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Effect of Body Size on Methylmercury Concentrations in Shoreline Spiders: Implications for Their Use as Sentinels

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Abstract: Shoreline spiders have been proposed as sentinels to monitor aquatic contaminants including methylmercury (MeHg). The present study examined the effect of spider body size on MeHg concentrations in shoreline spiders. We collected 6 taxa of spiders belonging to 4 families (orb-weavers [Araneidae], long-jawed orb weavers [Tetragnathidae: *Tetragnatha* sp.], jumping spiders [Salticidae], and wolf spiders [Lycosidae: *Pardosa* sp., *Rabidosa* sp., and *Schizocosa* sp.]) from the shorelines of 14 human-made ponds at the Lyndon B. Johnson National Grasslands in north Texas (USA). As a proxy for body size, we measured leg length (tibia + patella) of each spider. Spider taxa differed by 3-fold in mean MeHg concentration, and MeHg concentrations in 4 of 6 spider taxa increased significantly with leg length. The present study is the first to demonstrate that shoreline spider MeHg concentrations increase as a function of spider body size. Because spider size may account for some within-taxa variation in MeHg concentrations, future studies that utilize spiders as sentinels of aquatic contamination by MeHg or other biomagnifying contaminants should take spider size into account. *Environ Toxicol Chem* 2021;40:1149–1154. © 2020 SETAC.

Keywords: Spiders; Mercury; Body Size; Sentinel; Bioaccumulation

INTRODUCTION

Because of widespread atmospheric deposition of inorganic mercury (Hg), all waterbodies contain food webs that are contaminated with Hg (Chen and Driscoll 2018). The level of Hg contamination within waterbodies varies across the landscape due to variations in atmospheric deposition of inorganic Hg and the biogeochemical factors that affect the transport and methylation of inorganic Hg to form toxic and bioaccumulative methylmercury (MeHg; Evers et al. 2007; Hsu-Kim et al. 2018). One efficient and inexpensive way to monitor Hg contamination of aquatic food webs is to quantify MeHg concentrations in a sentinel organism (Mason et al. 2005; Eagles-Smith et al. 2020), defined as an organism that accumulates contaminants in its tissues without significant adverse effects (Beeby 2001). Ideally, sentinel organisms should be widely distributed, found in high abundance in the environment, easy to collect and identify and their tissue concentrations should reflect levels of a contaminant in the environment (Beeby 2001).

Shoreline spiders have been proposed as sentinels for monitoring aquatic contaminants including MeHg (Walters et al. 2008; Otter et al. 2013; Tweedy et al. 2013; Gann et al. 2015). Shoreline spiders are exposed to MeHg when they consume MeHg-contaminated emergent aquatic insects (Tweedy et al. 2013; Speir et al. 2014; Ortega-Rodriguez et al. 2019), and concentrations of MeHg differ among taxa of shoreline spiders, reflecting the proportion of aquatic prey in their diet (Ortega-Rodriguez et al. 2019). Because organisms can bioaccumulate MeHg as they age and grow (Wiener et al. 2003), we hypothesized that, for any given taxa of spider, the concentrations of MeHg within their tissues would increase with body size. In the present study we show that average concentrations of MeHg differ among shoreline spider taxa and that, within some taxa, the concentration of MeHg increases as a function of body size. Our results suggest that studies using spiders as sentinels to assess MeHg contamination in the

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environment should include data on spider body size to account for within-taxa variance in MeHg concentrations.

MATERIALS AND METHODS

Study site

Shoreline spiders were collected from 14 human-made ponds at the Lyndon B. Johnson National Grasslands, Wise County, Texas, USA (Supplemental Data, Figure S1). The Lyndon B. Johnson National Grasslands encompasses 8000 ha of prairie and hardwood forest in noncontiguous units managed by the US Department of Agriculture Forest Service and is used for recreation, livestock grazing, and wildlife management (Blackwell and Drenner 2009). Small human-made ponds are the numerically dominant type of lentic water body in the southeastern Great Plains (Chumchal et al. 2016). Many small dams (and consequently hundreds of ponds) were constructed in the Lyndon B. Johnson National Grasslands during the mid-to-late 1970s for erosion control and to provide water for livestock (Blackwell and Drenner 2009). Most of these ponds are <2000 m^2 in surface area and dry up periodically (Blackwell and Drenner 2009). The Lyndon B. Johnson National Grasslands is located in north central Texas in an ecoregion with moderate amounts of atmospheric Hg deposition (9–10 $\mu g/m^2/yr$; Drenner et al. 2013). The primary source of Hg in this region is atmospheric deposition (National Atmospheric Deposition Program 2020), and no known point source of Hg exists at the Lyndon B. Johnson National Grasslands. Previous studies have found that the aquatic food webs and emergent aquatic insects in ponds at the Lyndon B. Johnson National Grasslands are contaminated with Hg (Blackwell and Drenner 2009; Henderson et al. 2012). Concentrations of Hg in macroinvertebrates can vary among ponds (Blackwell and Drenner 2009).

Collection of spiders

We collected spiders from the ground and vegetation within 2 m from the water's edge of the ponds. Spiders were collected by hand during the night on 5, 11, and 20 June 2018, and by sweep nets during the day on 14 May and 6 July 2018. Spiders collected by hand were placed directly into new Nalgene bottles with fresh ethanol (see the Supplemental Data for information about preservation of spiders with 95% denatured ethanol). Spiders collected by nets along with incidentally collected plant material were placed into clean 2-gal zip-top plastic bags by inverting the net directly into the bag. The contents of the bag were then preserved with fresh ethanol for transport to the laboratory. In the laboratory, spiders collected using sweep nets were separated from plant material using clean forceps and transferred to new glass vials with fresh ethanol. To examine whether our sampling methods could have led to cross-contamination of spider samples, we compared the data in the present study with data from several previous studies (see the Supplemental Data). Despite differences in field and analytical methodologies between the present study and previous studies, MeHg concentrations and %MeHg values were similar across all studies, suggesting that our sampling methods did not affect Hg concentrations in spider samples (see the Supplemental Data).

Spider identification and measurement

In the laboratory, we identified spiders to family or genera using published keys (Ubick et al. 2017). We handled each spider with clean instruments, placed them in a clean Petri dish with fresh ethanol, and examined them with a dissecting microscope. After identification, we placed the spiders into clean vials with fresh ethanol with other spiders from the same taxa, pond, and sampling date. We collected 680 spiders of the following taxa: orb-weavers (Araneidae), 3 genera of wolf spiders (Lycosidae: *Pardosa* sp., *Rabidosa* sp., and *Schizocosa* sp.), jumping spiders (Salticidae), and long-jawed orb weavers (Tetragnathidae: *Tetragnatha* sp.; Table 1).

After identification, we determined the body size of each spider. We used clean instruments to place spiders in a clean Petri dish with fresh ethanol and measured them using a Zeiss Stemi 305 microscope and Zeiss Labscope measurement application (Ver 2.8.0, 2018). Measurements of sclerotized body parts (leg length, carapace width, and carapace length) have been used to quantify spider body size because they do not change with feeding or reproductive status like other body size measurements (e.g., mass or abdomen size; Hagstrum 1971; Jakob et al. 1996; Danielson-François et al. 2002). We measured "tibia + patella length" on the first leg as a proxy for body size (termed "leg length" hereafter; Higgins 1992). In addition to leg length, we measured 2 other proxies for spider body size: carapace width and carapace length (Hagstrum 1971; Elgar et al. 1990; Jakob et al. 1996). We determined that leg

TABLE 1: Taxa and numbers of shoreline spiders collected from 14 human-made ponds[a]

		Pond identification code													
Family	Genus	14A	15B	16A	27C	27D	38A	38B	38C	38D	39D	45A	45AA	76A	76B	Total
Araneidae		0 (0)	0 (0)	0 (0)	5 (1)	7 (2)	4 (1)	4 (2)	10 (3)	0 (0)	7 (1)	4 (2)	0 (0)	0 (0)	0 (0)	41 (12)
Lycosidae	*Pardosa* sp.	0 (0)	12 (2)	5 (1)	52 (2)	19 (2)	39 (3)	28 (2)	23 (2)	29 (2)	29 (2)	14 (2)	4 (1)	14 (1)	41 (3)	309 (25)
	Rabidosa sp.	11 (4)	4 (2)	6 (3)	1 (1)	2 (2)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1)	0 (0)	28 (14)
	Schizocosa sp.	0 (0)	1 (1)	0 (0)	3 (1)	0 (0)	0 (0)	1 (1)	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)	8 (3)	0 (0)	15 (7)
Salticidae		16 (2)	11 (1)	23 (2)	6 (1)	10 (1)	0 (0)	9 (2)	8 (2)	4 (1)	6 (1)	4 (1)	4 (1)	4 (1)	5 (1)	111 (17)
Tetragnathidae	*Tetragnatha* sp.	9 (1)	0 (0)	19 (3)	14 (2)	13 (2)	23 (2)	15 (2)	15 (2)	0 (0)	36 (3)	12 (2)	13 (2)	0 (0)	7 (1)	176 (22)
	Total	38 (8)	28 (6)	53 (9)	81 (8)	51 (9)	66 (6)	58 (10)	56 (9)	36 (4)	78 (7)	34 (7)	21 (4)	29 (6)	53 (5)	680 (97)

[a]Values in parentheses are the number of composite samples used for total Hg (THg) analysis.

length was positively correlated with both carapace width and carapace length in all 6 taxa of spiders (Supplemental Data, Figures S2 and S3). We used leg length as our body size measurement because measuring it with accuracy required less handling time compared with measuring carapace width or length. After measurement, each spider was placed in its own new glass vial with fresh ethanol.

To ensure adequate biomass for Hg analysis, we created composite samples of spiders of similar size (Table 1 and Supplemental Data, Table S1). For each pond, spiders were pooled by taxa and grouped by leg length such that the largest individual spider in each group had a leg length no more than 80% larger than that of the smallest individual spider (mean number of spiders/composite sample = 7 ± 0.6; range 1–35 spiders). This grouping scheme allowed us to create more composite samples with sufficient biomass for analysis than would have been possible by grouping spiders into predetermined size classes. Using this approach, we created 97 composite samples (mean number of composite samples/taxa = 16.2 ± 2.7; Table 1). Prior to combining spiders into their composite groups, spiders were dried at 60 °C for 72 h. All spiders in a composite group were then placed in a clean stainless-steel cylinder and homogenized to a fine powder using a ball-mill grinder. Homogenized tissues were transferred using clean utensils to new glass vials for storage until analyses of total Hg (THg; inorganic Hg + MeHg) and MeHg.

THg analysis

Composite spider samples were analyzed for THg using a Milestone DMA-80 Direct Hg Analyzer, which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy (US Environmental Protection Agency method 7473; 1998a). Quality assurance included reference standards (National Research Council of Canada Institute for National Measurement Standards), method blanks (empty quartz sample boats), and duplicate samples. Reference standards (DORM-4) were analyzed every 10 samples, and the average recovery percentage for DORM-4 was 90.2 ± 1.26% (mean ± standard error [SE]; range 81.0–101%; $n = 18$). The mean mass of THg in blanks was 0.12 ng (range 0.0–0.2 ng; $n = 17$). Duplicate samples were analyzed every 20 samples, and the average relative difference percentage was 4.3% (range 3.6–5.9%; $n = 5$). All samples were above the method limit of detection of 0.26 ng THg calculated by adding the limit of blank to 1.645× the standard deviation of low concentration samples (Ambruster and Pry 2008).

MeHg analysis

A subset of composite samples (*Tetragnatha* sp., $n = 15$; *Pardosa* sp., $n = 13$; *Rabidosa* sp., $n = 11$; Araneidae, $n = 9$; Salticidae, $n = 10$; and *Schizocosa* sp., $n = 7$) were analyzed for MeHg concentration at the Wildlife Toxicology Laboratory at the University of Alaska Fairbanks (USA). Dried and homogenized composite samples of spiders in glass vials were shipped to Alaska and arrived in good condition (all vials unbroken with lids sealed). Samples (~5 mg) were digested with 3 mL of 30% HNO_3 for 20 h in a water bath at 65 to 70 °C. Cooled digests were adjusted to 7 mL with ultrapure water (resistivity ≥18 MΩ/cm) and stored in the dark at room temperature until analysis within 48 h. To quantify MeHg, 100- to 200-µL aliquots of the sample digests were added to individual glass vials containing ultrapure water and acetate buffer to achieve a pH of 4.5. A 40-µL aliquot of 1% $NaBEt_4$ in 2% KOH was added to each vial, and the total volumes were adjusted to 40 mL with ultrapure water. Reference samples (DORM-4 and International Atomic Energy Agency (IAEA)-86, human hair) were digested and analyzed with the samples. Additional quality assurance included the analysis of check standards (10 pg MeHg), blank spikes (10 pg MeHg), matrix spikes (10 pg MeHg), duplicate samples, and reagent blanks. Reagent blanks consisted of 30% HNO_3 without the addition of sample. Spider and quality assurance samples were analyzed for MeHg using a Brooks Rand MERX®-M Automated MeHg Analytical System, which uses purge and trap, gas chromatography, and cold vapor atomic fluorescence spectroscopy (US Environmental Protection Agency 1998b).

All samples were analyzed in triplicate. Any samples with a coefficient of variation among replicates >15% were reanalyzed until <15% was achieved. The mean value of triplicates was used for statistical comparisons. Mean recovery percentages for DORM-4 and IAEA-86 were 92.7% (range 80.8–103.0%; $n = 6$) and 106.3% (range 84.7–125.2%; $n = 7$), respectively. The mean recovery percentages of MeHg from check standards, blank spikes, and matrix spikes were 99.8% (range 94.4–114.4%; $n = 18$), 101.2% (range 91.7–117.2%; $n = 6$), and 98.4% (range 90.0–102.9%; $n = 6$), respectively. The mean relative difference percentage between duplicates was 5.4% (range 0–10.8%), and the mean recovery was 96.1% (range 90.2–102.2%; $n = 6$). The mean mass of MeHg in digestion blanks was 0.05 pg (range 0.0–0.1; $n = 10$). All samples were above the method detection limit of 0.25 pg MeHg, calculated by adding the mean of reagent blanks to 3× the standard deviation of the same blanks.

Due to high analytical costs, MeHg could not be measured directly for all composite samples. To estimate MeHg concentrations in individual samples, we determined the average percentage of THg that was MeHg for each spider taxa using the subset of composite samples analyzed for MeHg ($n = 65$; Supplemental Data, Table S1). The average %MeHg for each respective spider taxa was then used to estimate MeHg concentrations from THg concentrations in the spider composite samples not directly analyzed for MeHg ($n = 32$; Supplemental Data, Table S1).

Statistical analysis

To determine whether average concentrations of MeHg differed among spider taxa, we used a one-way analysis of variance (ANOVA), followed by a Tukey–Kramer post hoc test. For each of the 6 taxa of spiders, we used linear regression to

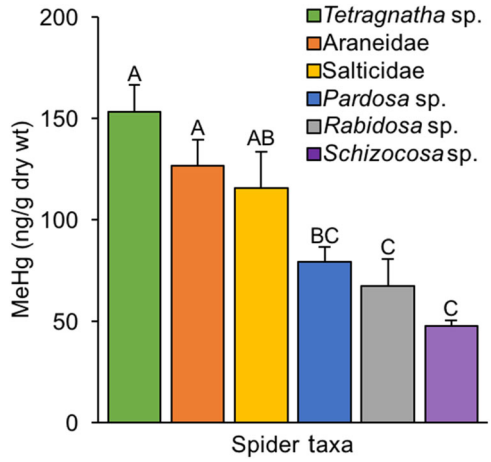

FIGURE 1: Mean methylmercury (MeHg) concentrations (mean ± standard error) for 6 taxa of shoreline spiders collected from 14 human-made ponds. Significant differences in mean MeHg concentration among taxa are indicated by different upper-case letters.

determine the relationship between mean spider leg length and MeHg concentration. Concentrations of MeHg were $\log_{10}$-transformed before analysis to improve homogeneity of variance and normality, but untransformed data are presented in the figures for ease of interpretation. Statistical significance was determined at $p < 0.05$, and statistical tests were performed using JMP Ver 15.

RESULTS

Mean concentrations of MeHg (mean ± SE) in spider taxa ranged from 47.5 ± 2.8 to 153 ± 16.9 ng/g dry weight in *Schizocosa* sp. and *Tetragnatha* sp., respectively, and were significantly different among spider taxa (Figure 1; ANOVA, $F_{5,91} = 11.2$, $p < 0.001$). Mean MeHg concentrations in *Tetragnatha* sp. and Araneidae were significantly higher than mean MeHg concentrations in *Pardosa* sp., *Rabidosa* sp., and *Schizocosa* sp. (Tukey–Kramer post hoc test, $p \leq 0.032$). The mean MeHg concentration of Salticidae was significantly higher than those of *Rabidosa* sp. and *Schizocosa* sp. (Tukey–Kramer post hoc test, $p \leq 0.014$).

Concentrations of MeHg increased significantly with mean spider leg length in 4 of the 6 spider taxa we examined (Table 2 and Figure 2). The mean percentage of variation in MeHg concentration explained by leg length (i.e., r^2) was 34% and ranged from 22 to 50% for *Pardosa* sp. and Salticidae, respectively (Table 2). The slope of the relationship between $\log_{10}$-MeHg and average leg length represents the rate at which MeHg increases with size, with a positive slope indicating that MeHg increased with spider size. We used the inverse log of the slope (Table 2) to determine that, on average, the concentration of MeHg increased by 1.3× with each mm of leg length (range 1.09–1.5× increase in MeHg concentration/mm of leg length in *Tetragnatha* sp. and Salticidae, respectively).

DISCUSSION

In the present study, the 6 taxa of shoreline spiders common around ponds at Lyndon B. Johnson National Grasslands exhibited 3-fold differences in average MeHg concentrations. Among spider taxa, average MeHg concentration is positively correlated with the proportion of aquatic prey in the diet (Ortega-Rodriguez et al. 2019). Therefore, we hypothesize that the taxonomic variation in MeHg concentration observed in the present study may be explained in part by differences in spider diet. For example, *Tetragnatha* sp. build webs over water, consume a high proportion of aquatic prey (Tweedy et al. 2013), and have high MeHg concentrations compared with ground hunting taxa that consume a mix of terrestrial and aquatic prey (e.g., Lycosidae: Michalko and Pekár 2016; Ortega-Rodriguez et al. 2019).

The present study is the first to demonstrate that MeHg concentration increases with size in shoreline spiders. Concentrations of MeHg were found to increase significantly with spider size in 4 of 6 taxa. Because the spiders in our analysis were collected from different ponds and the MeHg concentrations in spiders varied between ponds (see the Supplemental Data), our study represents a conservative evaluation of the effects of spider size on MeHg concentration. Furthermore, our ability to detect a significant impact of size in the majority of taxa, despite differences between ponds, suggests that the relationship between MeHg concentration and spider size is robust. Between-pond variation in MeHg contamination may explain why we did not observe a significant relationship between spider size and MeHg concentration in all taxa, and we hypothesize that studies that assess the effect of size on spider MeHg concentrations from a single location will find an even stronger effect of size on MeHg concentration.

Although an increase in MeHg concentration with size is consistent with age-related bioaccumulation (Wiener et al.

TABLE 2: Significance values, coefficients of determination, and regression equations for the relationship between spider leg length (mm) and $\log_{10}$-methylmercury (MeHg) concentrations (ng/g dry wt) for 6 taxa of shoreline spiders collected from 14 human-made ponds[a]

Family	Genus	*p* value	r^2	Regression equation
Araneidae		0.044	0.35	$\log_{10}(\text{MeHg}) = 1.8 + 0.05$ (leg length)
Lycosidae	*Pardosa* sp.	0.017	0.22	$\log_{10}(\text{MeHg}) = 1.4 + 0.18$ (leg length)
	Rabidosa sp.	0.23		
	Schizocosa sp.	0.077		
Salticidae		0.002	0.50	$\log_{10}(\text{MeHg}) = 1.6 + 0.18$ (leg length)
Tetragnathidae	*Tetragnatha* sp.	0.009	0.29	$\log_{10}(\text{MeHg}) = 1.9 + 0.04$ (leg length)

[a]Sample sizes reported in Table 1.

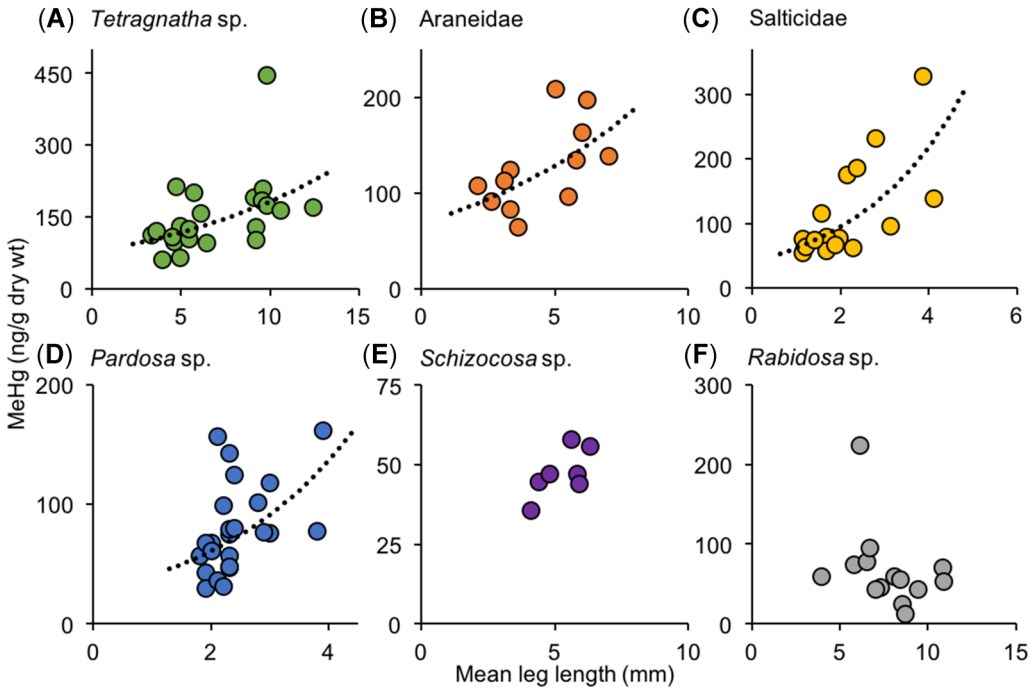

FIGURE 2: (**A–F**) Relationships between concentrations of methylmercury (MeHg) and leg length for 6 taxa of shoreline spiders collected from 14 human-made ponds. Note: *x* and *y* axes are not consistent across taxa.

2003), an increase in MeHg with spider size would also be expected if larger spiders consumed larger, and potentially more MeHg-contaminated, prey than smaller spiders (Chumchal and Hambright 2009; Bartrons et al. 2015). Regardless of the mechanism, on average MeHg concentration increased in 4 shoreline spider taxa by a factor of 1.3× with each mm of spider leg length. Because spider size may account for some within-taxa variation in MeHg concentrations, future studies that utilize spiders as sentinels of aquatic contamination by MeHg or other biomagnifying contaminants should take spider size into account.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4964.

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Author Contribution Statement—M.P. Hannappel, M.M. Chumchal, and R.W. Drenner designed the study. M.P. Hannappel, M.M. Chumchal, R.W. Drenner, F.M. Willoughby, and L.P. Trauffler conducted field work. M.P. Hannappel, identified, measured, and prepared all specimens for analysis with the assistance of J.H. Kennedy, A.R. Nolan, and L.P. Trauffler. M.P. Hannappel and A.R. Nolan conducted THg analysis. B.D. Barst and J.M. Castellini conducted MeHg analysis. M.P. Hannappel wrote the manuscript with the assistance of M.M. Chumchal, R.W. Drenner, J.H. Kennedy, B.D. Barst, and J.M. Castellini. Funding was acquired by M.M. Chumchal, R.W. Drenner M.P. Hannappel, L.P. Trauffler, and A.R. Nolan.

Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (mphannappel2@gmail.com).

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