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Variation of total mercury concentrations in pig frogs (*Rana grylio*) across the Florida Everglades, USA

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Abstract

The Pig Frog (*Rana grylio*) is an aquatic frog that is an abundant component of the Everglades ecosystem. South Floridians recreationally and commercially hunt pig frogs in marshes throughout Water Conservation Areas (WCA) and Big Cypress National Preserve (BCNP) in South Florida. Most of these areas are under fish consumption advisories because of high levels of methylmercury present in game fish tissues. It is important to understand how mercury is distributed throughout Pig Frog populations because their consumption from certain areas may present a risk to human health. We sampled 88 pig frogs along a north-south transect through the Florida Everglades. There were substantial differences in total mercury (THg) concentrations from leg muscle tissue among sites. Total mercury in frog leg tissue was highest from areas protected from harvest in Everglades National Park (ENP), with a maximum concentration of 2051 ng/g wet mass. The THg levels in *R. grylio* leg tissue from most harvested areas are below Federal advisory limits. However, many pig frogs collected near Frog City, and one from WCA 3B and 3AN, harvested sites, had THg levels above the USEPA 0.3 mg/kg Fish Tissue Residue Criterion. Spatial patterns in the mercury found among pig frogs were similar to those of other wildlife species from the Everglades. We found frogs to have high THg levels in areas where alligators and mosquito fish also have high THg. THg in ENP frogs had an exponential relationship to SVL, we found no other relationship in frogs from other sites. Our data suggests that pig frogs should not be harvested or consumed from sites that exceed federal limits.

Keywords: *Rana*; Pig frogs; Frog legs; Total mercury; Everglades; Spatial distribution

1. Introduction

Several freshwater marshes in South Florida's Everglades are extensively contaminated with mercury (Hg) (Sundlof et al., 1994; Stober et al., 1998; Krabbenhoft, 2000; Rumbold et al., 2002a; Williams

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et al., 2001). Over two million acres of wetlands in South Florida are currently under fish consumption advisories or bans because of mercury contamination in top predators such as Large-mouth Bass (*Micropterus salmoides*), Bowfin (*Amia calva*) and Snook (*Centropomus undecimalis*) (Ware et al., 1990; Stober et al., 1998). Mercury toxicity has adverse effects on wildlife and humans that include decreased reproductive success, developmental abnormalities, brain damage, and death (Aulerich et al., 1974; Eisler,

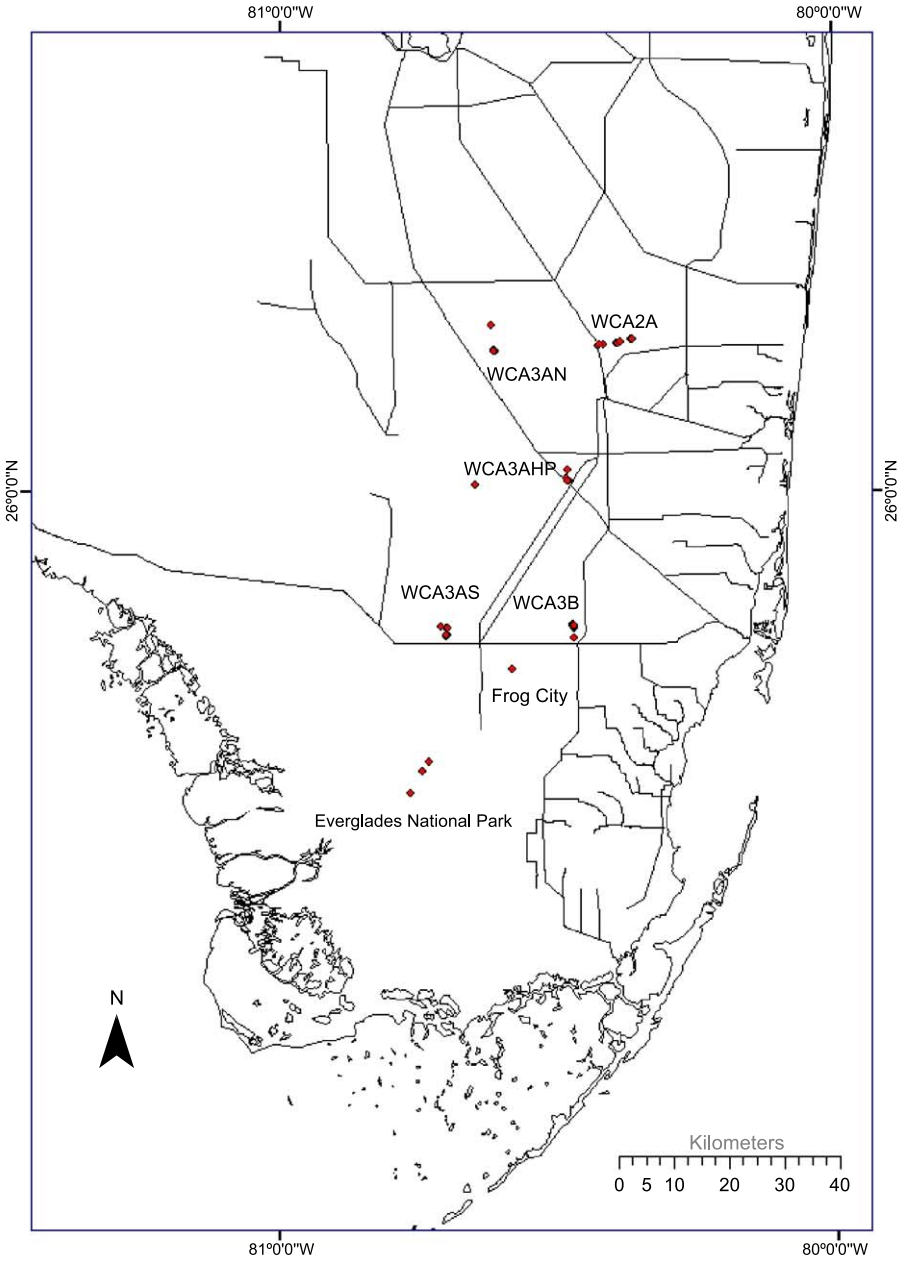

Fig. 1. Location of *R. grylio* collection areas throughout the Florida Everglades.

1987; Kanamadi and Saidapur, 1992; Punzo, 1993; Britson and Threlkeld, 1998; Stober et al., 1998; Lacerda and Fitzgerald, 2001; Netting, 2001; USEPA, 2001). Researchers are currently trying to understand how bioaccumulated methylmercury (MeHg) is distributed throughout the Everglades aquatic food web (Krabbenhoft, 2000; Bemis et al., 2002; Rumbold et al., 2002b; Axelrad et al., 2004); however, it is not known how MeHg transfer occurs among intermediate trophic levels in South Florida wetlands (Stober et al., 1998; Loftus, 2000; Leady and Gottgens, 2001).

Most studies of mercury levels in wildlife have focused on mammals, birds, and fish, whereas amphibians and reptiles have received little attention (Sundlof et al., 1994; Gerstenberger and Pearson, 2002). Amphibians, however, are a major component in many wetland systems, and sometimes comprise more vertebrate biomass than either birds or small mammals (Burton and Likens, 1975). Pig frogs are an integral component of the greater Everglades food web and are also harvested regularly by humans. The Pig Frog, consumes a variety of insects, crustaceans, amphibians, and even birds (Ligas, 1960, 1963; Lamb, 1984; Ugarte unpublished data). It is also a source of prey for a variety of wading birds (Rodgers, 1982), raptors (Coward, 1984), snakes (Craighead, 1968), amphibians (Ligas, 1960), fish (Loftus, personal communication), and alligators (Barr, 1997). People harvest pig frogs for their edible legs in South Florida in the Water Conservation Areas (WCA), the Miccosukee Indian Reservation, and Big Cypress National Preserve (BCNP). Some of these areas (WCA 3A and WCA 3B) are known to have fish contaminated with high levels of mercury (greater than 1.5 μg/g). Baseline United States Environmental Protection Agency (USEPA) sampling data in 1995 and 1996 found that areas with high levels of total mercury in Mosquitofish (*Gambusia holbrooki*) overlap with high levels found in White Egret (*Egretta alba*) chick feathers throughout the South Florida Water Conservation Areas and Everglades National Park (Sundlof et al., 1994; Stober et al., 1998, 2001). Similar patterns of mercury concentration may exist among pig frogs, a harvested species.

We examined the total mercury (THg) concentration in leg muscle tissue of pig frogs collected along a north–south gradient throughout the Florida Everglades (Fig. 1). The main objectives of this study are to assess the variation in total mercury in pig frogs within and among harvested and protected areas; and to determine the relationship between mercury level and snout-vent length (SVL) of frogs. This information will be useful to frog harvesters for determining areas that may have frogs with high mercury concentrations, and if any size-classes of frogs should be avoided, and in understanding spatial patterns of mercury across the Everglades.

2. Materials and methods

2.1. Area descriptions

We collected 88 pig frogs throughout five areas of South Florida during October 2001, and September and October 2002. These areas included WCA 2A, WCA 3A, WCA 3B, and Shark River Slough (ENP) and Frog City in ENP (Fig. 1). Frogs were collected from long hydroperiod marshes dominated by water lilies (*Nymphaea odorata*) or spikerush (*Eleocharis* spp.).

2.2. Preliminary survey of mercury

We collected 20 frogs from WCA 3A on 1 October 2001, as a preliminary survey of mercury in these frogs. We searched for frogs during 20-min sampling periods. Frogs were captured at night by hand from an airboat and their location was marked with a Garmin 76 map GPS. Once captured, the frogs were placed on ice, and transported to a laboratory. Leg muscle was examined in all specimens. A sterile skin biopsy tool was used to remove the tissue. Plugs were removed from various parts of the legs for each sample. Samples were weighed and analyzed at the Southeastern Environmental Research Center (SERC), at Florida International University. Total Mercury (THg) was determined using a PSA Merlin Plus Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) mercury analysis system.

2.3. Survey of mercury distribution

To examine the variation of mercury in these frogs across the Everglades, we captured 68 frogs at night from an airboat at the sites shown in Fig. 1.

These were kept on ice, and transported to the laboratory. Samples were analyzed at the U.S. Geological Survey, Florida Integrated Science Center-Aquatics Resources Studies. Frogs were weighed with a 300-g pesola scale, sexed, and dorsal SVL measured with a ruler to 0.5 mm. A sterile scalpel and fresh gloves were used for each individual. Leg tissue was removed and placed in a labeled vial. Wet weight was recorded using a Mettler Toledo AG204 Delta range scale, and we used a Direct Mercury Analyzer (DMA-80, Milestone) to analyze concentrations of THg in Pig Frog leg muscle tissues. The DMA-80 is an integrated system that specializes in analyzing solids or liquids. The tissue is dried and decomposed and mercury vapors are captured in a gold amalgam trap. Once the vapors are desorbed, total mercury is quantified using atomic absorption spectrometry at 254 nm (Milestone, 1998). This system requires no preparation of tissues. Samples were placed in nickel boats and analyzed at a drying temperature of 300 °C for 60 s, followed by a decomposition temperature of 850 °C for 180 s and amalgam heating for 12 s.

3. Results

3.1. Quality assurance

In the preliminary study, spiked samples, standard reference material (DORM), and blanks were used. All replicate samples were within the accepted 10% relative percent difference. We also analyzed a small sub-sample of five frogs for both THg and MeHg. We found a significant linear relationship between MeHg and THg ($F_{1,3}$=22.57, R^2=0.88, *P<0.01). Although our sample size was small, Arnold (2000) also found a linear relationship between THg and MeHg in pig frogs. She found that MeHg constitutes 95% of the THg in pig frogs. Similar relationships have been found in various fish species; therefore, we sampled THg in lieu of MeHg (Lasorsa and Allen-Gil, 1995; Goldstein et al., 1996; USEPA, 2001).

In the broader sampling of this study, laboratory quality control (QC) consisted of blanks and standard reference material. Three replicates were analyzed for each individual, with blanks analyzed before and after each triplet, and a standard reference (TORT-CRM) analyzed every nine samples. Samples were larger than the calculated detection limit of 0.008 μg/g. Recovery of THg from certified reference standards averaged 99%±(96–102%, n=20). Relative percent difference between at least one set of duplicates per sample was within 10%. In one run, a standard reference was outside 20% of the true value. In this case, we reanalyzed all samples that were analyzed prior to this reference.

We also sent seven of the 68 samples analyzed using the Direct Mercury Analyzer (DMA-80, Milestone) to the Florida Department of Environmental Protection, Central Chemical Laboratory. This was done to ensure the accuracy of the results using this relatively new method. The FDEP used cold vapor AA spectroscopy to analyze samples. Recovery of THg from Laboratory Fortified Blanks was 100%—relative percent difference between duplicate samples was 0.06%, within the acceptable range. We used t-tests to compare mean THg from DMA-80 samples and cold vapor samples. Results obtained by the two methods were not significantly different (FDEP $\bar{x}$=0.267±0.117, DMA-80 $\bar{x}$=0.257 0.111, $t_{1,6}$=0.147).

Data were analyzed using SPSS standard version 11.5 (Lead technologies). We used a t-test to compare mean differences in THg concentration (ng/g wet weight) between frogs collected from WCA 3AS in 2001 and 2002. We used a type III two-way analysis of variance (ANOVA) to assess the effects of sex and site on THg levels in Pig Frog leg muscle tissues. Total mercury data was fourth-cube transformed to normalize data and meet the homogeneity of variance assumption. Finally, we used simple linear regression to determine if the variance in body size explained the variance in THg. All data from the preliminary survey and the more extensive 2002 sampling effort were pooled to examine linear relationships between SVL and THg.

3.2. Tissue residues

We analyzed 20 individuals (1 male, 2 females, 17 juveniles) in our preliminary study. Mercury from site 3AS frogs varied little among individuals $\bar{x}$=121.40± 31.5 ng/g wet weight. A positive linear relationship exists between MeHg and THg (R^2=0.88, *P<0.01). This has also been found in pig frogs sampled from

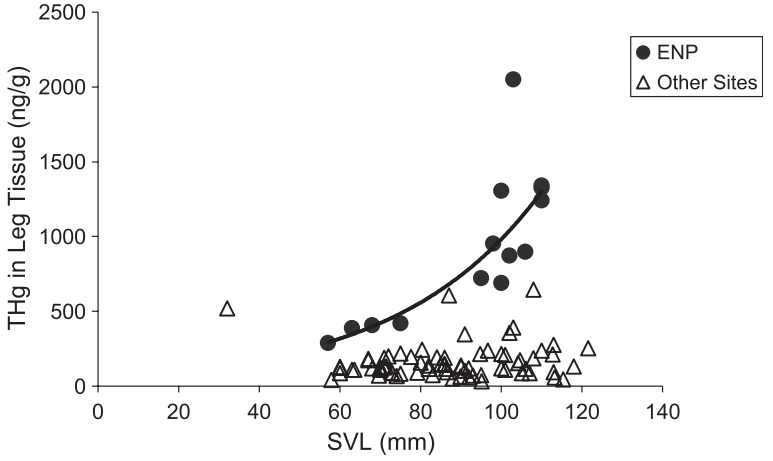

Fig. 2. Total mercury (ng/g) concentration in *R. grylio* leg muscle tissue by site.

Okeefenokee, GA (Arnold, 2000). Although mean THg in 3AS frogs collected during 2002 (n=10) was lower than during 2001 (n=20), the difference in mercury levels between years was not significant ($t_{1,28}$=0.55, P=0.464) (Table 1).

We sampled 68 frogs (25 males, 23 females, and 20 juveniles) along a north-southtransect in 2002. There were substantial differences in THg concentrations from leg muscle tissue among sites ($F_{1,6}$=43.713, $^{**}P$<0.001) (Table 1). Tukey's HSD Post-Hoc Test revealed that frogs from Shark Slough sites in Everglades National Park had significantly higher levels of THg than the other sites. Frog City in ENP, and WCA 3B frogs had higher levels of THg than did frogs from WCA 3AS, and WCA 3A HP, WCA 2A. WCA 3AN had significantly lower levels than Frog City and ENP (Fig. 3). We found levels above the EPA human health fish tissue criterion of 300 mg/kg in three sites, primarily ENP, FC, and one frog each in WCA 3B and WCA 3A (USEPA, 2001). Males and females did not have significantly different levels in leg tissue, but there was a significant interaction between site and sex ($F_{1,12}$=3.442, $^{**}P$<0.001). Mercury levels were higher in males than in females at WCA 3AN, the opposite pattern was observed at WCA HP (Table 1).

Although there was no linear relationship between SVL and THg concentration among all frogs (Fig. 2), there was an apparent pattern in frogs from one site, ENP. An exponential curve best fit this data (x̄ Pig

Table 1
Mean Total Mercury levels (ng/g) wet weight in *R. grylio* by site and sex-size class

Site	Total N	Sample size			Mean THg (pooled sex-size classes)±SE (ng/g)			Mean THg (ng/g)	Average SVL (mm)	Average weight (g)
		Male	Female	Juvenile	Male	Female	Juvenile			
3AS 2001	20	1	2	17	148.18	132.49±7.15	118.52±33.28	121.39±31.52	75	43
3AS 2002	10	3	4	3	100.63±38.26	122.26±21.46	109.30±20.24	111.9±26	77	44
3AN	7	2	2	3	238.48±168.1	97.1±15.14	159.72±53.34	164.3±91	103	115
3A HP	9	4	3	2	45.565±13.33	87.90±29.06	167.49±107.03	86.8±63.2	94	85
2A	8	3	3	2	123.14±100.27	116.21±61.64	80.58±40.63	109.9±65	95	79
3B	11	4	4	3	209.22±26.60	247.17±107.85	192.03±9.18	218.3±64.7	97	92
ENP	14	6	4	4	1234.71±448.14	999.28±340.31	376.42±60.11	911.5±464	93	86
FC	9	3	3	3	480.54±251.11	279.43±65.85	288.83±200.32	349.6±184	85	67
Total	88	26	25	37	440.69±522.09	309.55±357.59	212.57±129.02	329.24±391.38		

Sites. 3AS (years 2001 and 2002): WCA 3A South, between Tamiami Trail and I-75; 3AN: WCA 3A North of I-75; 3A HP: WCA 3A near Holiday Park; 2A: WCA 2A; WCA 3B; ENP: Everglades National Park, Shark River Slough; FC: Frog City.

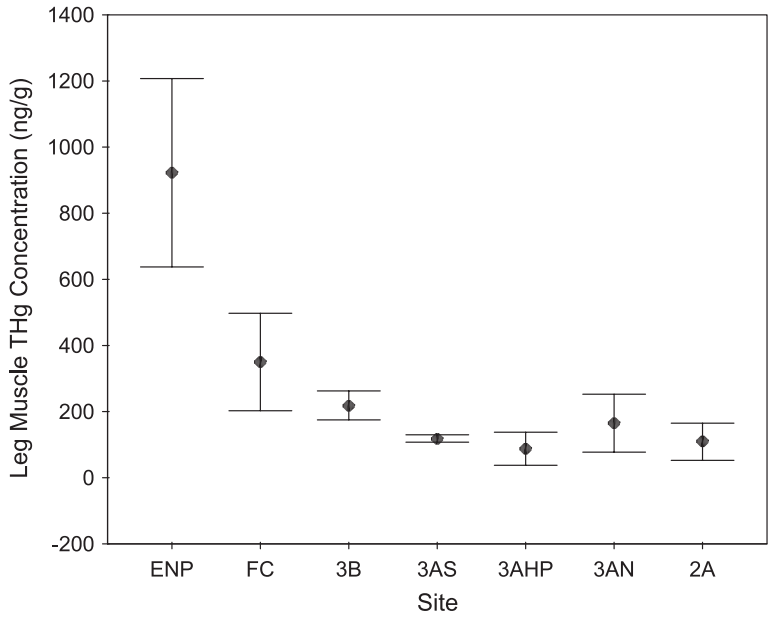

Fig. 3. The relationship between THg concentration (ng/g) and snout-vent length (SVL) in *R. grylio* from the Everglades.

Frog THg=58.688*$e^{SVL*0.0282}$) ($P<0.001$, $R^2=0.82$, $F_{1,2}=53.81$).

4. Discussion

Frogs are generally considered to be short-lived consumers that occupy an intermediate trophic position within wetland systems. Therefore, it has been assumed that their accumulated mercury levels would be low (Ware et al., 1990). Our data suggest that pig frogs may be important in the transfer of MeHg through parts of the Everglades food web. We found substantial variation in THg in *R. grylio* (33.07–2051 ng/g) across the South Florida Everglades. Levels in some frogs from ENP (Shark Slough), Frog City, WCA 3AN and 3B were higher than the USEPA human health fish tissue criterion of 0.300 mg/kg (USEPA, 2001). In a small sample of pig frogs, we also found levels of THg in the liver were two to five times the levels of THg found in leg-muscle tissue (Table 2). This high concentration of THg in leg muscle and additional organs suggests that these frogs also exceed the methylmercury criterion for the protection of piscivorous wildlife (0.077 mg/kg), and therefore may pose a substantial risk to wildlife within some areas (e.g., ENP, WCA 3B, Frog City, WCA 3AN) (USEPA, 1997). High levels of Hg at these intermediate levels may help explain high levels of Hg in top predators (Loftus, 2000; USEPA, 2001). Although it appears that liver levels are consistently higher than in leg tissues of the same individual (Table 2), additional sampling is necessary to validate the pattern we observed.

We found a north–south trend in THg among these frogs, with higher concentrations occurring in the southern populations. Frogs south of Tamiami Trail had higher mean THg concentration levels than other

Table 2
Mean wet weight levels of total mercury in different tissues of *R. grylio* (three replicates except for * which consisted of one specimen)

Frog	Tissue	Mean THg level (ng/g)	SE
A	Liver	544.41	6.05
A	Leg	139.60	6.58
B	Liver	291.74	32.28
B	Brain*	61.64	15.23
B	Leg	127.45	3.32
C	Liver	253.24	14.59
C	Leg	143.21	10.4
D	Liver	269.03	44.55
D	Leg	87.43	2.98

geographic areas we sampled. Spatial patterns of THg levels are similar to those found in alligators (Rumbold et al., 2002a). Average levels and variation in THg within specifically ENP were high, and are similar to results obtained for alligators (Rumbold et al., 2002a). High THg levels have been found in *Gambusia* from parts of ENP, and the central part of WCA 3A (Stober et al., 1998). We also found high THg levels in ENP, but did not find significantly high levels of THg in leg muscle tissue from some parts of WCA 3A, where previous studies detected a large mercury hotspot. Although, recent research suggests that this "hotspot" has been reduced due to emission, DOC and sulfate control (Axelrad et al., 2004), other studies have not found directional north–south trends in THg levels, (Stober et al., 1998; Arfstrom et al., 2000).

Stober et al. (2001) analyzed THg and MeHg along a north-to-south nutrient gradient in the greater Everglades. They suggest that food web complexity (detrital and primary productivity-based pathways), in addition to hydropattern and habitat alteration influence mercury methylation across the system. More recently however, it is believed that sulfates are an important factor in facilitating the methylation process, and that release of sulfates from agricultural areas (EAA) into canals and the WCA's may be responsible for trends in methylmercury production across the Everglades (Axelrad et al., 2004).

Diet, sex, weight, and age have not been considered sufficient factors to explain high mercury levels, in some Everglades fauna (Rumbold et al., 2002a). Patches of invertebrate or vertebrate Pig Frog prey with high levels of THg may explain some of the observed variability in THg (Loftus, 2000). We can find distinct differences in diet prey among sites, for example, in 3B and ENP, crayfish and amphibians were common food items, whereas hemipterans are a common item for pig frogs in WCA 3AS. Linking diet of pig frogs and THg is difficult however, since their diet is dynamic and varies within and across each of the geographic areas we sampled seasonally and probably annually (Ugarte et al., in preparation).

Long-lived organisms typically have higher levels of MeHg than short-lived organisms (Leady and Gottgens, 2001). Inclusively, in some species such as fish, older individuals accumulate more mercury than younger individuals (USEPA, 1997). *Rana grylio* has variable age structure across the Everglades and can live for at least three years, and probably more (Ugarte et al. in prep), since bullfrogs, a close relative of the Pig Frog, live to at least sixteen years (Bury and Whelan, 1984). The fact that Pig Frog body size did not correlate well in most areas with THg concentration is not surprising, because size is not always a good indicator of age in amphibians, especially in reproductively mature frogs (Maiorana, 1976; Platz and Lathrop, 1993; Wake and Castanet, 1995). The exponential relationship between SVL and THg in Shark Slough, ENP, the protected area, suggests that these frogs are older than frogs of the same size from other harvested areas (WCAs and FC). Heavy harvest pressure from the other areas (southern WCA 3A) may remove older individuals from populations. Consequently, ENP frog populations, which are protected from harvest, would include older frogs with higher mean Hg levels than frog populations at harvested sites. In the other areas, it is also possible that the relationship between size and THg may not emerge unless THg levels are sufficiently high.

Although many studies have examined mercury concentrations in organisms consumed by humans and wildlife (e.g., fish, alligators, wading birds), few have investigated mercury concentrations in wild populations of harvested frogs (Ware et al., 1990; Gerstenberger and Pearson, 2002). Gerstenberger and Pearson (2002) measured mercury in *Rana catesbeiana* from Nevada near active mines and found low levels of mercury. Ware et al. (1990) found low levels in whole bodies of *R. grylio*; however, frogs were collected from one area, WCA 3A North. Arnold (2000) found high levels of total mercury (1000 ng/g) in *R. grylio* from Okeefenokee Swamp.

Because vertebrates from the Everglades are known to have high levels of mercury, it is important to determine THg levels in *R. grylio* and the organisms they consume (Roelke, 1990; Ware et al., 1990; Sundlof et al., 1994; Loftus, 2000; Rumbold et al., 2002a). This information will be especially useful for assessing the health risks to humans and clarifying the role of pig frogs within the transfer of Hg in the aquatic food web.

5. Conclusion

Leg tissue of THg was highest in protected areas of Everglades National Park, where harvesting is prohibited. The THg levels in *R. grylio* leg tissue from

most harvested areas are below the USEPA 0.3 mg/kg human Health Fish Tissue Criterion (USEPA, 2001). However, the average concentration of THg in frogs collected near Frog City, and some from WCA 3B and 3AN, harvested sites, had THg levels above this criterion. Most frogging in South Florida is believed to be recreational (Enge, 1992), therefore economic impacts should be minimal. Froggers, both recreational and commercial should avoid frogging in areas with high THg concentrations. Although our current understanding of Hg transfer throughout the Everglades food web is not complete, our data provides information about a harvested, commercially available food source for humans. Further collections of these frogs may help refine our understanding of mercury spatial patterns within WCA 3A, and clarify patterns of additionally high levels of Hg in frogs.

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