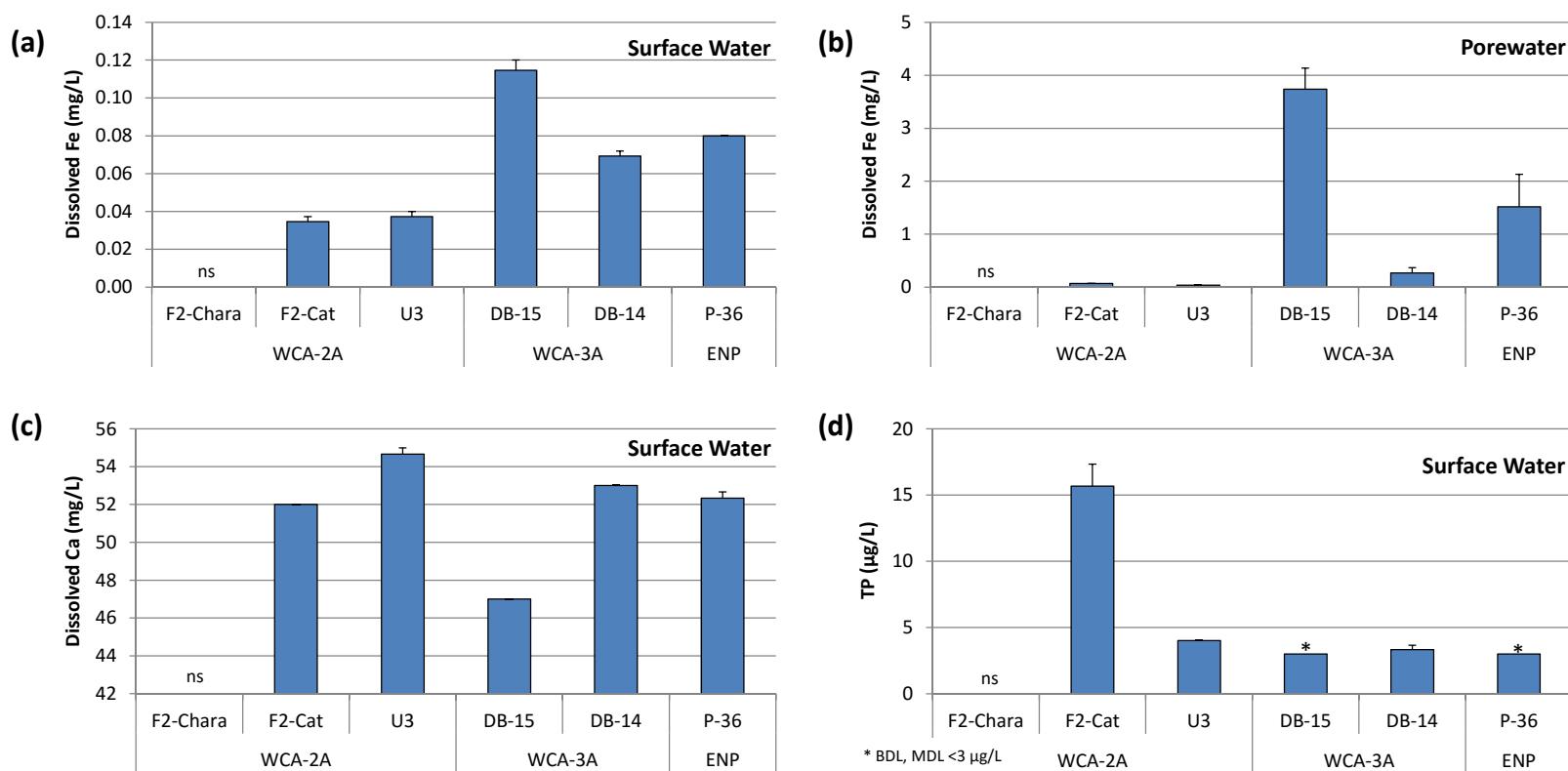


Figure 20. The mean (+1 S.E.) dissolved surface (panel a) and pore (panel b) water dissolved iron (Fe), dissolved surface water calcium (Ca, panel c,) and surface water total phosphorus (TP, panel d) concentrations among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 - December 2, 2015. N=3. ns = not sampled.

Fish

There was a clear separation in the THg concentrations in the eastern mosquitofish, *Gambusia holbrooki*, with lower levels measured in fish collected in WCA-2A and higher concentrations in the fish from WCA-3A and ENP (Figure 21a). The MeHg concentrations in the food consumed by *G. holbrooki* were directly correlated with the THg concentrations in the whole fish ($r=0.95$; $n=6$; cf. Figure 21a and b). These patterns are consistent with previous findings to be presented later in this report, and indicate that *G. holbrooki* derive their Hg content from their diet. It therefore follows that the MeHg concentrations in the food resources at a site to be an influential variable in determining the Hg body burden in *G. holbrooki*.

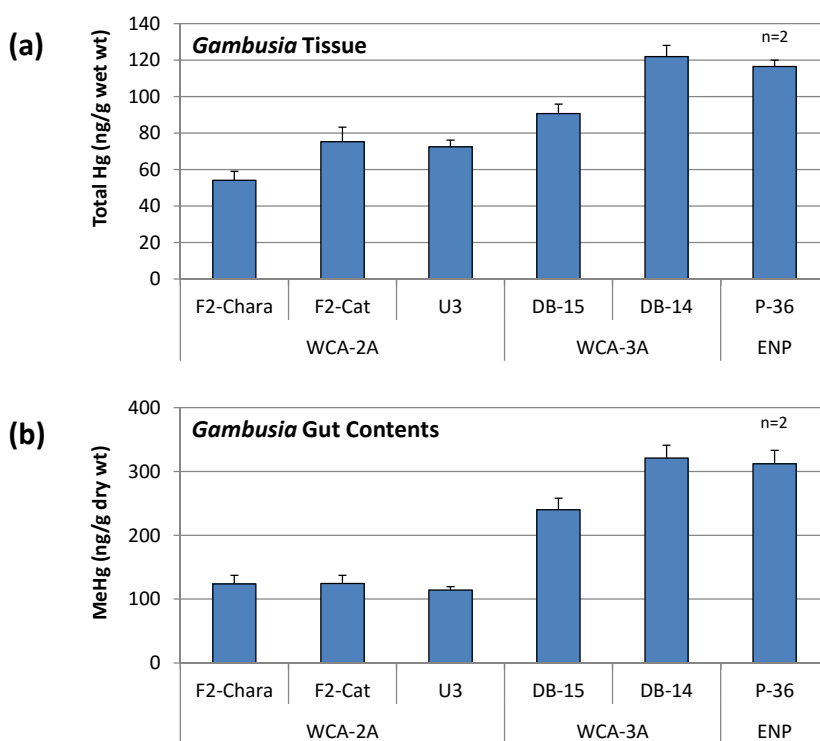


Figure 21. The mean (+1 S.E.) total mercury (Hg) concentrations in *Gambusia holbrooki* (panel a) and the methylmercury (MeHg) concentrations in their gut contents (panel b) among six sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3 unless otherwise noted.

We selected two predominantly herbivorous fish species, *Jordanella floridae* (flagfish) and *Poecilia latipinna* (sailfin molly), to compare Hg levels with the THg content of the omnivorous *G. holbrooki*. Two findings from the analyses are noteworthy. First, the general pattern of THg and MeHg concentrations of the herbivorous fish (WCA-2A < WCA-3A sites; Figure 22a and b) was similar that of THg concentrations in *G. holbrooki* (Figure 21a). This indicates the likelihood that differences in MeHg content of the basal food resources (e.g., plants and algae) at a location

accounted for the ultimate Hg concentrations in *G. holbrooki*. The second finding is that the MeHg:THg concentration ratio in the herbivorous fish was > 0.8 at all locations (Figure 22c), demonstrating that either the basal food items consumed by these herbivorous fish consist almost entirely of MeHg (and only minor amounts of inorganic Hg), or that the inorganic Hg present within their food items is rapidly excreted from the digestive tracts of the fish.

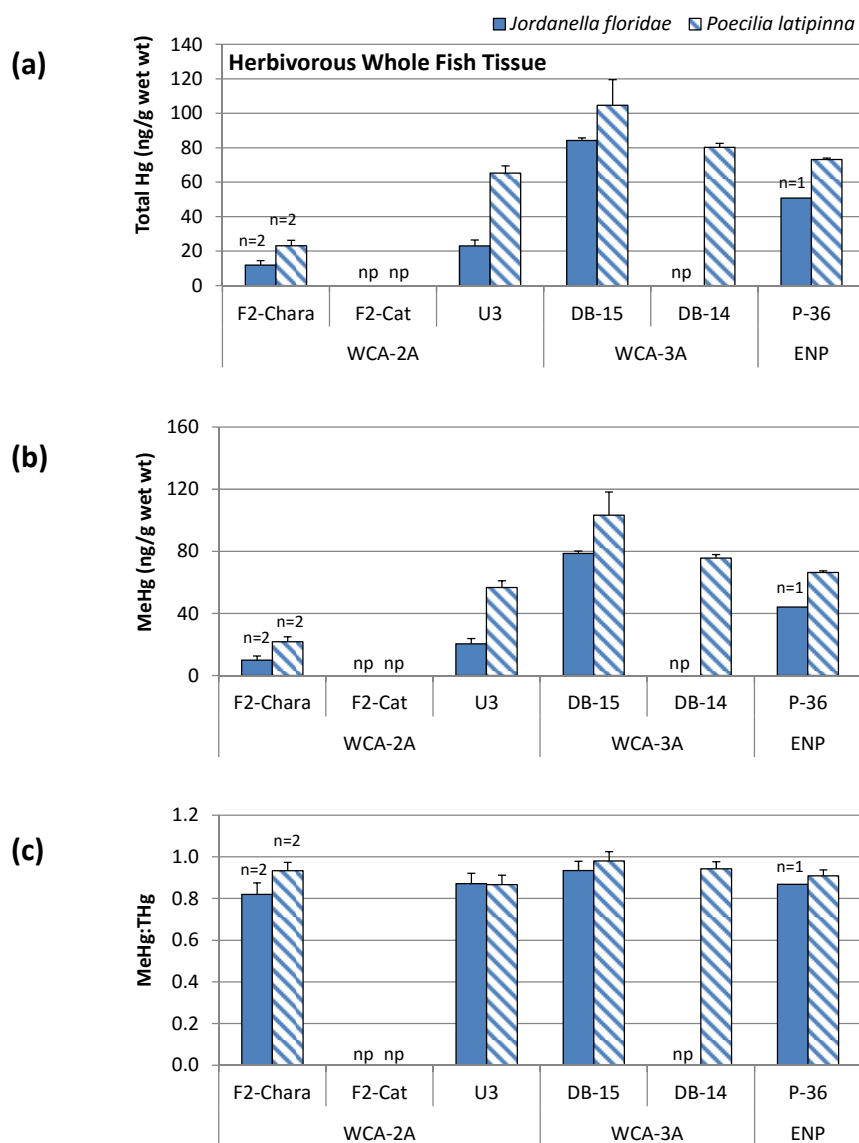


Figure 22. The mean (+1 S.E.) total mercury (Hg, panel a) and methylmercury (MeHg, panel b) concentrations, and the ratio of MeHg to THg (panel c), in *Jordanella floridae* (flagfish) and *Poecilia latipinna* (sailfin molly), among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3 unless otherwise noted. np=not present.

Periphyton

Epiphytic periphyton MeHg concentrations among the four sampled locations were comparable (2.89-3.81 ng/g dry wt) (Figure 23a). However, periphyton collected at U3 was higher in Ca (as calcium carbonate, CaCO_3) than at the two WCA-3A and the single ENP stations (Figure 23b), which lowered the total organic carbon (TOC) content in the periphyton at U3 (Figure 23c). Since MeHg is associated with the living and dead organic matter within the periphyton epiphytic “sleeves” (and not the CaCO_3 matrix), we normalized the MeHg concentration to the TOC content by dividing the measured MeHg concentration (Figure 23a) by the TOC concentration (Figure 23c) at each station to obtain the MeHg content based on the amount of organic matter present in the periphyton (Figure 23d). However, the normalization did not change the MeHg content in the periphyton among the sampling sites in a meaningful way, except for lowering the MeHg concentration at DB-14 relative to the three remaining stations.

The total nitrogen (TN) and total phosphorus (TP) concentrations in the U3 periphyton were also lower at the other three stations (Figure 24a and b), again because of the higher CaCO_3 content. Presenting TN and TP each as a ratio to the TOC concentration lowers the variability among stations towards a narrower range for each nutrient (Figure 24c and d), with $\text{U3} > \text{DB-15} > \text{P-36} > \text{DB-14}$.

Utricularia

The MeHg content in *Utricularia purpurea* was 3 to 8 times higher than the MeHg concentrations in periphyton at the corresponding location (cf. Figures 23a and 25a). While the overall CaCO_3 levels were lower in *U. purpurea* than in periphyton (cf. Figures 23b and 25b), there were nevertheless marked CaCO_3 differences in *U. purpurea* among locations (Figure 25b), which affected the TOC concentration (Figure 25c). As was performed for MeHg in periphyton, we normalized the MeHg concentrations in *U. purpurea* according to their TOC values (Figure 25d). The normalization increased the MeHg concentration at DB-15 relative to the non-normalized value (Figure 25a), making the normalized concentration comparable to those at DB-14 and P-36.

Besides containing higher MeHg concentrations than periphyton, *U. purpurea* also had higher N and P concentrations than periphyton (Figures 24a and b and 26a and b). While the TOC:TN ratio was higher in *U. purpurea* (14:1 to 20:1) than in periphyton (14:1 to 16:1) (cf. Figures 24c and 26c), the *U. purpurea* contained lower TOC:TP ratios (875:1 to 1020:1) than did periphyton (1180:1 to 1770:1) (cf. Figures 24d and 26d). The higher N content of within the periphyton may be attributable to nitrogen fixation (Inglett et al. 2004), while the P enrichment in *U. purpurea* may have been due to phosphatase activity within the bladders. Phosphatase activity has been measured in bladders of other *Utricularia* species outside of the Everglades (Sirová et al. 2003).

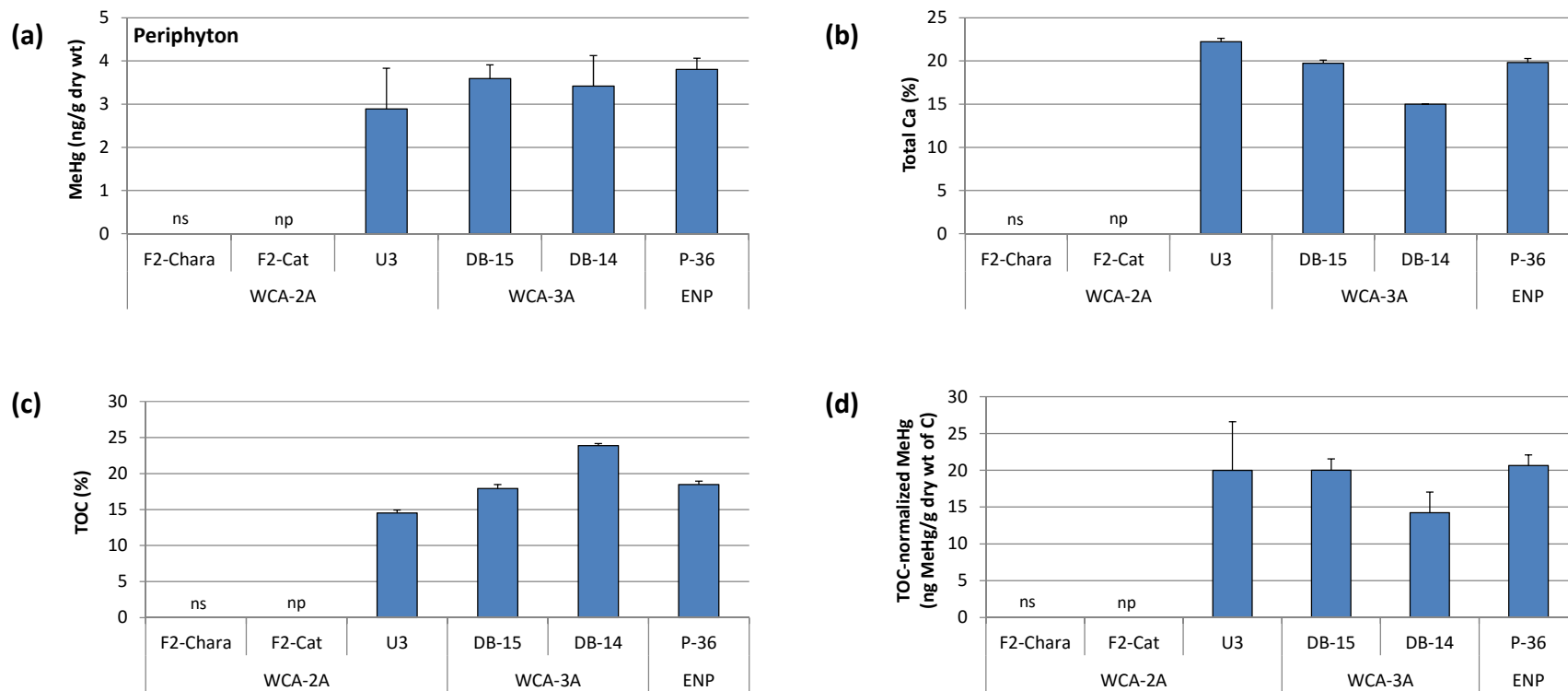


Figure 23. The mean (+1 S.E.) methylmercury (MeHg, panel a), total calcium (Ca, panel b), total organic carbon (TOC, panel c), and TOC-normalized MeHg (panel d) concentrations in periphyton among four sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled; np=not present.

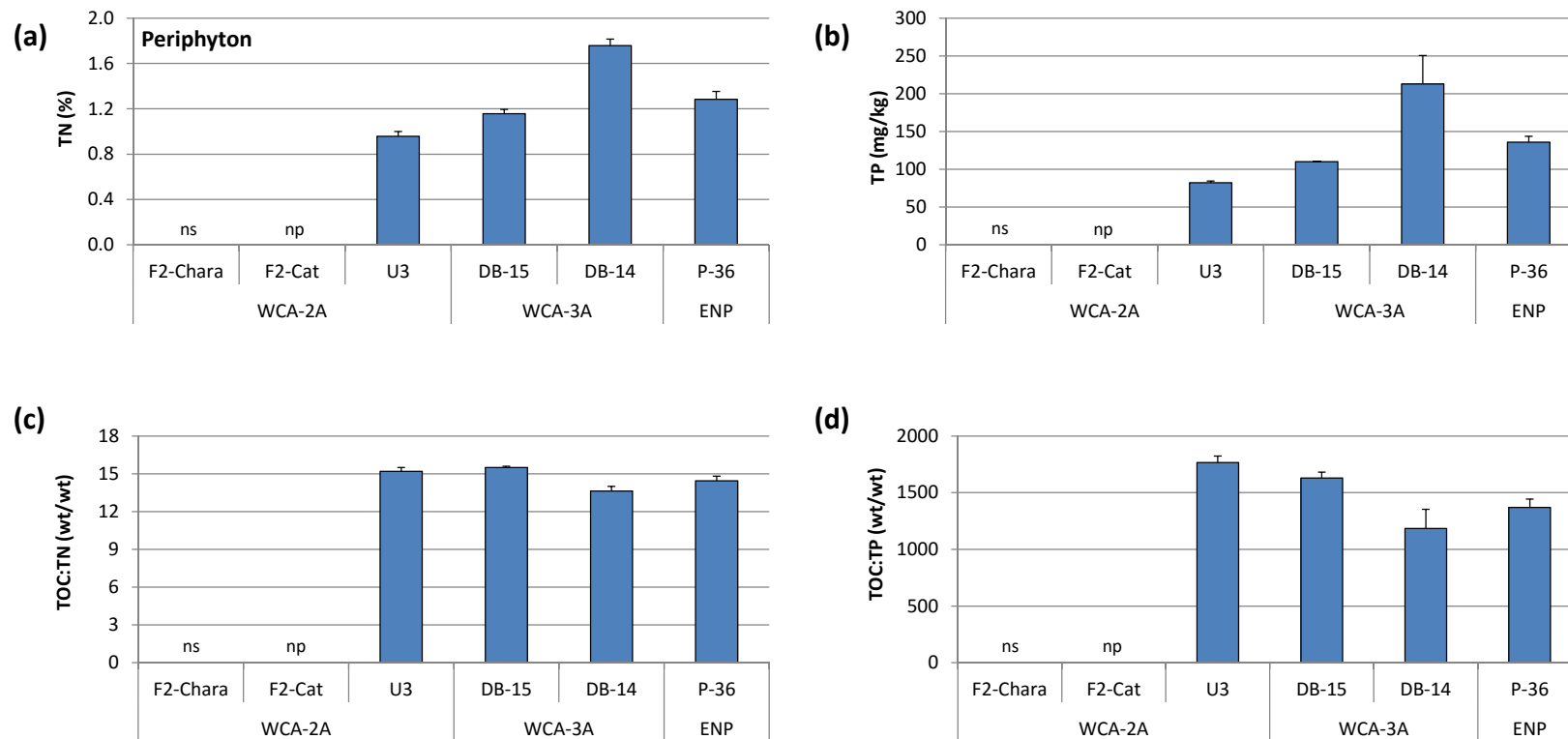


Figure 24. The mean (+1 S.E.) total nitrogen (TN, panel a) and total phosphorus (TP, panel b) concentrations, and the total organic carbon (TOC) to TN (panel c) and TOC to TP (panel d) ratios in periphyton among four sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled; np=not present.

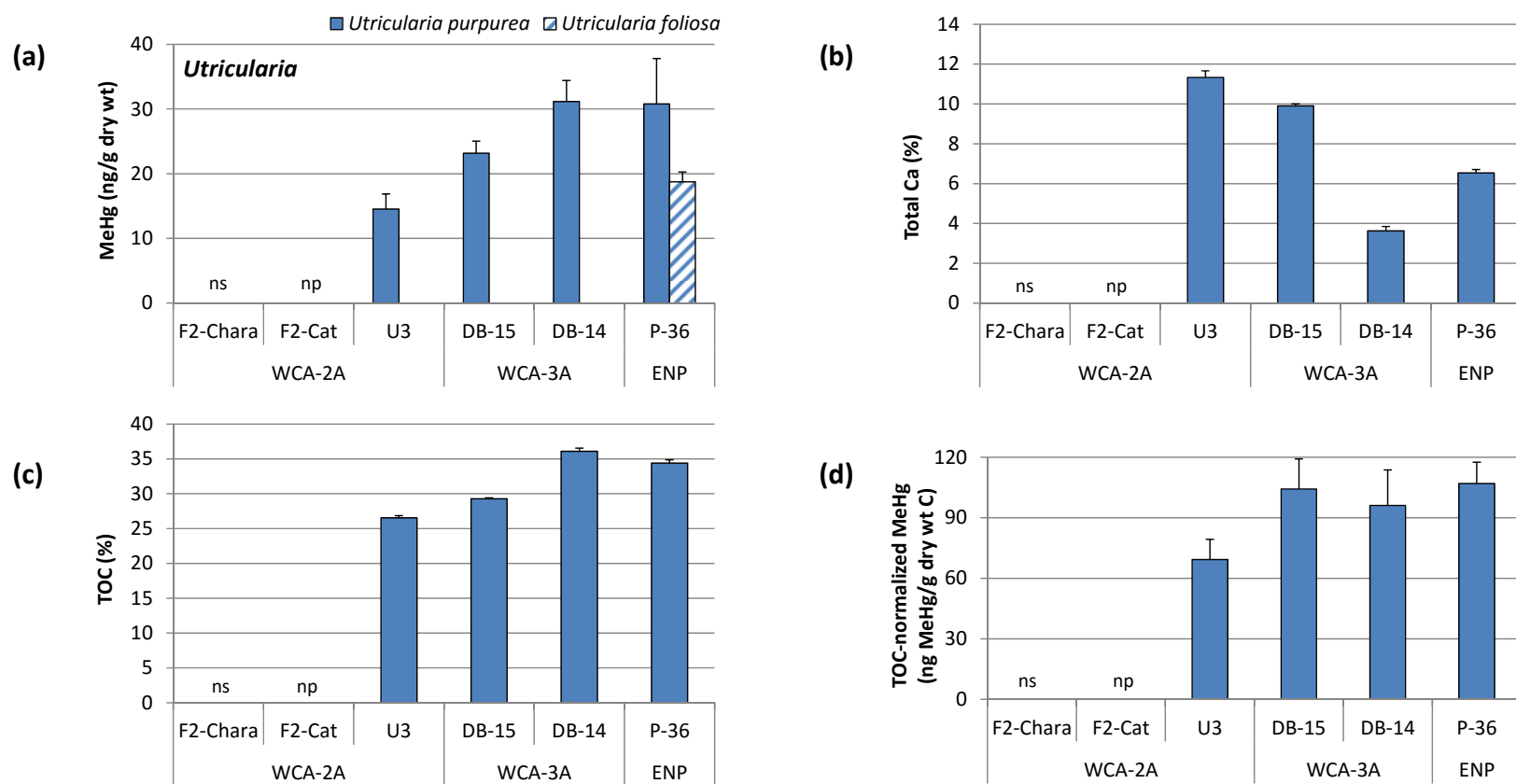


Figure 25. The mean (+1 S.E.) methylmercury (MeHg, panel a), total calcium (Ca, panel b), total organic carbon (TOC, panel c), and TOC-normalized MeHg (panel d) concentrations in *Utricularia* among four sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled; np=not present.

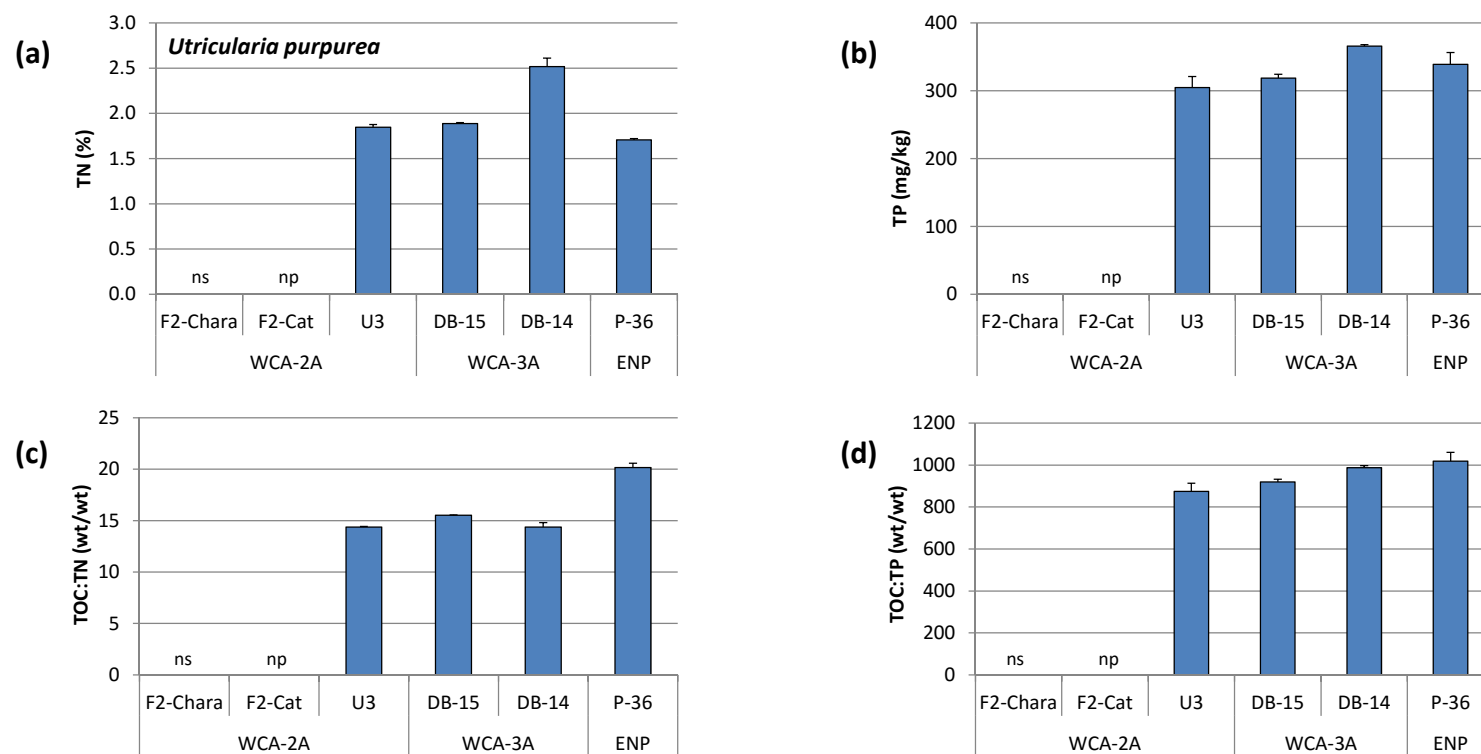


Figure 26. The mean (+1 S.E.) total nitrogen (TN, panel a) and total phosphorus (TP, panel b) concentrations, and the total organic carbon (TOC) to TN (panel c) and TOC to TP (panel d) ratios for *Utricularia purpurea* among four sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled; np=not present.

Detritus

The MeHg concentration in detritus (Figure 27a) exhibited a similar pattern as in periphyton (Figure 23a) and *U. purpurea* (Figure 25a): lower concentrations for WCA-2A than in WCA-3A and ENP. Although somewhat lower, the absolute MeHg detrital concentrations were more closely aligned with those analyzed in the periphyton than *U. purpurea* (cf. Figures 23a, 25a, and 27a).

The Ca content of the detritus varied widely, with low values of ~ 2% at F2-Cat and DB-14, to an elevated value of 24% at U3 (Figure 27b). The pattern among locations was similar to the Ca concentrations measured in periphyton (Figure 23b) and *U. purpurea* (Figure 25b). As a consequence, the TOC concentrations in detritus (Figure 27c) followed the same relative rankings as in periphyton (Figure 23c) and *U. purpurea* (Figure 25c): DB-14 > P-36 ≈ DB-15 > U3.

Detrital TN was highest at DB-14 (Figure 28a), whereas the TP concentration was highest at F2-Cat (Figure 28b) due to the history of P-enrichment. As found for periphyton (Figure 24a and b) and *U. purpurea* (Figure 26a and b), TN and TP concentrations in detritus at DB-14 were highest while the lowest concentrations were usually found at U3. The TOC:TN ratio in the detritus was approximately 9:1 among stations, excluding the nutrient enriched F2-Cat (Figure 28c). Detritus from sites P-36 and DB-14 contained approximately 1100 times more TOC than P on a mass basis, whereas the ratios at DB-15 and U3 were ≈ 853:1 to 925:1 (Figure 28d). Again, F2-Cat was excluded from the comparison. The overall TOC:TP ratio for detritus at U3, DB-15, DB-14, and P-36 (853:1 to 1120:1) was similar to the range for *U. purpurea* (875:1 to 1020:1) among the same sites.

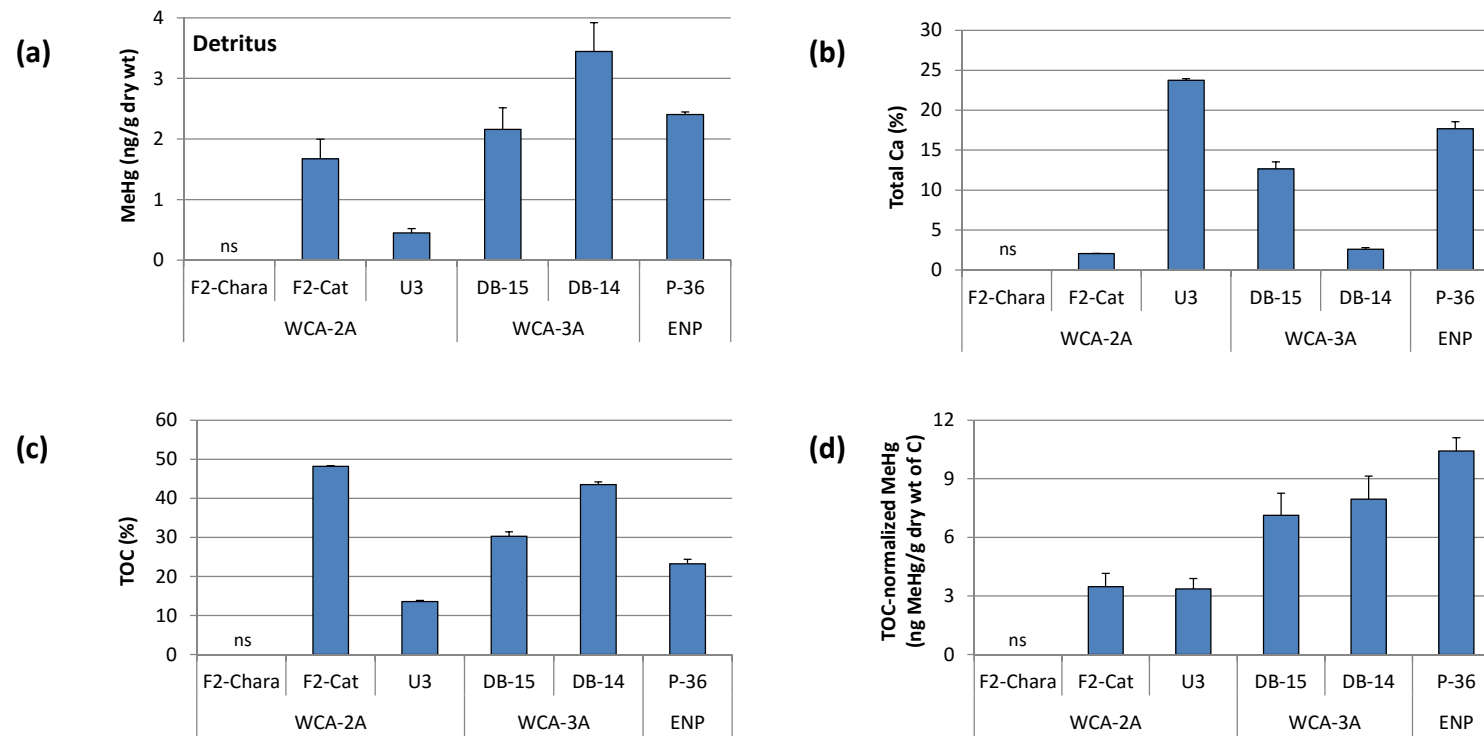


Figure 27. The mean (+1 S.E.) methylmercury (MeHg, panel a), total calcium (Ca, panel b), total organic carbon (TOC, panel c), and TOC-normalized MeHg (panel d) concentrations in detritus among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled.

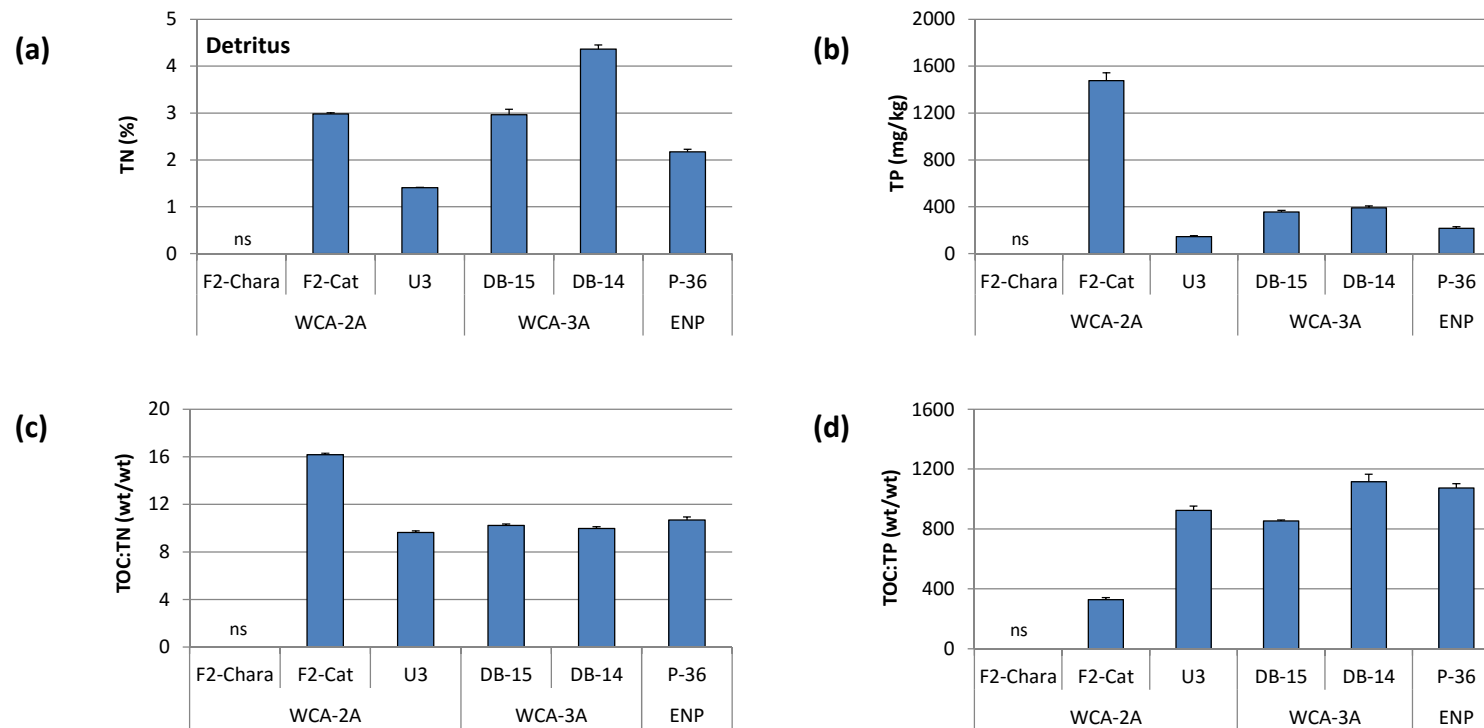


Figure 28. The mean (+1 S.E.) total nitrogen (TN, panel a) and total phosphorus (TP, panel b) concentrations, and the total organic carbon (TOC) to TN (panel c) and TOC to TP (panel d) ratios for detritus among five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3. ns=not sampled.

Stable Isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) Analyses of Food Webs and Trophic Levels

Despite the consistent and distinct differences in water, periphyton and soil chemistry between the Everglades regions, and even at sites within regions, none of these clearly explains the spatial differences in Hg contamination of fish. An alternate hypothesis is needed. We conjecture that the length and nature of food webs supporting *G. holbrooki* and other higher consumers varies across the Everglades, and may contribute to variation in Hg levels. Several investigators have studied the distribution, diet and trophic position of invertebrates and mosquitofish in the Everglades (e.g., Loftus 2000; King 2001; Williams and Trexler 2006; King and Richardson 2008; Hagerthey et al. 2014). Analysis of DB Environmental mosquitofish stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) data (Years Two and Three) with respect to these previous findings has shown interesting spatial and temporal trends. However, the utility and interpretation of stable isotope data collected in Years Three and Four for determining carbon flows and food web links required further exploration.

The $\delta^{13}\text{C}$ values for the invertebrates at F2-Cat, DB-15, DB-14, and P-36 ranged within -29.5‰ and -32.8‰ (Fig. A panel a), and point to detritus (at F2-Cat), *U. purpurea* and detritus (at DB-15 and DB-14), and *U. purpurea* (at P-36) as likely invertebrate food sources (Figure 29 panel b). The three major invertebrates and fish $\delta^{13}\text{C}$ signatures at U3 were considerably more enriched than at any of the other five locations (Figure 29 panel a), which suggests that *Utricularia purpurea* (bladderwort), *Eleocharis cellulosa* (spikerush), and *Cladium jamaicense* (sawgrass) were the primary food items for the invertebrates and fish at U3 (Figure 29 panel b).

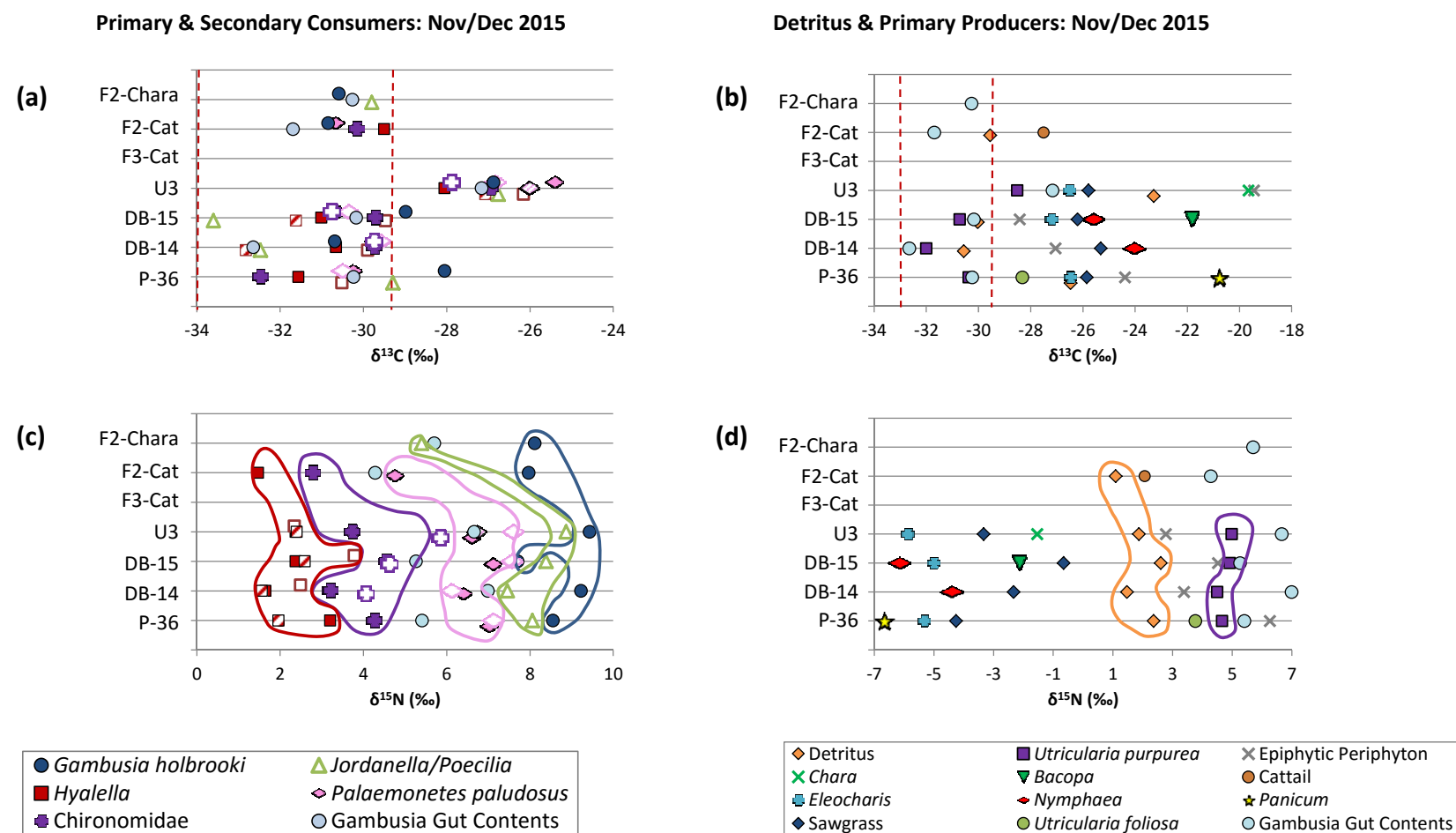


Figure 29. The isotopic composition of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in primary and secondary consumers (panels a and c) and detritus and primary producers (panels b and d) among six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in Nov/Dec 2015. The polygons circumscribe individual taxa measured across each location, and show how each taxon groups separately from other taxa. Invertebrates were collected from detritus in the slough (solid symbols) and in the ridge (hatched symbols), and within periphyton (open symbols) where applicable.

Whereas *Jordanella floridae* (flagfish) and *Poecilia latipinna* (sailfin molly) are considered to be herbivores (Table 1), *Gambusia holbrooki* (eastern mosquitofish) is an omnivore (Table 1). Gut analysis of *G. holbrooki* at sampling locations in WCA-2A have validated a diet consisting of plants, detritus, and invertebrates, with detritus occurring more frequently than primary producers at F2-Cat than F2-Chara or U3 (DBE 2014). This is consistent with the nearly exclusive basal food source of cattail litter at F2-Cat. At some locations (F2-Chara and U3 in WCA-2A), the $\delta^{13}\text{C}$ ratios of the herbivorous fish and *G. holbrooki* were closely aligned, whereas in locations within WCA-3A and ENP (DB-15, DB-14, and P-36) the herbivorous fish values were considerably less than the $\delta^{13}\text{C}$ for *G. holbrooki* (Figure 29 panel a), suggesting different food web pathways. Focusing on the detritus and primary producer food sources (Figure 29 panel b) for the herbivorous fish, *U. purpurea* and *U. foliosa* (P-36 only) $\delta^{13}\text{C}$ signatures most closely corresponded with those of the herbivorous fish at WCA-3A and ENP locations (Figure 29 panels a and b). According to $\delta^{13}\text{C}$ ratios, the herbivorous fish at U3 are more likely feeding on *Eleocharis* (spikerush) and *Cladium jamaicense* (sawgrass) plant fragments (Figure 29 panels a and b).

Periphyton did not appear to be a source of food for either the herbivorous fish or *G. holbrooki* with the exception of *G. holbrooki* at DB-15. Yet periphyton could still be a source of food for the fish if they are selectively grazing on different algal constituents (diatoms and filamentous green and cyanobacteria) of the periphyton community. Whereas we measured the stable isotopes on the whole periphyton biocenosis, each of the algal components would have its own unique $\delta^{13}\text{C}$ signature. Future work should gather information on the $\delta^{13}\text{C}$ signatures of the algal components of the periphyton community.

The $\delta^{13}\text{C}$ ratios for *G. holbrooki* varied considerably depending on location (Figure 29 panel a), indicating the feeding behavior of the fish to be habitat-specific. The highest $\delta^{13}\text{C}$ value was -26.9‰ for *G. holbrooki* at U3, whereas the fish at F2-Chara, F2-Cat, and DB-14 contained the lowest $\delta^{13}\text{C}$ contents ranging from -30.6‰ to -30.8‰. Considering that the $\delta^{13}\text{C}$ values at DB-15 and P-36 were higher than those for any of the invertebrate groups (Figure 29 panel a), the *G. holbrooki* at those locations are likely feeding less on invertebrates and more on a combination of plants and detritus, which exhibited a greater range of $\delta^{13}\text{C}$ content than did the invertebrates (cf. Figure 29 panels a and b). Note that at F2-Cat, where there is a near absence of SAV, the detritus (primarily derived from *Typha* (cattail)) $\delta^{13}\text{C}$ value is closely aligned to those of *G. holbrooki* and the invertebrates at that station. This finding is consistent with the gut taxonomic analysis of *G. holbrooki* at F2-Cat (DBE 2014).

The station that displayed the most deviation in $\delta^{13}\text{C}$ among all the measured animal populations was U3 (Figure 29 panel a), where $\delta^{13}\text{C}$ concentrations ranged from -25.4‰ to -27.9‰ and where invertebrate values are in close proximity to *G. holbrooki* gut and tissue $\delta^{13}\text{C}$ concentrations. This could indicate that *G. holbrooki* is consuming more invertebrates at U3 than at DB-15 or P-36, where there is a significant gap between the *G. holbrooki* and invertebrate $\delta^{13}\text{C}$ ratios (Figure 29 panel a).

Table 1. The feeding behavior of *Jordanella floridae*, *Poecilia latipinna*, and *Gambusia holbrooki* as reported in several literature sources.

Literature Source	Location	Feeding Behavior		
		<i>Jordanella floridae</i> (flagfish)	<i>Poecilia latipinna</i> (sailfin molly)	<i>Gambusia holbrooki</i> (eastern mosquitofish)
Browder et al. 1994	Everglades	Herbivore* (primarily algae and plant fragments)	Herbivore* (primarily algae and plant fragments)	Omnivore* (algae & invertebrates)
Hill & Cichra 2005	Florida	Omnivore (algae, plant material, detritus, zooplankton, and insects)		
Loftus and Kushlan 1987	southern Everglades; open marshes	bottom material (sediment)	algae-grazers and bottom material	
Loftus 2000	Everglades	Herbivore*	Herbivore*	Omnivore* (algae and animal prey)
Pyke 2005	unspecified			Insects, spiders, crustaceans, worms, molluscs, aquatic invertebrates larvae and pupae, algae and other plant material, small fish and diatoms*
Rawlik et al. 2002	Everglades		Herbivore	Omnivore
Smith and Trexler 2004	Everglades		Herbivore	Omnivore (primarily invertebrates in wet season, algae in the dry)

*via stomach content analysis

The $\delta^{15}\text{N}$ values for each of the primary (invertebrates and herbivorous fish (*J. floridae* and *P. latipinna*) and the secondary (*G. holbrooki*) consumer groups were distinct (Figure 29 panel c). This indicates increasing trophic position for *Hyalella* > Chironomidae > *Palaemonetes paludosus* > herbivorous fish > *G. holbrooki*.

Although not as distinctly separated, the $\delta^{15}\text{N}$ values for detritus and primary producer categories were also segregated (Figure 29 panel d). Detritus (1.09-2.60‰) and *U. purpurea* (4.50-4.98‰) values were each very similar among the stations, whereas epiphytic periphyton and *C. jamaicense* (sawgrass) signatures were more diffuse. Note that *U. purpurea*, a carnivorous plant, contained the most enriched $\delta^{15}\text{N}$ ratio at each station where it was found, except at P-36 where epiphytic periphyton was higher (Figure 29 panel d).

Hyalella $\delta^{15}\text{N}$ values were the lowest of any invertebrate or fish among the sampling sites (Figure 29 panel c), and were confined to a narrow range (1.47-3.78‰). However, their $\delta^{13}\text{C}$ values ranged from -32.8‰ at DB-14 to -26.2‰ at U3 (Figure 29 panel a). Since $\delta^{13}\text{C}$ enrichment is typically conserved across trophic strata, it can indicate the origin of the food resource (Post 2002). Thus, the similar $\delta^{13}\text{C}$ values observed for *Hyalella*, detritus and *U. purpurea* suggest the latter as likely food sources for the former (Figure 29 panel b).

Although the $\delta^{13}\text{C}$ data for herbivorous fish and *G. holbrooki* indicated that *U. purpurea* and detritus were more likely food sources than invertebrates at DB-15 and P-36, these two basal food sources could also have contributed to the fish diet at the remaining stations according to the $\delta^{13}\text{C}$ signatures (Figure 29 panels a and b). Indeed, if a combination of *U. purpurea* and detritus comprised the main C sources of herbivorous fish and *G. holbrooki* at all locations excluding F2-Chara, then the $\delta^{15}\text{N}$ signatures for both fish groups (7.97-9.43‰ for *G. holbrooki* and 7.46-8.87‰ for herbivorous fish) would comply with the $\delta^{15}\text{N}$ values of *U. purpurea* and detritus after the theoretical 3.4‰ per trophic level increase in $\delta^{15}\text{N}$ (Cabana and Rasmussen 1994; 1996; Vander Zanden and Rasmussen 1999). The implication is that if *U. purpurea* and detritus are principal C sources for food chains supporting these fish, then the variation in the MeHg measured in *U. purpurea* and detritus within each station could be an explanation for the differences in the fish MeHg concentrations across the sites.

Looking back to Year 3 and the $\delta^{15}\text{N}$ stable isotope compositions of detritus, primary producers, and primary and secondary consumers covering Jan/Feb 2015, May 2015, and July 2015, there is a constancy in the isotopic values within each plant and animal taxon throughout four sampling periods, including Nov/Dec 2015 in Year 4 (cf. Figures 29, 30, 31, 32 panels c and d). This consistency suggests that the $\delta^{15}\text{N}$ contents of primary and secondary consumers, and their food sources, are not only independent of season, but also somewhat independent of location. It also lends credence to using the $\delta^{15}\text{N}$ isotope as a means for comparing modeled *G. holbrooki* trophic position (TRPO) through time.

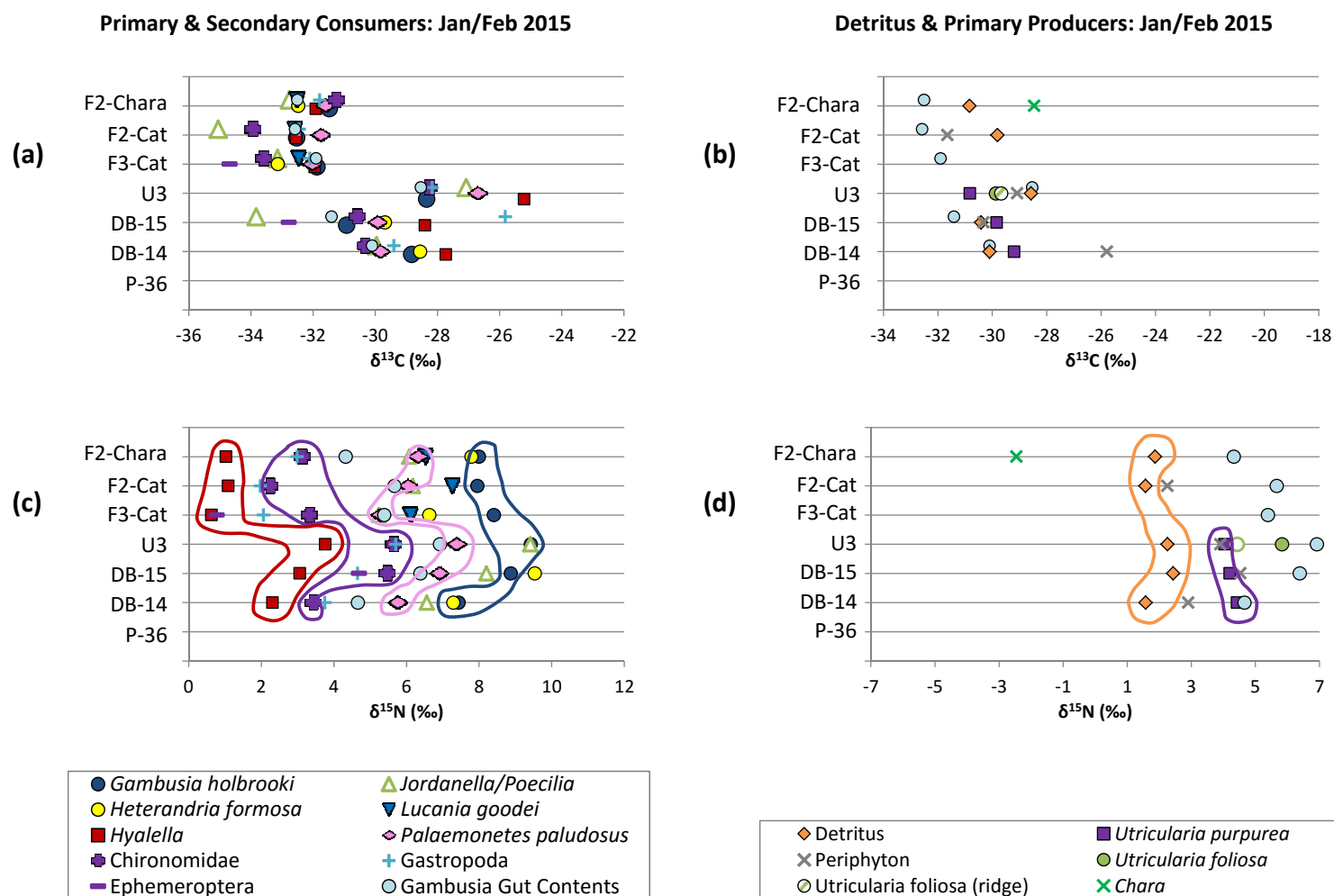


Figure 30. The isotopic composition of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in primary and secondary consumers (panels a and c) and detritus and primary producers (panels b and d) among six locations throughout the Water Conservation Areas (WCA) 2A and 3A in Jan/Feb 2015. The polygons circumscribe individual taxa measured across each location, and show how each taxon groups separately from other taxa. Invertebrates were collected from either floc, periphyton, or macrophytes depending on the station.

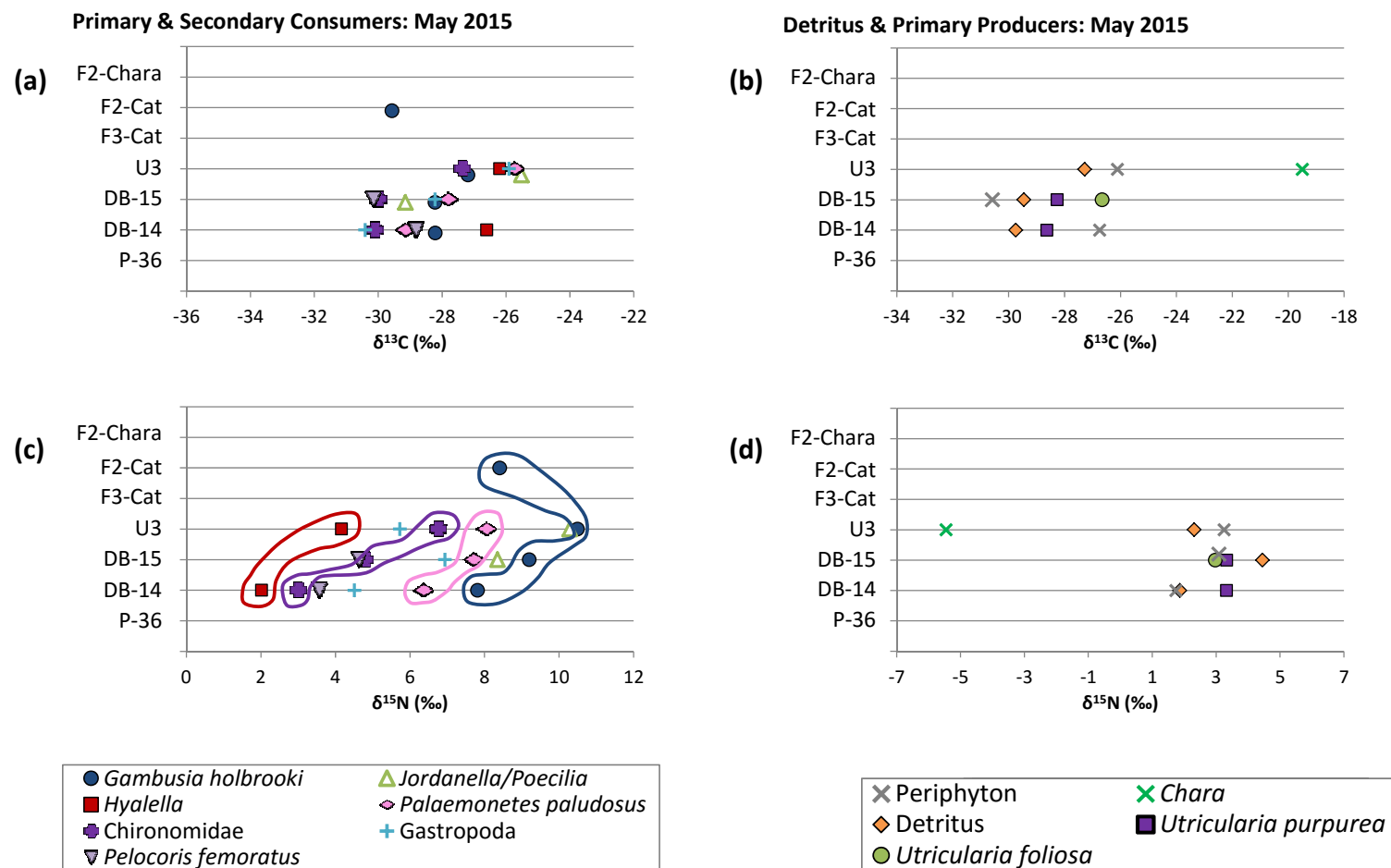


Figure 31. The isotopic composition of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in primary and secondary consumers (panels a and c) and detritus and primary producers (panels b and d) among four locations throughout the Water Conservation Areas (WCA) 2A and 3A in May 2015. The polygons circumscribe individual taxa measured across each location, and show how each taxon groups separately from other taxa. Invertebrates were collected from either floc, periphyton, or macrophytes depending on the station.

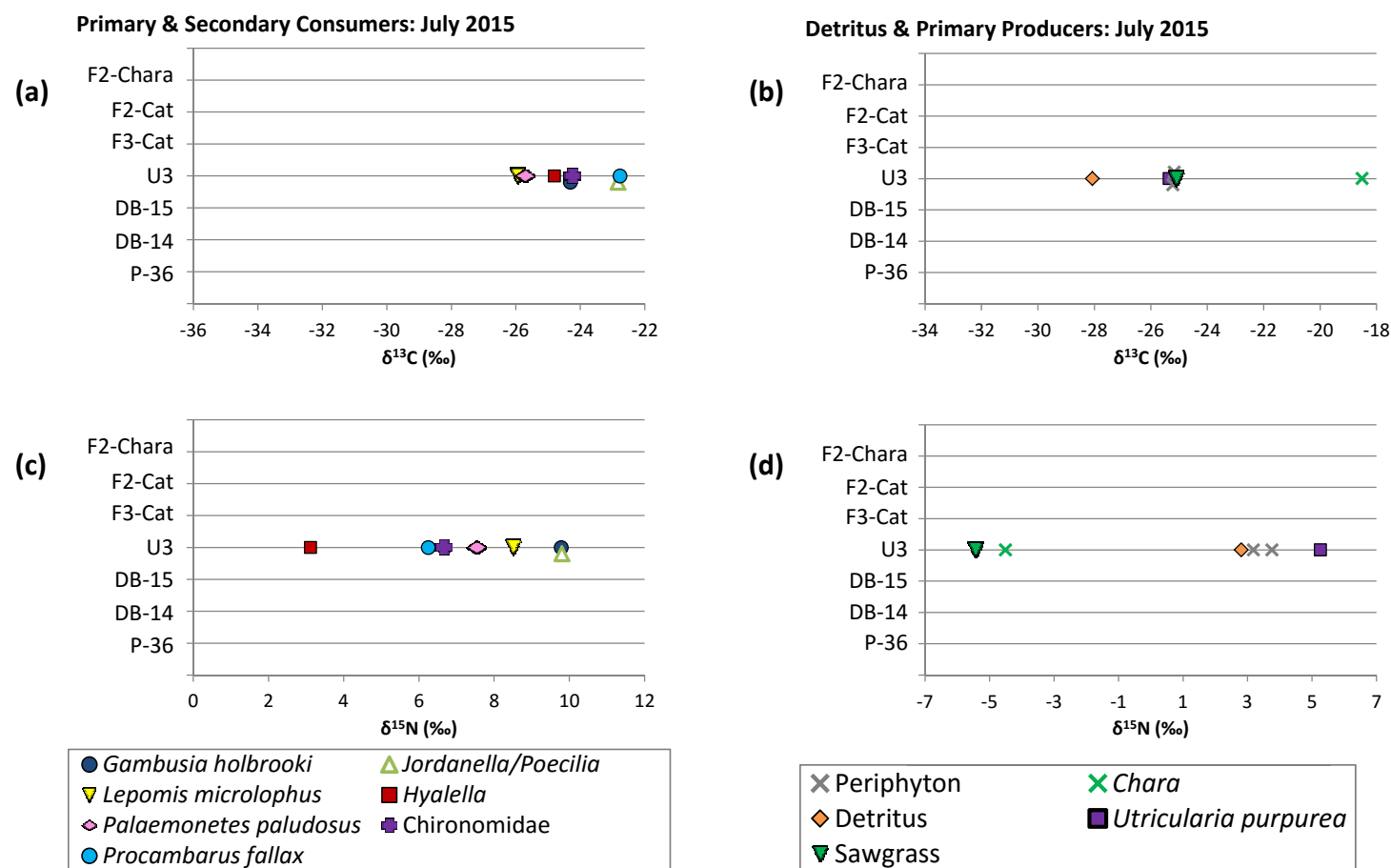


Figure 32. The isotopic composition of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in primary and secondary consumers (panels a and c) and detritus and primary producers (panels b and d) at U3 in Water Conservation Areas (WCA) 2A in July 2015. Detritus samples were fumigated, except for July where detritus samples were acid-washed, prior to analysis for stable isotopes. Periphyton samples were fumigated prior to analysis. Invertebrates were collected from either floc or periphyton.

Index of Detritivory (I_D), Trophic Position (TRPO), and Food Chain Length (FCL) for *G. holbrooki*

The Index of Detritivory, I_D, of a secondary consumer such as *G. holbrooki*, relies on the δ¹³C values of an algal-based and a detritus-based consumer (Williams and Trexler 2006). The I_D is used in the determination of TRPO to segregate how much of a secondary consumer's diet originates as algal-based vs. detritus-based food chains.

One of our working hypotheses is that *G. holbrooki* occupy higher trophic positions (TRPO) at some sites than others in the Everglades; consequently, the longer food chain lengths (FCLs) associated with the higher TRPOs will result in greater Hg biomagnification rates. We employed two different approaches to determining the I_D, TRPO, and FCL for *G. holbrooki* among sampled locations.

The first was by determining the Index of Detritivory (I_D) and trophic position (TRPO) of *G. holbrooki* at each location using the δ¹³C and δ¹⁵N values for primary and secondary consumers (including *G. holbrooki*) in a two end-member mixing model (Williams and Trexler 2006; Post 2002).

$$I_D^{2ndcons} = \frac{\delta^{13}C^{2ndcons} - \delta^{13}C^{algalbasal}}{\delta^{13}C^{detrbasal} - \delta^{13}C^{algalbasal}}$$

where:

$I_D^{2ndcons}$ = Index of detritivory for a secondary consumer
(i.e., *Gambusia holbrooki*)

$\delta^{13}C^{2ndcons}$ = δ¹³C value for the secondary consumer

$\delta^{13}C^{algalbasal}$ = δ¹³C value for the algal-based consumer
(i.e., herbivorous fish)

$\delta^{13}C^{detrbasal}$ = δ¹³C value for the detrital-based consumer (i.e., *Hyaella* spp.)

The I_D ranges from 0 (100% of C originated from primary producers) to 1 (100% of C originated from detritus).

TRPO^{2ndcons} =

$$2 + \frac{\{\delta^{15}\text{N}^{2ndcons} - [(\delta^{15}\text{N}^{detrbasal} \times I_D^{2ndcons}) + (\delta^{15}\text{N}^{algalbasal} \times (1 - I_D^{2ndcons}))]\}}{3.4}$$

where:

TRPO^{2ndcons} = Trophic position of a secondary consumer
(i.e., *Gambusia holbrooki*)

$\delta^{15}\text{N}^{2ndcons}$ = $\delta^{15}\text{N}$ value for the secondary consumer

$\delta^{15}\text{N}^{algalbasal}$ = $\delta^{15}\text{N}$ value for the algal-based consumer
(i.e., herbivorous fish)

$\delta^{15}\text{N}^{detrbasal}$ = $\delta^{15}\text{N}$ value for the detrital-based consumer (i.e., *Hyaella* spp.)

2 = reflects the assumed trophic position of the baseline consumer

3.4 = is the enrichment in $\delta^{15}\text{N}$ per trophic level

The model does have some built-in assumptions:

1. there are only two carbon (C) sources supporting the *Gambusia* food web; and
2. the basal consumers are fairly strict consumers of their respective food (C) items (i.e., not mixing C sources)

Gambusia spp. are omnivores, feeding on a wide variety of plants, detritus, invertebrates, and even small fish (Table 1). Depending on food abundance and source, *Gambusia* can alter the amounts and types of items consumed in environments where more than two C sources underpin the food web, in violation of assumption #2 above. A multitude of factors dictate the abundance of food items for *Gambusia*, including biological (food web structure), seasonal (light, temperature), and hydrologic (hydroperiod, water depth, days since recession) variables (Loftus 2000).

The second approach involved using only the $\delta^{15}\text{N}$ data collected among trophic levels at a location. (Ouédraogo et al. 2015). Consumers become enriched in ^{15}N relative to their food by ~ 3.4‰ (Cabana and Rasmussen 1994; 1996; Vander Zanden and Rasmussen 1999). As a consequence, sequential consumers become enriched with ^{15}N in a stepwise manner, which can serve as a time-integrated indicator of trophic position. However intra-system variation in $\delta^{15}\text{N}$ values characterizing organisms at the base of the food web can pose a significant problem (Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1999). The $\delta^{15}\text{N}$ of primary

producers and primary consumers from different habitats within the Everglades varies (Figures 29 – 32), such that the $\delta^{15}\text{N}$ of any one primary consumer differs from the same or other primary consumers. Thus, an absolute measure of trophic position requires that the $\delta^{15}\text{N}$ of consumers be interpreted relative to an appropriate baseline $\delta^{15}\text{N}$ value (Vander Zanden and Rasmussen 1999).

In this method, FCLs and TRPO are calculated using mean $\delta^{15}\text{N}$ of all primary consumer taxa collected as the site-specific baseline. The assumption is that organisms represented by higher trophic levels, such as the omnivorous *G. holbrooki*, feed equally on the primary consumers.

$$\delta^{15}\text{N}_{\text{adj}} = \delta^{15}\text{N}_{\text{Gambusia}} - \delta^{15}\text{N}_{\text{Primary Consumers}}$$

$$\text{TRPO} = \lambda + \delta^{15}\text{N}_{\text{adj}} / \Delta_n$$

$\lambda = 2$ (trophic position for a basal consumer)

$\Delta_{n=3.4}$ (the enrichment in $\delta^{15}\text{N}$ per trophic level)

$$\text{FCL} = S^{15}\text{N}_{\text{Gambusia}} - S^{15}\text{N}_{\text{baseline}} / (\Delta_n - \lambda)$$

There are sources of error in modeling I_D and TRPO that were addressed by us:

1. *Isotopic discrimination of ^{13}C between trophic levels.* Although the ^{13}C isotopic discrimination between trophic levels is usually considered to be negligible, there nevertheless can be significant differences between carnivores and herbivores in $\delta^{13}\text{C}$ composition (Vander Zanden and Rasmussen 2001). Thus ^{13}C may be an imperfect tracer of C sources.

2. *The constancy of the $\Delta^{15}\text{N}$ increment of 3.4‰ between consumer trophic levels.* Although a $\Delta^{15}\text{N}$ of 3.4‰ is generally used for the ^{15}N isotopic fractionation between trophic levels (Havens et al. 2003), there is variability bias depending on whether the animal is a herbivore (lower $\Delta^{15}\text{N}$ increment) or carnivore (higher $\Delta^{15}\text{N}$ increment) (Vander Zanden and Rasmussen 2001). This can lead to an error in the TRPO, but is considered to be minor provided that primary consumers were used as baseline indicators (Vander Zanden and Rasmussen 2001). Since *G. holbrooki* is an omnivore, there is uncertainty about the appropriate $\Delta^{15}\text{N}$ increment to use in the modeling equations for TRPO. Moreover, the $\delta^{15}\text{N}$ of the basal food sources for herbivores or omnivores can vary widely, which adds further uncertainty.

Fortunately, the severity of the errors and the limitations of the models are known, and thus corrective actions can be taken to limit the errors and implied assumptions:

i. Since detritus- and primary producer-based pathways are the two main basal resources available to *G. holbrooki* and their prey, the goal is to select a strict detritivore and a strict algavore to serve as end members representing each C source in the Mixing Model. We have devoted much attention in establishing the appropriate end members, such that their $\delta^{13}\text{C}$ values are sufficiently distinct (Vander Zanden and Rasmussen 2001), in calculating the I_D and TRPO for *G. holbrooki* in the two-end member mixing model. Usage of this model in the Everglades was initially based on *Hyaella* and Seminole ramshorn snail (*Planorbella duryi*) as the two-end member primary consumers representing a detritivore and algavore, respectively (Williams and Trexler 2006). We initially (Jan/Feb 2015) used *Hyaella* and a variety of snails as the two end members in the mixing model, but found that snails were unsatisfactory. First, the Seminole ramshorn snail was not present at our sampling stations in sufficient quantities. Second, an attempt to substitute the ramshorn snail with a variety of snails was unsuccessful in that the $\delta^{13}\text{C}$ isotope ratios among different species were widely distributed, making it difficult for us to select any one of them as being solely an algavore. Instead of snails, we determined that algae- or plant-consuming fish would be an appropriate surrogate for the algavore end member; we retained *Hyaella* as the detritivore end member due to its ubiquity, numbers, literature references, and prior use in the Everglades as the detritivore end member in mixing models.

ii. Using the primary consumers, rather than the primary producers, as the baseline trophic level of $\delta^{15}\text{N}$ limits the error in the $\Delta^{15}\text{N}$ -based estimates of TRPO (Vander Zanden and Rasmussen 2001). All of the estimates in TRPO used in this report relied on the $\delta^{15}\text{N}$ of primary consumers.

To convince ourselves that the herbaceous fish, *Poecilia latipinna* (sailfin molly) and *Jordanella floridae* (flagfish), were indeed solely (or mostly) herbivores, we undertook a literature search on their feeding habits that included gut contents and observations on feeding behavior. The results of the literature search (Table 1) indicated that *P. latipinna* and *J. floridae* were primarily herbivorous, although they can occasionally resort to detritivory (Loftus 2000).

We also compared the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios between *P. latipinna* and *J. floridae* within each location to evaluate the homogeneity of both species with respect to diet (represented by $\delta^{13}\text{C}$) and trophic level (represented by $\delta^{15}\text{N}$) (Figure 33). Comparison of the closed symbols representing *J. floridae* with the open symbols representing *P. latipinna* for a given date and location revealed very minor differences between the two herbivorous fish species with respect to diet ($\delta^{13}\text{C}$) or trophic position ($\delta^{15}\text{N}$).

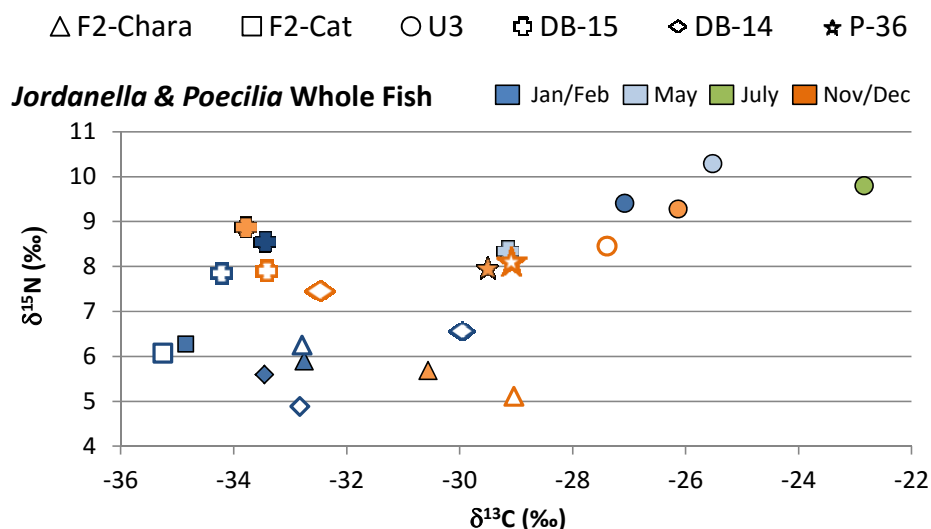


Figure 33. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios for *J. floridae* (closed symbols) and *P. latipinna* (open symbols) whole fish at selected locations within the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015. N=1-3. Note that both species were not always present at a location on a sampling date.

Index of Detritivory (I_D)

The range of values for I_D was from 1.0 (indicating an exclusive detrital food base) to 0.0 (indicative of a primary producer basal food source) (Figure 34). The *Gambusia* I_D for Jan/Feb at the cattail- and *Chara*-dominated F stations in WCA-2A indicated the fish were feeding on prey items based on a detritus food source. *Gambusia* from U3 varied from a diet of primary producer-based prey in Jan/Feb to one that was detritus-based in May, with mixtures of detritus- and primary producer-based prey or food items in July and Nov/Dec. Carbon sources contributing to the diets of fish retrieved from DB-15 and DB-14 in WCA-3A ranged from mixed (Jan/Feb) to completely detrital food sources in Nov/Dec. Lastly, the *G. holbrooki* at P-36 were feeding exclusively on prey items consuming primary producers, or the consuming the primary producers directly (or a combination of both).

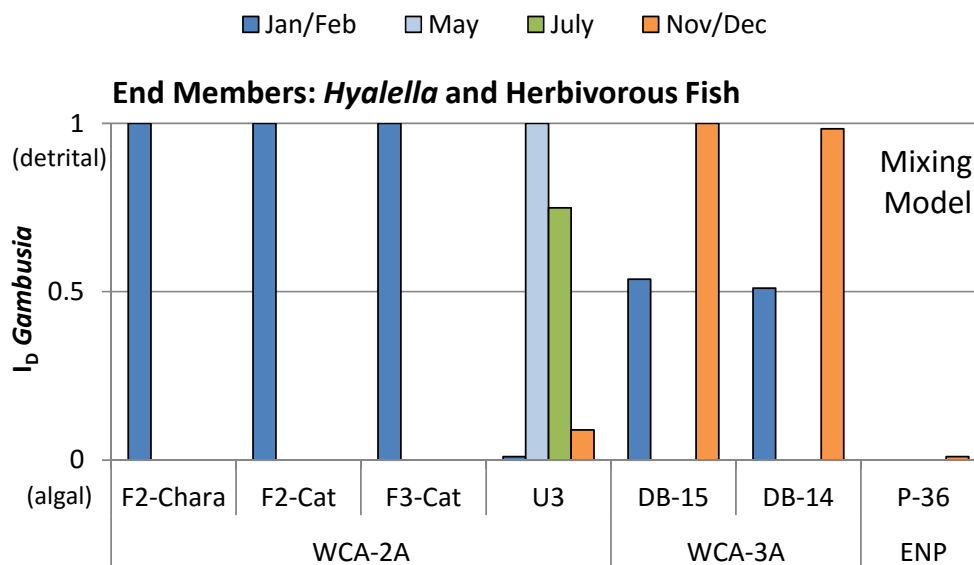


Figure 34. The mean Index of Detritivory (I_D) for *Gambusia* at seven locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015. The herbivorous fish were *Poecilia latipinna* (sailfin molly) and *Jordanella floridae* (flagfish).

Trophic Position (TRPO)

Regardless of which model (Mixing or $\delta^{15}\text{N}$ Normalization), our analyses suggest that the trophic positions of the *Gambusia* at the F sites in WCA-2A were higher (levels 3-4) than at U3, WCA-3A, and ENP sites (levels 2-3) (Figure 35 panels a, b, and c). Depending on which model (Mixing or $\delta^{15}\text{N}$ Normalization) was employed, there were differences between trophic levels within stations in WCA-2A and ENP, but minimal differences existed between the two model outputs for *G. holbrooki* at DB-15 and DB-14. The TRPO for *G. holbrooki* was lower by 0.5 and 1.0 trophic levels at the F stations in WCA-2A, and P-36 in ENP was higher by a full trophic level, using the $\delta^{15}\text{N}$ Normalization model compared to the end member mixing model. *G. holbrooki* captured at U3 displayed the most variability between the two models, varying from +1 trophic level higher to -1 trophic level lower during the four sampling dates (Figure 35 panels a and b)

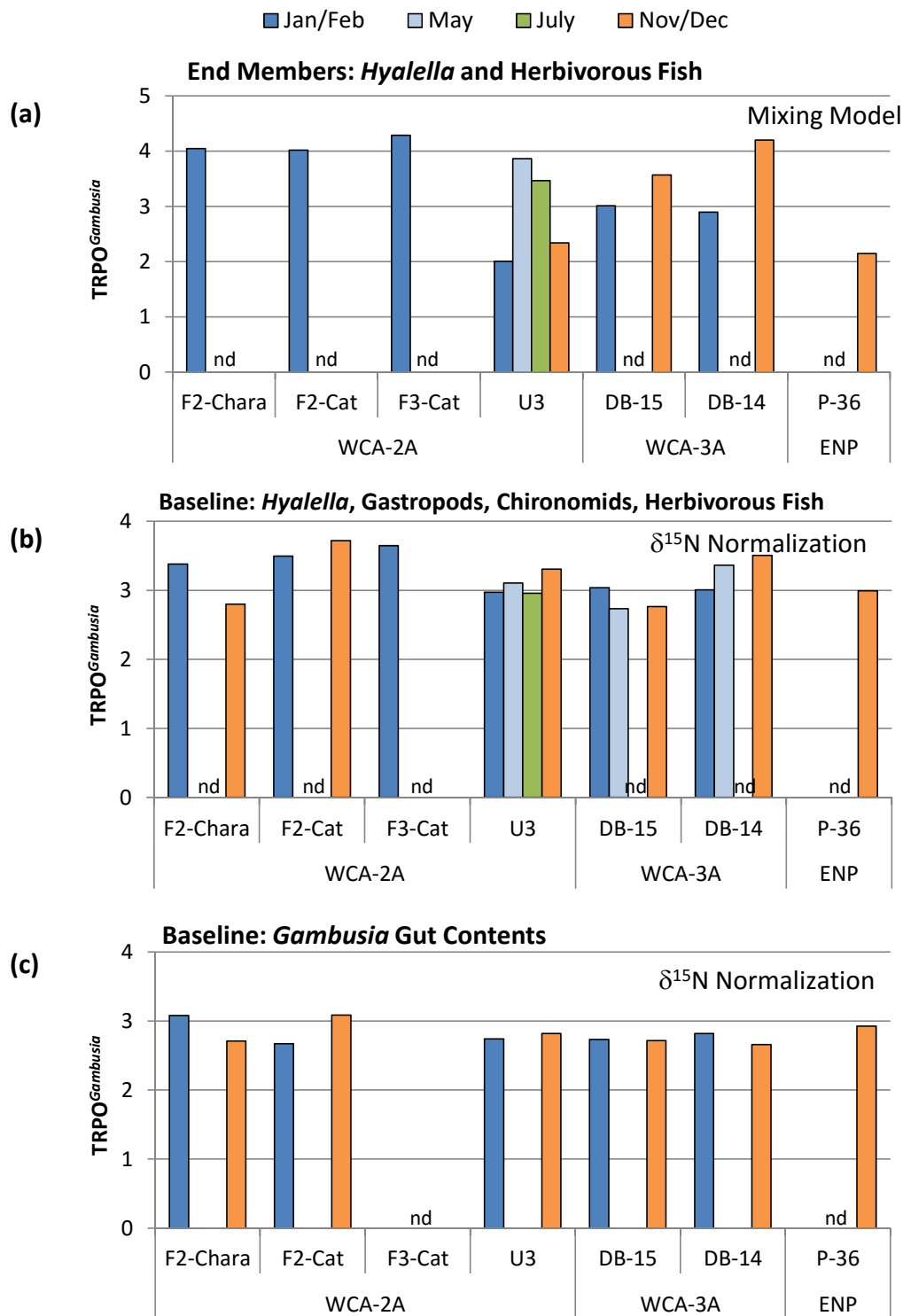


Figure 35. The mean trophic position (TRPO) for *Gambusia* at seven locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015. The herbivorous fish were *Poecilia latipinna* (sailfin molly) and *Jordanella floridae* (flagfish). nd = no data.

Food Chain Length (FCL)

Using invertebrates and herbivorous fish as the baseline in the $\delta^{15}\text{N}$ Normalization model yielded higher FCLs across all sites and dates compared to relying on the *G. holbrooki* gut content as the baseline in the model (Figure 36). Nevertheless, the relative rankings among the stations between the baseline categories (invertebrates and herbivorous fish vs. *G. holbrooki*) used indicated *G. holbrooki* collected at the F stations in WCA-3A represented longer food chains, which is consistent with the higher trophic levels measured for the F stations than the remaining stations (Figure 35).

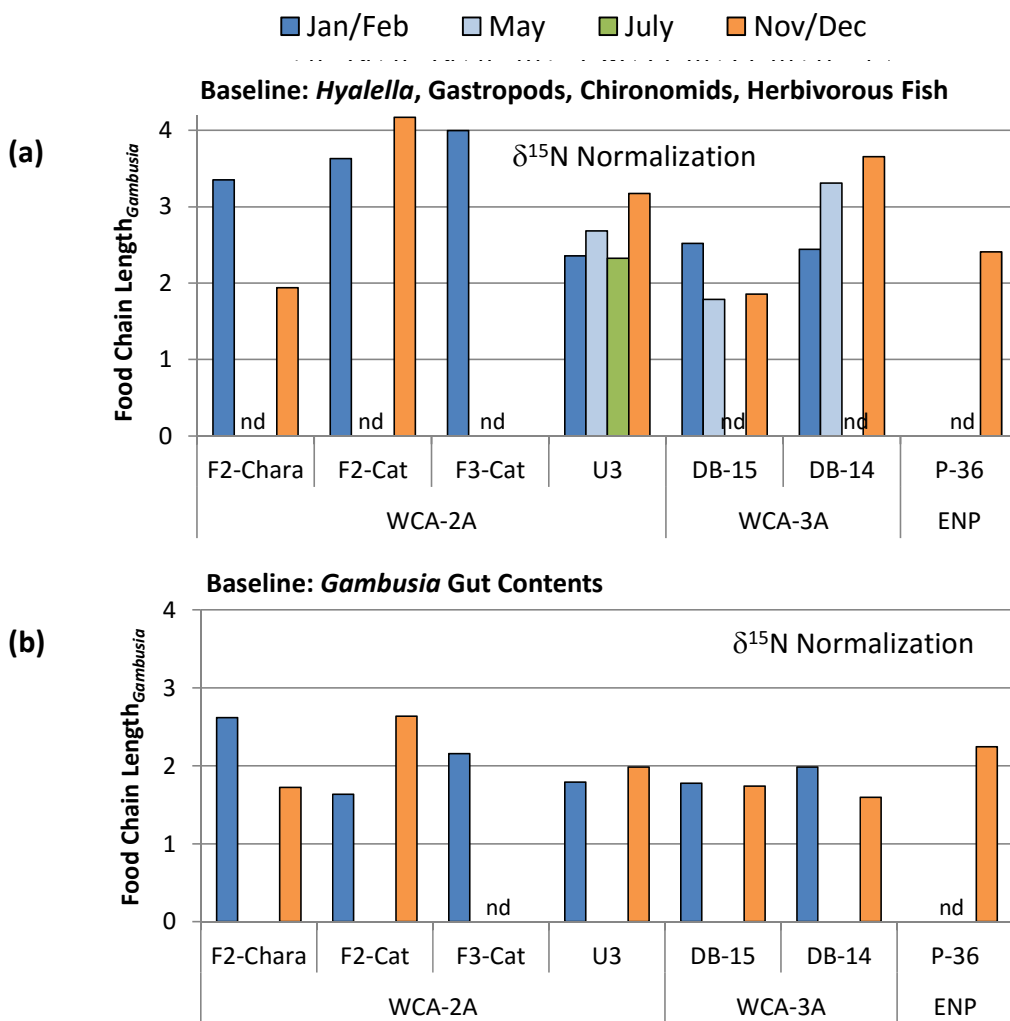


Figure 36. Food chain lengths calculated by the $\delta^{15}\text{N}$ Normalization model using either a) all invertebrate and herbivorous fish or b) *G. holbrooki* gut contents as the baseline. Assumed trophic position of the baseline indicator was 2. nd = no data.

Hagerthey et al. (2014) state that “the higher density of emergent macrophytes typically formed in eutrophic wetlands creates a physical barrier to predators, limiting the trophic transfer of energy, presumably to piscivorous birds and carnivorous fish”. Indeed, the lengths and mass of *Gambusia* in F2-Cat and F3-Cat are greater than in U3, DB-15, and DB-14 (DBE 2014), suggesting a more protected habitat in the cattail allows for larger, and perhaps longer-lived, *Gambusia*, which may explain the higher trophic position and FCL of *Gambusia* at F2 and F3 than at U3, DB-15, and DB-14. According to the I_D , the *Gambusia* at F2 and F3 are feeding on detritus and prey that consume detritus rather than primary producers as calculated for U3, DB-15, and DB-14. Thus the nutritional content of the litter in the nutrient-enriched F sites may also contribute to larger *Gambusia* populations than at the more oligotrophic slough sites

Bioconcentration (BCF) and Bioaccumulation (BAF) Factors

The bioconcentration factor (BCF) is the MeHg concentration in a plant or detritus divided by the MeHg concentration in SW. The highest BCFs for periphyton was for data collected in Jan/Feb and Nov/Dec (Figure 37a), whereas the BCF was highest in detritus collected in Jan/Feb (Figure 37b). The BCFs for *Utricularia* were rather evenly distributed across all the sampling dates (Figure 37c).

Examining the BCFs among the three substrates within sampling dates, *Utricularia* had the highest BCFs compared to either periphyton or detritus at all stations for each of the sampling dates (Figure 38). This aligns with the finding that *Utricularia* contains the highest MeHg concentrations than either periphyton or detritus.

The bioaccumulation factor (BAF) is the ratio of the THg concentration in *G. holbrooki* to the MeHg concentration in the SW. The BAFs for *G. holbrooki* sampled in Jan/Feb were higher than the other dates across all the stations (Figure 39). This was likely due to the higher BCFs in the basal food items consumed by *G. holbrooki* and their prey in Jan/Feb (Figure 37).

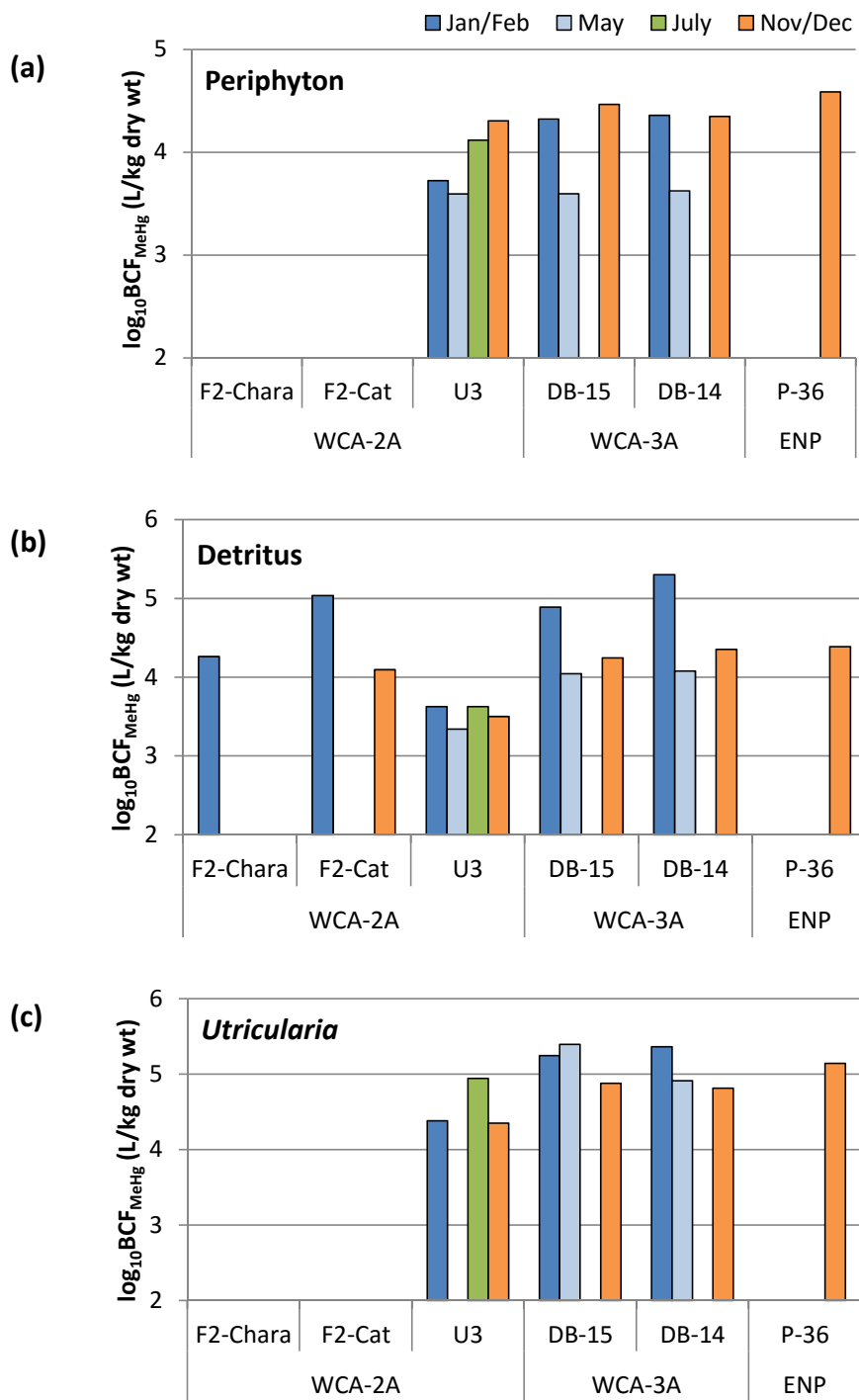


Figure 37. The log of the bioconcentration factor (BCF) for methylmercury (MeHg) in three basal food resources (periphyton, panel a; detritus, panel b, and *Utricularia*, panel c) at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015.

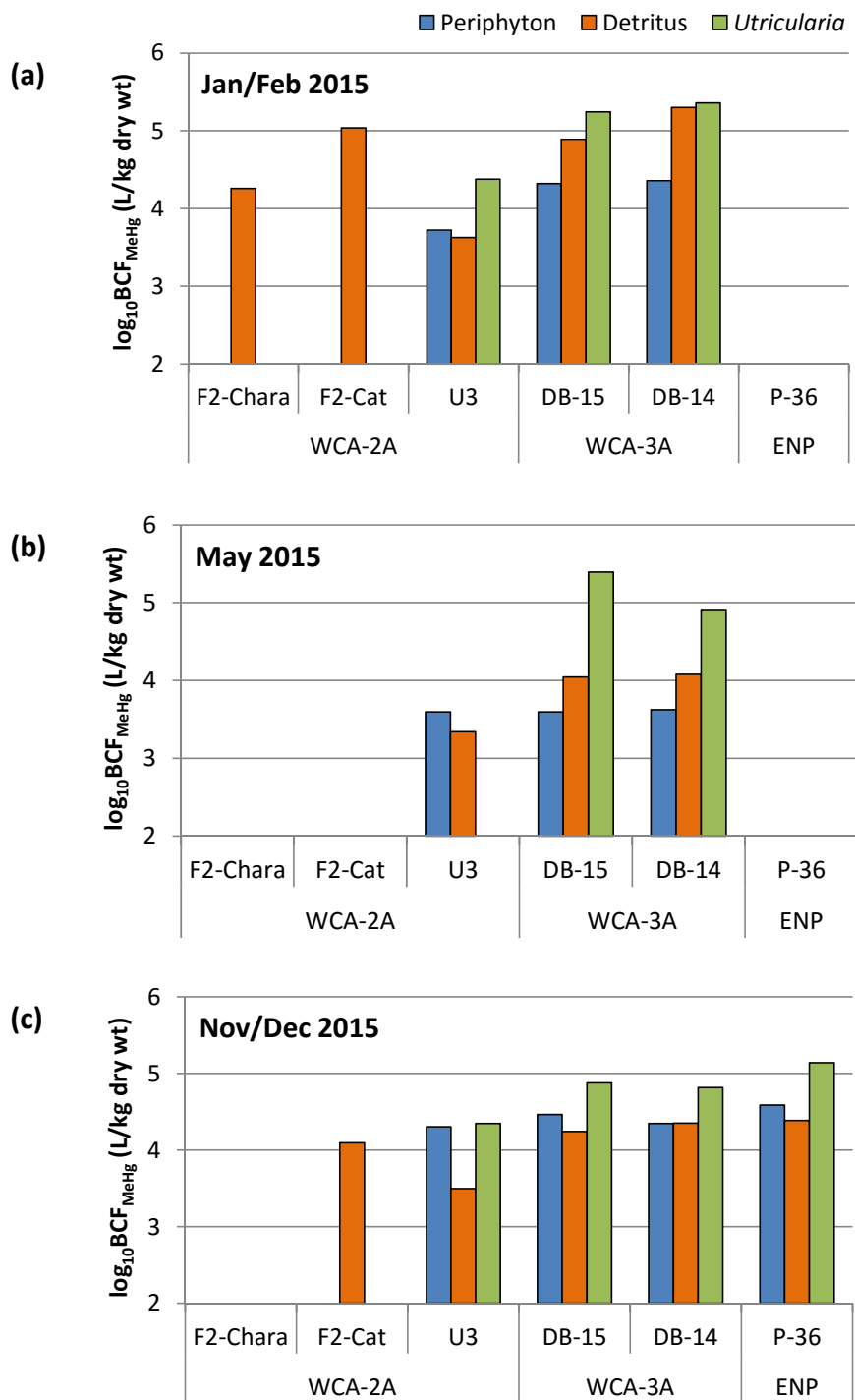


Figure 38. The log of the bioconcentration factor (BCF) for methylmercury (MeHg) in three basal food resources (periphyton, detritus, and *Utricularia*) at six locations on three sampling dates (Jan/Feb, panel a; May, panel b; and Nov/Dec, panel c) in 2015 throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP).

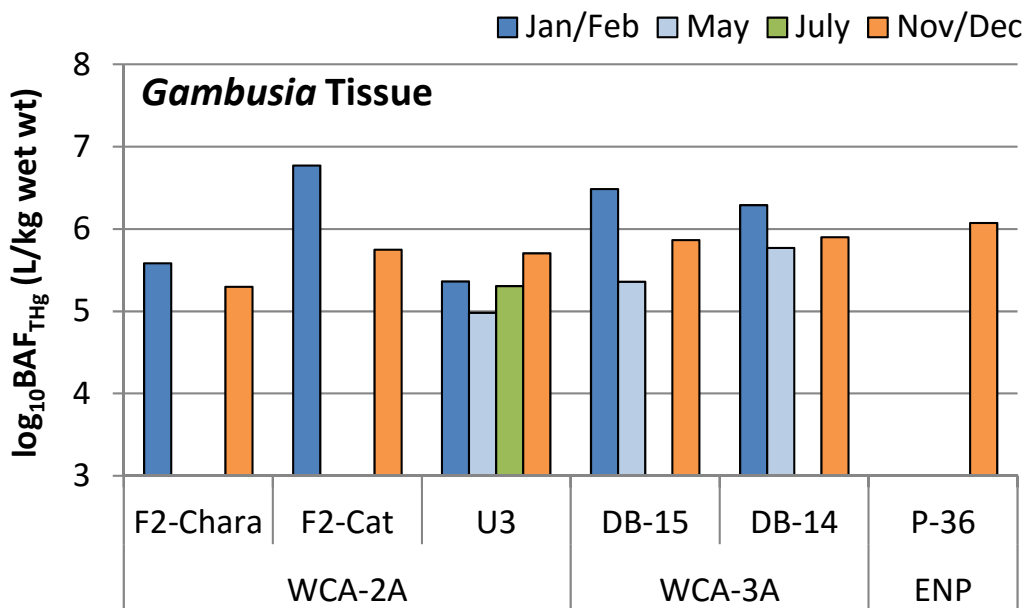


Figure 39. The log of the bioaccumulation factor (BAF) for total mercury (THg) in whole *Gambusia holbrooki* (minus gut contents) at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015.

Relationship between Food Source Hg, *Gambusia holbrooki* Hg, and Stable Isotope Content

The $\delta^{13}\text{C}$ of three major invertebrate taxa (*Hyalella*, Chironomidae, and *Palaemonetes paludosus*) sampled at all locations except at U3 predominantly grouped within a narrow range of -29‰ to -33‰ (Figure 40), indicating that they were feeding on a mix of different substrates that produced similar weighted $\delta^{13}\text{C}$ averages. There was also a hierarchy in the $\delta^{15}\text{N}$ concentrations within the invertebrate groups, with *Hyalella* having the lowest values followed by Chironomidae, and lastly *Palaemonetes paludosus* containing the highest $\delta^{15}\text{N}$ isotope ratios (Figure 40). Based on the $\delta^{15}\text{N}$ results, *Hyalella* can be considered more of a basal herbivore or detritivore than either Chironomidae or *Palaemonetes paludosus*, which are more likely omnivorous.

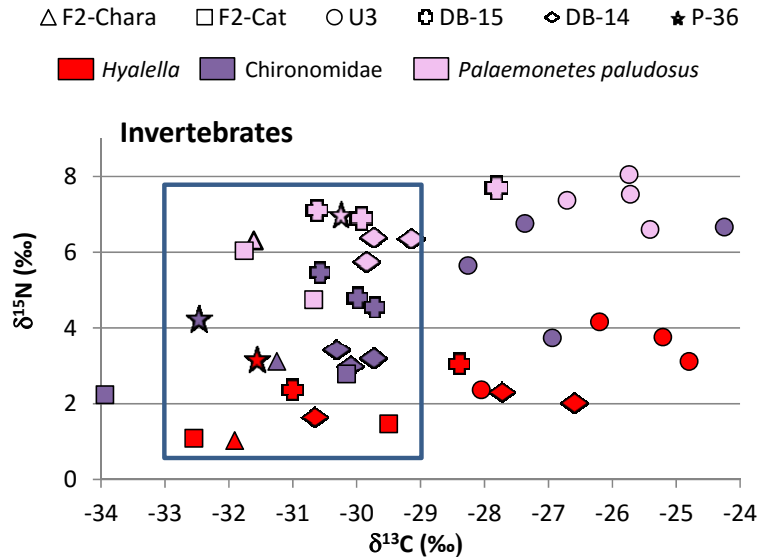


Figure 40 The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios for *Hyalella*, Chironomidae, and *Palaemonetes paludosus* at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015. The box contains the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges wherein a majority of the invertebrate data points lie except for those invertebrates collected at U3.

The $\delta^{13}\text{C}$ range for *G. holbrooki* gut contents and whole fish without gut contents (within the vertical lines in Figure 41) coincides with the $\delta^{13}\text{C}$ range for the invertebrates shown in Figure 40 (again excluding U3), signifying that the three major invertebrate groups are likely part of the diet of *G. holbrooki*. This also suggests that there is no discrimination of C isotopes during the digestion and assimilation of consumed food items and incorporation into fish tissues, so $\delta^{13}\text{C}$ appears to be a reliable tracer of C origin in the Everglades. A distinct segregation also occurred in the $\delta^{15}\text{N}$ values between gut contents and whole body *G. holbrooki*, with incrementally higher $\delta^{15}\text{N}$ ratios of $\Delta^{15}\text{N}$ of 2.5‰ to 4‰ associated with the whole body than the gut contents, an expected outcome since $\delta^{15}\text{N}$ isotopic enrichment occurs by ~ 3-5‰ for each trophic level along the food chain gradient (Post 2002).

Whereas SW diss MeHg concentrations were not correlated with *G. holbrooki* THg concentrations (Figure 42), the relationship between MeHg concentrations in the gut contents and the THg in the whole body (minus the gut contents) of *G. holbrooki* was highly correlative (Figure 43a and b). The whole body THg was approximately two times higher than the MeHg gut content on a dry weight basis (Figure 43b).

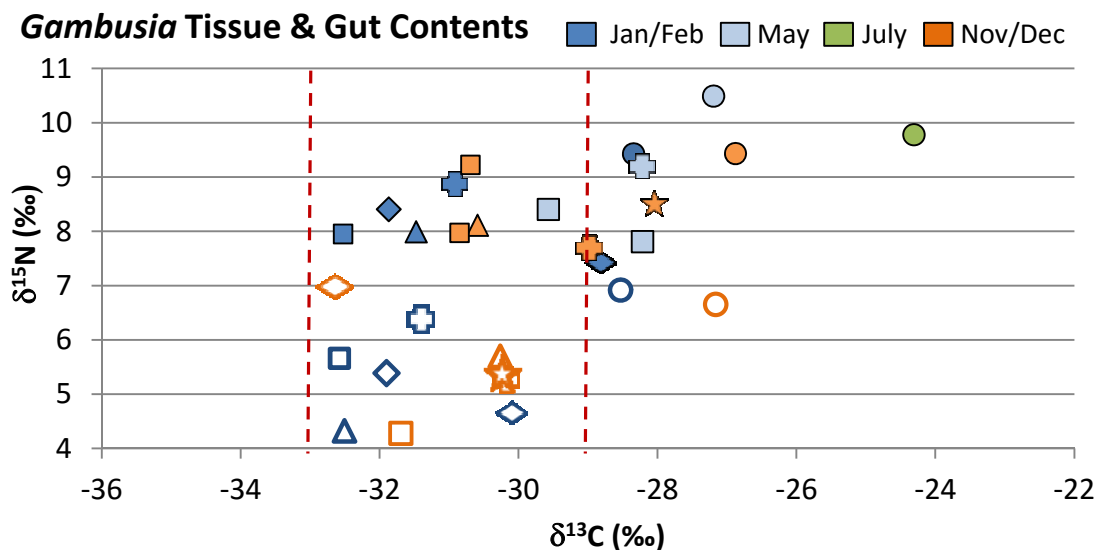


Figure 41. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios for *G. holbrooki* gut contents (open symbols) and whole fish without gut contents (closed symbols) at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015. See Figure 40 legend for identifying which symbols represent each station. The vertical dashed lines represent the $\delta^{13}\text{C}$ range wherein a majority of the data points lie except for the *G. holbrooki* collected at U3. Note that gut contents were not analyzed in May and July.

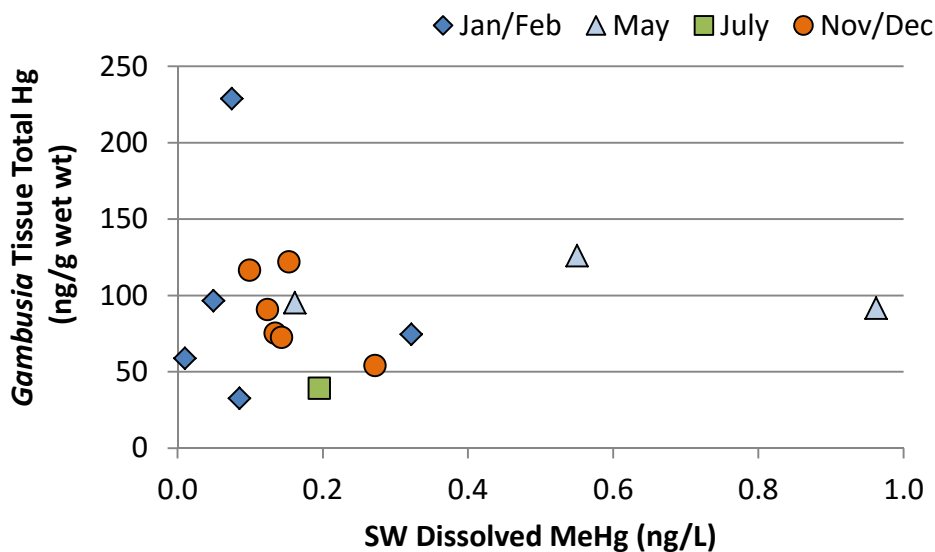


Figure 42. The relationship between surface water (SW) dissolved methylmercury (MeHg) and *G. holbrooki* total mercury (THg) concentrations at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015.

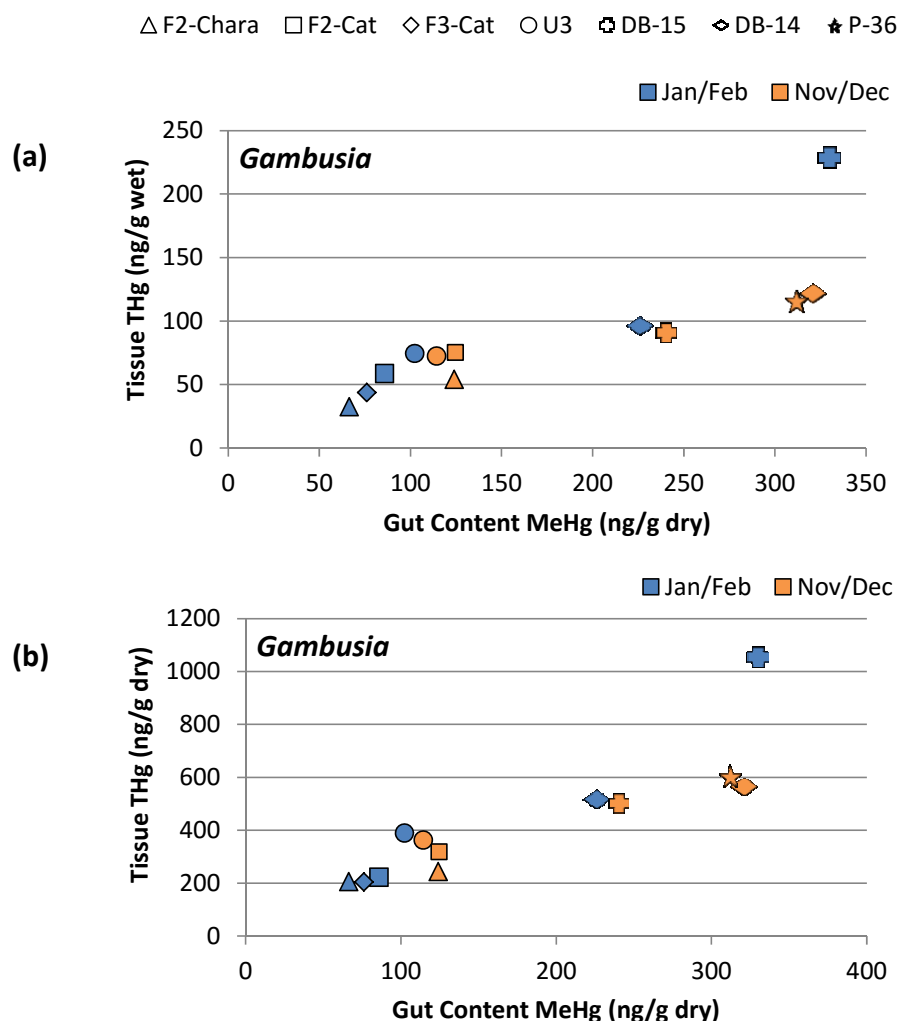


Figure 43. Comparisons in the mean methylmercury (MeHg) concentrations in the gut contents of *G. holbrooki* and the whole fish (minus gut contents) total mercury (THg) concentrations expressed on a a) wet weight and b) dry weight basis at seven locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in Jan/Feb and Nov/Dec 2015. N=3.

The $\delta^{13}\text{C}$ isotope signatures indicated that detritus and *Utricularia* served as food items for the herbivorous fish, *J. floridae* and *P. latipinna* (Figure 29a and b). A plot of the MeHg concentration in the primary producer and detritus food groups versus herbivorous fish MeHg concentration produced a high correlation for detritus, but not for *Utricularia* (Figure 44a). Correlations were weaker between the MeHg concentrations in either of these two food items and *G. holbrooki* THg concentrations (Figure 44b), likely due to the more omnivorous nature of *G. holbrooki*. The lack of a correlation between fish THg and MeHg in *Utricularia* may be due to the high MeHg concentrations in the plant across all stations, which may be above a threshold where there is not a linear relationship between fish Hg concentration and the MeHg concentration of its food

source. Of course, it could be that neither the herbivorous fish or *G. holbrooki* are consuming *Utricularia* at some locations, notwithstanding the $\delta^{13}\text{C}$ evidence that they are.

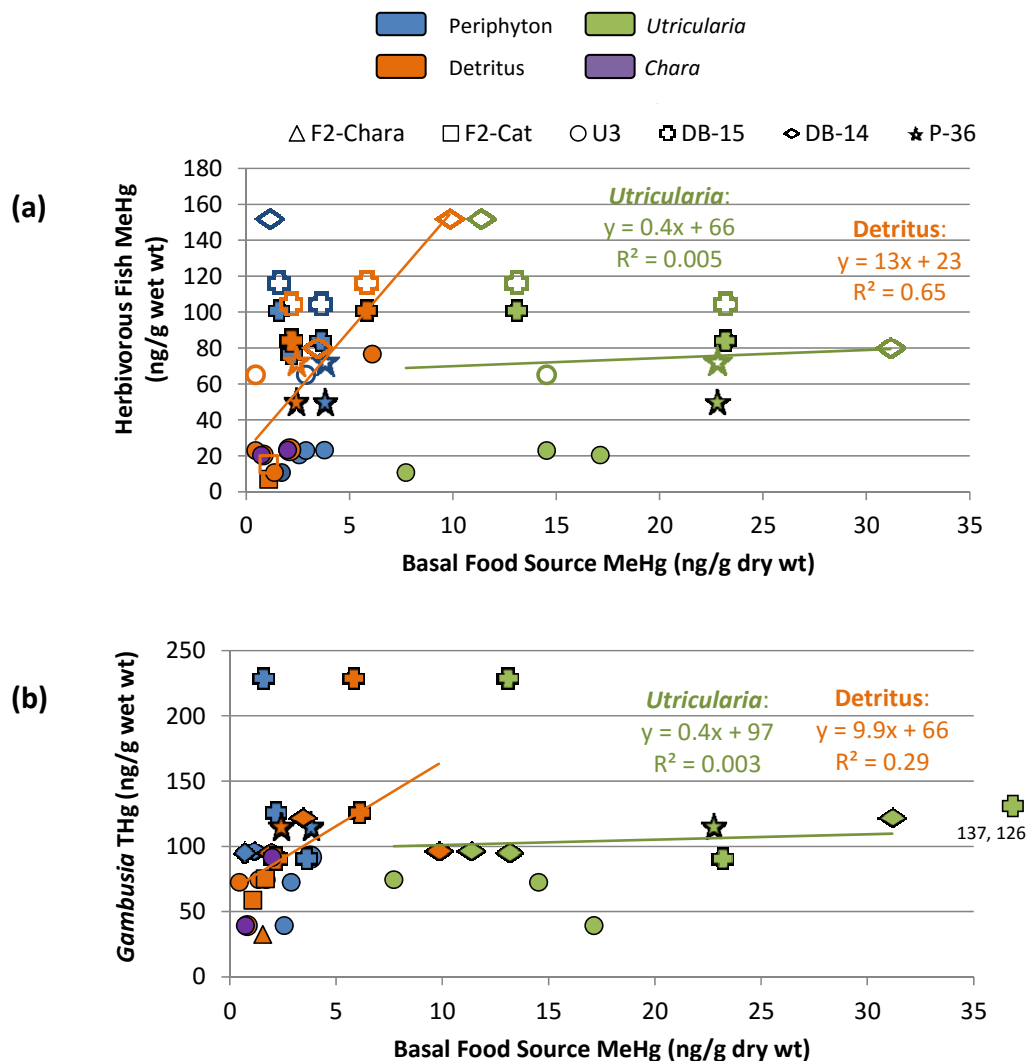


Figure 44. The relationship between methylmercury (MeHg) concentrations in various primary producer and detritus food groups and a) the MeHg concentration in the herbivorous fish, *J. floridae* and *P. latipinna*, and b) total mercury (THg) in *G. holbrooki* at six locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) in 2015.

The calculated *Gambusia* trophic positions resulting from the application of the Two-End Member Mixing or $\delta^{15}\text{N}$ Normalized models to the stable isotope data for Jan/Feb collection events did not support the hypothesis that higher trophic positions for *Gambusia* at the slough sites in WCA-3A and ENP contribute to the greater tissue Hg concentrations at those sites, compared to the WCA-2A F sites (F2-Cat, F2-Chara, F3-Cat) (Figures 35 and 36). However, trophic position and food chain length is only one component of food web structure; the other

two components are food web complexity and food web base. The data from these sampling events suggest that higher Hg concentrations at the base of the food web at slough sites, which subsequently are biomagnified in prey species, more than compensates for the modeled longer food chains at the F sites. This results in the higher *Gambusia* Hg concentrations at U3, DB-15, DB-14, and P-36 than at the F stations.

Note that the *Utricularia* spp., which was prevalent in the water at the higher Hg-containing *Gambusia* (including their gut contents) harvested at U3, DB-15, DB-14, and P-36, were particularly high in MeHg content relative to other basal food sources (detritus and periphyton). Troxler and Richards (2009) found *Utricularia* to be the primary source of flocculent materials in the deep-water sloughs of WCA-3A, although periphyton was not always present at their study sites and was not considered. If *Utricularia* is a primary food source for intermediate consumers occupying trophic positions below *Gambusia*, as well as serving as a direct food source to *Gambusia*, then the higher MeHg content of the plant could explain a lot of the variation in *Gambusia* Hg levels among the sites.

Figure 45 is a plot of the $\delta^{15}\text{N}$ versus MeHg concentrations in the tissues of the major invertebrate and fish populations measured during Jan/Feb and Nov/Dec 2015. As expected, there is a positive relationship between the $\delta^{15}\text{N}$ content and MeHg concentration among the species along the trophic level gradient within a particular location, with r^2 -values ≥ 0.86 for all the regression lines. Comparisons of the regression line slopes for each sampling period indicate the relationship is similar (i.e., slopes were parallel) among all locations (i.e., the increase in MeHg concentration per increment in $\delta^{15}\text{N}$ is approximately the same along the trophic level gradient), which is indicative of similar biomagnification factors between trophic levels among the diverse vegetation communities. The slopes of the regression lines for all stations except DB-15 were higher in Jan/Feb than in Nov/Dec (Figure 45), meaning that there was a higher biomagnification factor present throughout the food chain in Jan/Feb 2015 than in Nov/Dec 2015. However, because of the variation in the $\delta^{15}\text{N}$ values of the food sources (Figures 29 and 30), the high degree of omnivory, and varying MeHg levels in the food resources (Figure 44), there are significant separations in the trophic end-members among locations. As an example, *Hyalella*, representing the lowest trophic level, is almost an order of magnitude lower in MeHg concentration at F2-Cat than at any other location during Jan/Feb; the MeHg concentration differences for this species among the stations were less during Nov/Dec (Figure 45).

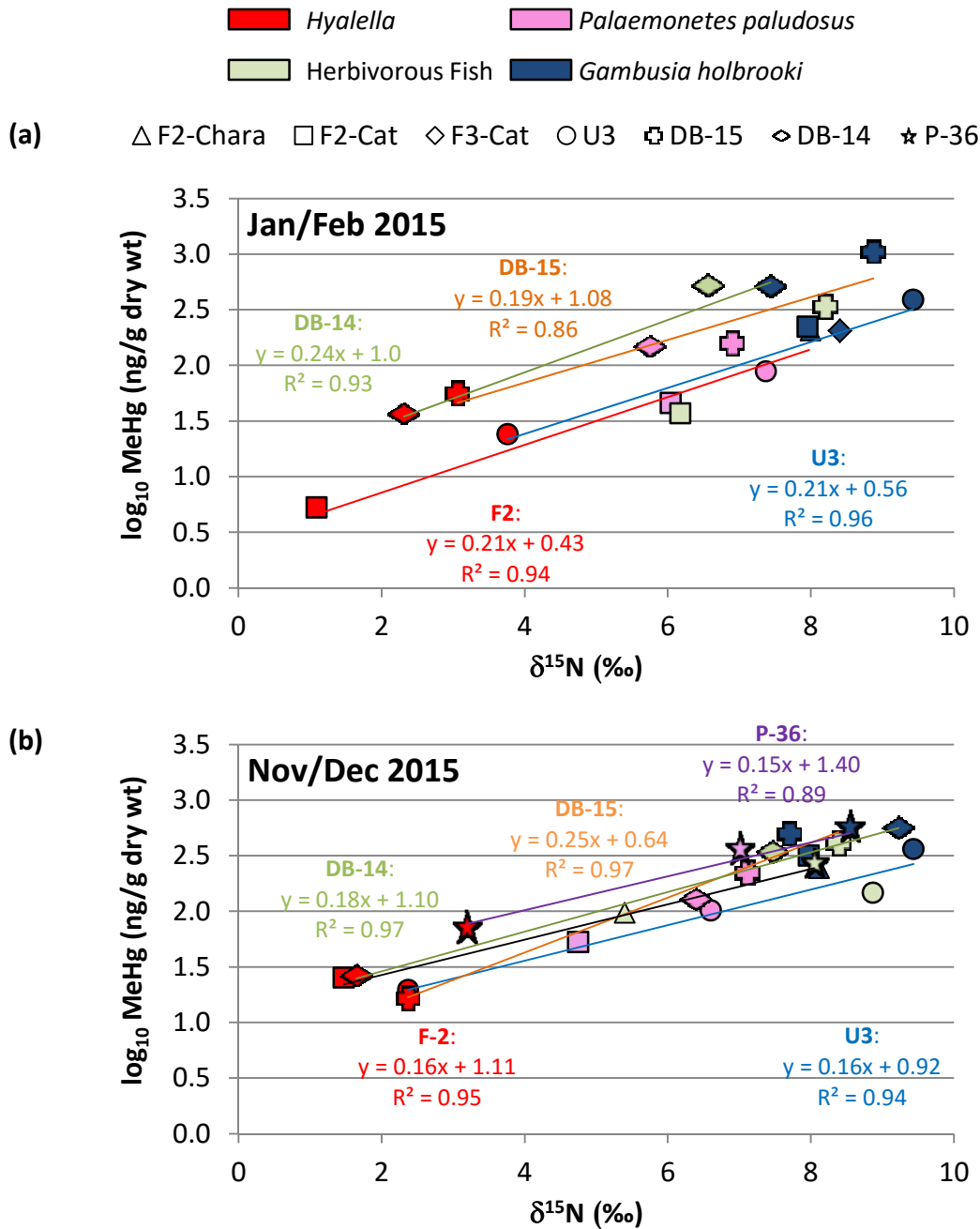


Figure 45. Relationship between methylmercury (MeHg) concentration and $\delta^{15}\text{N}$ in two species of invertebrates (*Hyaella* sp. and *Palaemonetes paludosus*), two species of herbivorous fish (*Poecilia latipinna* and *Jordanella floridae*), and the omnivorous *Gambusia holbrooki* at two locations (F2 and U3) within WCA-2A and two locations (DB-15 and DB-14) within WCA-3A on a) Jan/Feb and b) Nov/Dec 2015. An additional location (P-36) in the Everglades National Park was sampled in Nov/Dec 2015. The total Hg concentrations measured for *G. holbrooki* was assumed to be equivalent to MeHg; all other fish and invertebrates were analyzed for MeHg. *Gambusia*: n=6 composited replicates (size classes averaged in Jan/Feb) and n=3 composited replicates in Nov/Dec; invertebrates: n=1 composite when enough sample mass was available for analysis; *P. latipinna*, and *J. floridae*: n=1-3 replicate composite samples. F2-Cat *Hyaella* sp. MeHg was BDL (<5.3 ng/g dry weight) in Jan/Feb.

As reported by Pickhardt et al. (2006), our data generally support that diet is the principal pathway of mercury uptake by *G. holbrooki* across our sampling sites, and that differences in dietary mercury exposure related to the food web explain some of the variability in the previously reported tissue mercury concentrations.

Conclusions

The variation in biotic Hg level across Everglades compartments could not be well explained by “conventional” biogeochemical factors. The length and nature of *G. holbrooki* food webs were considered as possible explanatory variables for patterns in Hg enrichment.

Although the trophic position of *Gambusia* can vary between levels 2 and 4 within a location, our finding in Year 3 that *Gambusia* from the enriched cattail area of WCA-2A occupied a higher trophic position than the fish in the sloughs of WCA-2A, WCA-3A, and the ENP was verified in Year 4. This conclusion was supported by two independent trophic state models. A separate model found that the food chain length was longer for the fish in P-enriched cattail communities than in P-limited ridge and slough areas, but that these differences did not account for differences in mosquitofish Hg levels.

Using an independent data set collected in Year 4, we confirmed the log-linear relationship between trophic position (as determined by the $\delta^{15}\text{N}$ concentration ratios) and the MeHg concentrations among invertebrates and fish that was reported in Year 3. The MeHg concentration increments among the trophic levels at different sites pointed to similar MeHg transfer up the food chain, indicating the importance of controlling factors affecting the net production, and subsequent bioavailability, of MeHg at the microbiological and molecular levels. The comprehensive data analysis in Task 1 suggested that detritus may be an important MeHg vector into Everglades food webs.

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Appendix

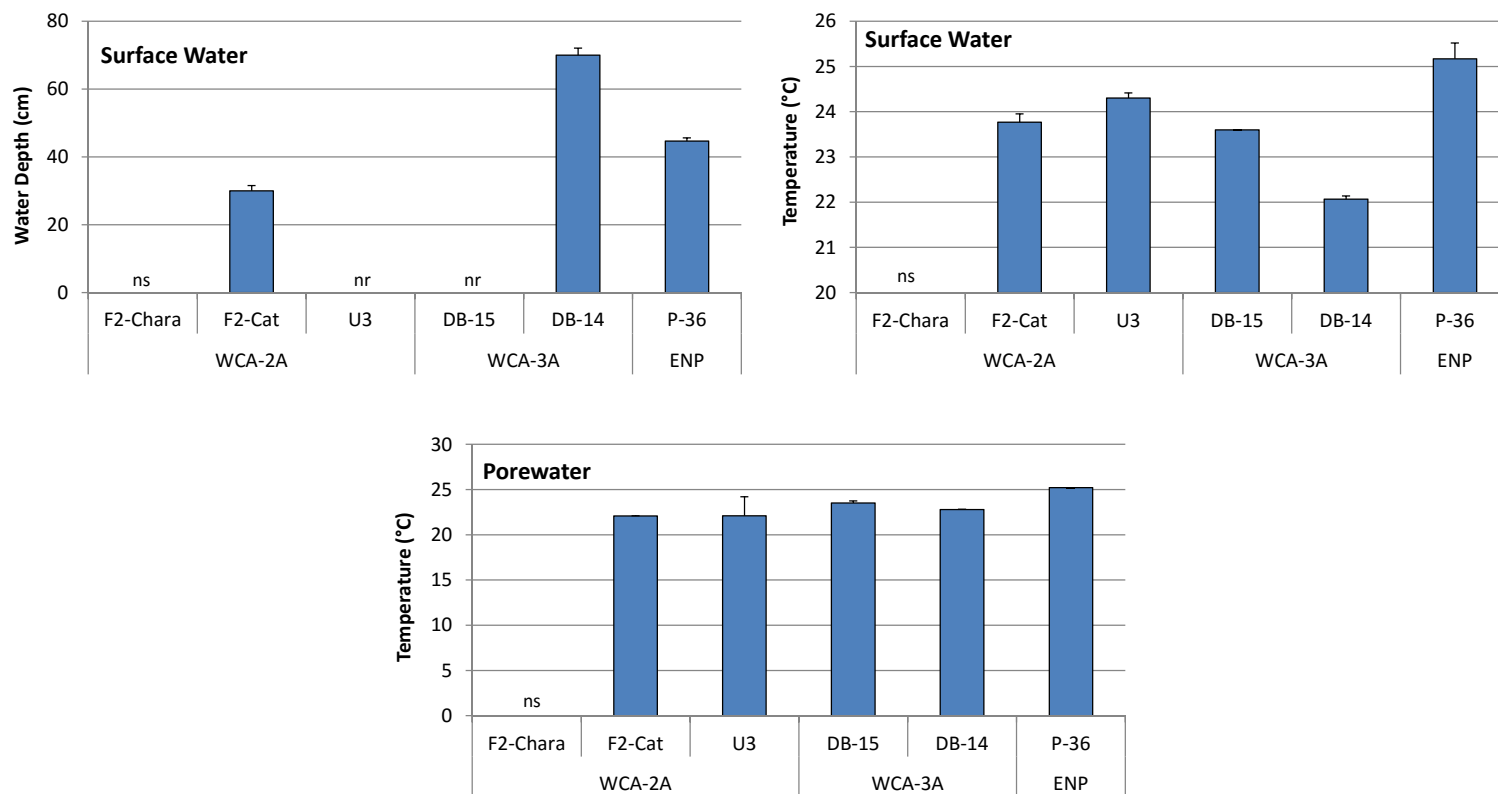


Figure A- 1. The mean (+1 S.E.) water depth (panel a), surface water temperature (panel b), and porewater temperature (panel c) among six sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 - December 2, 2015. N=3. ns=not sampled; nr=not reported.

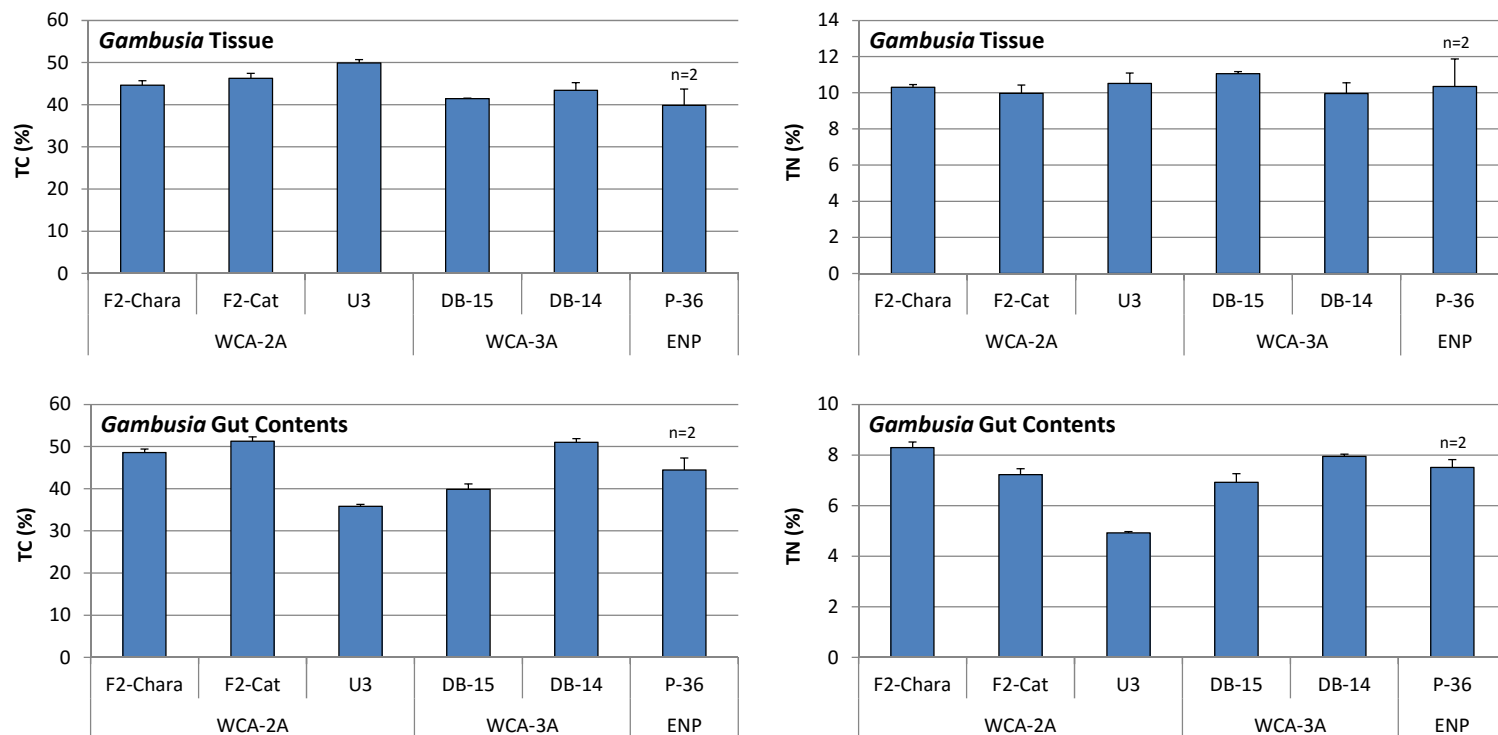


Figure A- 2. The mean (+1 S.E.) total carbon (TC) and total nitrogen (TN) concentrations in *Gambusia holbrooki* (top panels) and their gut contents (lower panels) among six sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3 unless otherwise noted.

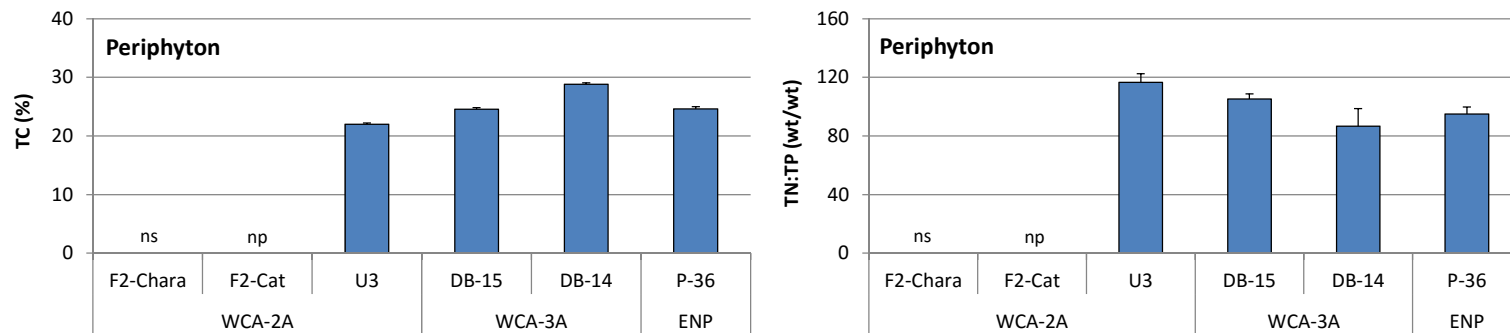


Figure A- 3. The mean (+1 S.E.) total carbon (TC) and total nitrogen (TN) concentrations in periphyton among four sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3 unless otherwise noted. ns=not sampled; np=not present.

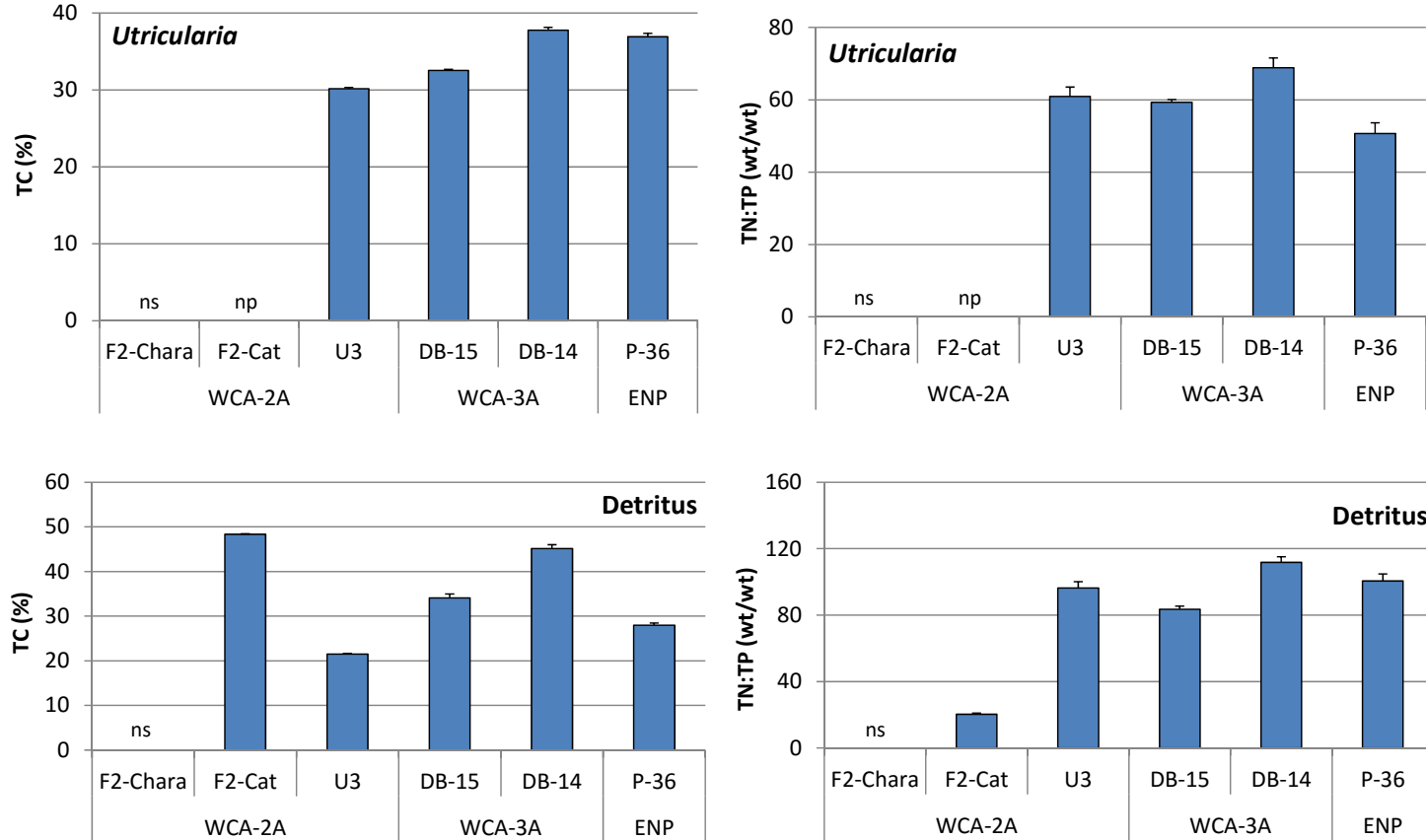


Figure A- 4. The mean (+1 S.E.) total carbon (TC) and total nitrogen (TN) concentrations in *Utricularia* (top panels) and detritus (lower panels) among four or five sampling locations throughout the Water Conservation Areas (WCA) 2A and 3A and the Everglades National Park (ENP) November 30 – December 2, 2015. N=3 unless otherwise noted. ns=not sampled; np=not present.