Science of the Total Environment 607–608 (2017) 1056–1064

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evaluating mercury concentrations and body condition in American alligators (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge (MINWR), Florida

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HIGHLIGHTS

- Mercury concentrations in blood of alligators were investigated (2007–2014).
- Index created for monitoring the health status of alligators using body condition.
- Alligators with low BMI coincided with elevated mercury concentrations.
- Size is a significant predictor of mercury concentration in female alligators.

GRAPHICAL ABSTRACT

An American alligator residing in Merritt Island National Wildlife Refuge, FL. Photo taken in front of NASA's Kennedy Space Center Vehicle Assembly Building by Louis J. Guillette Jr.

ARTICLE INFO

Article history:
Received 10 March 2017
Received in revised form 6 July 2017
Accepted 8 July 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Mercury
Seasonal
Alligator
Florida
Behavior
Health

ABSTRACT

Concentrations of mercury (Hg) are not well studied in free-ranging wildlife. Atmospheric deposition patterns of Hg have been studied in detail and have been modeled for both global and specific locations and often correlate to environmental impact. However, monitoring the impact of Hg deposition in wildlife is complicated due to local environmental conditions that can affect the transformation of atmospheric Hg to the biologically available forms (e.g., rainfall, humidity, pH, the ability of the environment to methylate Hg), as well as affect the accessibility to organisms for sampling. In this study, Hg concentrations in blood samples from a population of American alligators (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge (MINWR), FL, USA, over a seven-year period (2007 to 2014; $n = 174$ individuals) were examined to assess Hg variation in the population, as well as the difference in Hg concentration as a function of health status. While most of this population is healthy, 18 individuals with low body mass indices (BMI, defined in this study) were captured throughout the sampling period. These alligators exhibited significantly elevated Hg concentrations compared to their age/sex/season matched counterparts with normal BMI, suggesting that health status should be taken into account when examining Hg concentrations and effects. Alligator blood Hg concentrations were related to the

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http://dx.doi.org/10.1016/j.scitotenv.2017.07.073
0048-9697/Published by Elsevier B.V.

interaction of age/size, sex, and season. This study illustrates the value of a routinely monitored population of large predators in a unique coastal wetland ecosystem, and illuminates the value of long-term environmental exposure assessment.

1. Introduction

Mercury (Hg) is a naturally occurring elemental contaminant that is ubiquitous in the environment. Deposition of Hg across the planet and concentrations in the environment fluctuate based on local environmental parameters (Sexauer Gustin et al., 2012; Shia et al., 1999). Once Hg enters the environment, bacteria in soil and sediment can convert it to the organic form (methylmercury) that can enter the food web (Compeau and Bartha, 1985; Kerin et al., 2006). Since Hg biomagnifies, large, upper-trophic level predators may be subject to more severe symptoms of Hg toxicity (Atwell et al., 1998; Chumchal et al., 2011; Snodgrass et al., 2000). Mercury exposure in both wildlife and humans has been linked to complications in reproduction, neurological function, embryonic development, and increased risk of adult-onset diseases (Ceccatelli et al., 2010; Devlin, 2006; Frederick et al., 2011; Frederick and Jayasena, 2010; Jayasena et al., 2011; Kurland et al., 1960). In carnivorous reptiles, high Hg concentrations have been reported, but few deleterious effects of Hg exposure have been documented (Brisbin et al. 1998; Blanvillain et al., 2007; Burger, 2002; Nilsen et al., 2016).

Mercury contamination in Florida, USA, has been a consistent environmental concern since the 1980s, when large-mouth bass (*Micropterus salmoides*) were discovered to contain Hg concentrations in muscle tissue exceeding the limit safe for human consumption (1.5 μg/g) (Lange et al., 2000). Despite significant bioremediation efforts throughout the state, very high environmental concentrations of Hg persist in Florida and resident wildlife (Cleckner et al., 1998; Evans and Crumley, 2005; Mazzotti and Brandt, 1994; Mazzotti et al., 2009; Rumbold et al., 2002; Ugarte et al., 2005; Yanochko et al., 1997). Many site-specific studies have assessed Hg burden, with numerous studies revealing that the more southern sites in Florida, specifically the Everglades, have greater Hg burdens (Table 1) (Burger et al. 2000; Delaney et al. 1988; Heaton-Jones et al., 1997; Horai et al. 2014; Nilsen et al., 2016; Rumbold et al., 2002; Yanochko et al., 1997). Here, Hg concentrations in the American alligator population located at Merritt Island National Wildlife Refuge (MINWR), an important conservation area for several endangered and threatened wildlife species in Florida, are discussed (Breininger et al., 1991; Breininger et al., 1996; Provancha and Provancha, 1988). Horai et al. (2014) reported elevated Hg concentrations in the livers of American alligators (*Alligator mississippiensis,* hereafter referred to as 'alligators') at MINWR relative to two other sites in northern Florida, Lakes Apopka and Woodruff. Concentrations in liver samples at MINWR ranged from 90 to 864 ng/g, with the mean being 441 ng/g. Whereas, concentrations from Lake Woodruff and Lake Apopka were lower, ranging from 173 to 570 ng/g, with a mean of 348 ng/g; and 16–137 ng/g with a mean of 69 ng/g, respectively (Horai et al. 2014). Concentrations in liver samples at MINWR are well below the range recently reported for alligators of the same age class in the Everglades, 567–14,293 ng/g, with a median liver concentration of 3,594 ng/g (Nilsen et al., 2017). These values suggest that MINWR may be classified as a moderate site for Hg accumulation (Table 1).

The geographic region including MINWR, North central Florida, has seasonally varying concentrations of Hg deposition from the atmosphere with the highest deposition rates in the summer months (NADP, 2017). This variation is a reflection of increased seasonal rainfall, and increased wet deposition of Hg, which accounts for up to 90% of Hg contamination in inland water bodies (NADP, 2017). Southern Florida has previously been subject to a variety of anthropogenic Hg sources with concentrations far exceeding those in Northern Florida, but atmospheric deposition remains the greatest source of Hg throughout Florida, (Axelrad et al., 2009; Guentzel et al., 1995; NADP, 2017; Sexauer Gustin et al., 2012). The environment of MINWR exhibits unique features making it well suited for use as a model system to examine Hg exposure and accumulation in wildlife. MINWR is located within a typical coastal marsh ecosystem that has restricted access from the public. MINWR contains a robust population of alligators. Alligators are large predators known to live >70 years, facilitating bioaccumulation of Hg (and other persistent environmental contaminants) relative to shorter-lived predators and prey items (Axelrad et al., 2009; Lange et al., 2000, Wilkinson et al., 2016). Collectively, these features make MINWR an attractive site for studying the patterns and sub-lethal effects of chronic environmental Hg exposure in a long-lived sentinel species (Delany et al., 1988; Milnes and Guillette, 2008; Rumbold et al., 2002). Examination of seasonal Hg concentrations in large predators may provide insight into accumulation patterns, and may explain Hg exposure influence in the context of ecological, physiological, and behavioral characteristics. In addition, data on Hg concentrations in wildlife over consecutive months or years can supplement studies conducted during periods that are more limited (e.g., only a few

Table 1
A comparison of published values for mercury (Hg) in the tissues of American alligators (*Alligator mississippiensis*) in the state of Florida.

Location	[Hg] in mg/kg				Source
	Liver	Muscle	Scute	Blood	
Farm raised	0.1	0.1	0.08		Heaton-Jones et al. 1997
Florida sites					
Lake Apopka[1]		0.11			Delany et al. 1988
Lake Apopka[1]	0.108	0.057			Burger et al. 2000
Lake Apopka[1]	0.44				Horai et al. 2014[a,b]
Lake George[2]		0.04			Delany et al. 1988
Lake Hancock[3]		0.1			Delany et al. 1988
Lake Iamonia[4]		0.61			Delany et al. 1988
Lake Kissimmee[5]				0.417	Nilsen et al. 2016
Lake Lochloosa[6]				0.148	Nilsen et al. 2016
Lake Newnans[7]		0.27			Delany et al. 1988
Lake Orange[8]		0.37			Delany et al. 1988
Lake Rodman[9]		0.51			Delany et al. 1988
Lake Trafford[10]				0.198	Nilsen et al. 2016
Lake Trafford[10]		0.43			Delany et al. 1988
Lake Woodruff[11]	1.94				Horai et al. 2014
Merritt island NWR[12]	3.02				Horai et al. 2014[a,b]
Merritt island NWR[12]				0.152	This Study
St Johns River[13]				0.177	Nilsen et al. 2016
Everglades sites					
Big Cypress[13]	3.9	0.4			Rumbold et al. 2002
Big Cypress[13]	2.52	0.33	0.34		Heaton-Jones et al. 1997
Everglades National Park[14]	10.4	1.2			Rumbold et al. 2002
Holiday Park[15]	9.97	1.12	1.93		Yanochko et al. 1997[a]
WCA1[16]	1.7	0.3			Rumbold et al. 2002
WCA2A[17]				1.56	Nilsen et al. 2016
WCA2A[17]	4.5	0.75			Rumbold et al. 2002
WCA2A & 3A	39.99	2.61	1.03		Heaton-Jones et al. 1997
WCA3A[18]				1.33	Nilsen et al. 2016
WCA3A[18]	10.37	1.22	4.13		Yanochko et al. 1997[a]
WCA3A north	3.9	0.5			Rumbold et al. 2002
WCA3A south	2.3	0.5			Rumbold et al. 2002

Superscript numbers denote location on map in Fig. 1.
[a] Converted from dry weight.
[b] Converted based on 75% moisture as no value was provided.

weeks, months, or years), as well as provide a baseline for evaluating future seasonal studies (Lance, 2003).

In this study, the alligator population at MINWR was sampled to investigate the seasonal fluctuation of Hg concentration in alligators over multiple years. Due to the long time-scale of this study, the high number of individuals sampled, and the expanse of MINWR that the samples cover, the variation of Hg concentration in this population is assessed seasonally, annually, and spatially. Additionally, this population presented the opportunity to mathematically characterize the health status of alligators based on two common morphometric measurements: tail girth (TG) and snout-vent length (SVL). Once characterized, the health status was compared to Hg concentration.

2. Materials and methods

2.1. Sample collection

Researchers with the Florida Fish and Wildlife Conservation Commission and the Medical University of South Carolina handled alligators using methods approved by the American Society of Ichthyologists and Herpetologists (ASIH, 2004). At time of capture, morphometric measurements were made, including snout-vent length (SVL) and tail girth (TG), as well as a field assessment of body condition, prior to the collection of blood samples (Table S1). Blood samples were chosen, as this is a non-invasive sample that allows for routine and repeated collection from the same alligator. Blood Hg measurements also provide a proxy of internal tissue Hg concentrations (Nilsen et al., 2017). A total of 174 adult alligators (total length > 90 cm) were sampled from MINWR (Merritt Island, FL; 28.585596°N, −80.649514°W), including at least two males and two females, per season, per year, from summer 2007 to spring 2014 (Table S1). Alligators are sexually dimorphic and experience determinant growth, but reach sexual maturity at approximately the same SVL (approximately 100 cm) (Wilkinson et al., 2016). Thus, all alligators were classified as adults in this study. During this period, 19 individuals were recaptured, for a total of 193 samples (Table S1, S2). For each alligator, blood samples were collected via the post-occipital venous sinus with a sterile 18.5-gauge needle and 60 mL syringe immediately following capture (Myburgh et al. 2014). Blood samples were then transferred to a 10 mL lithium heparin Vacutainer collection tube (BD, Franklin Lakes, NJ) and kept on wet ice for no longer than 5 h before being frozen at −20 °C until analysis.

The Marine Environmental Specimen Bank at the National Institute of Standards and Technology stores the samples used in this project.

2.2. Body mass index calculation

Alligators were classified as having a low body mass index (BMI) based on their observed emaciation upon capture (Fig. S1). Then, a mathematical description of BMI was applied:

$$(\mathrm{BMI} = \mathrm{TG}/(\mathrm{SVL} \times 2)) \quad (1)$$

where tail girth (TG, in cm) is divided by 2× the snout to vent length (SVL, in cm) to yield the BMI. BMI of <0.18 equated to a 'low' BMI (18 individuals fit this description; Table S1). The TG and SVL are the circumference of the tail at the urogenital slit and the length from the snout to the urogenital slit, respectively.

2.3. Blood hg analysis

For analysis of Hg concentration, blood samples were thawed at room temperature and gently mixed by inversion for 30 s. The mass fraction of Hg was determined in one aliquot (100 μL) of whole blood with a direct Hg analyzer (DMA-80, Milestone, Shelton, CT). The National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3133, Mercury Standard Solution was used for external calibration with a sixteen-point curve ranging from 0.5 ng to 800 ng of Hg. The limit of detection for this curve was 0.5 ng of Hg. NIST SRM 955c levels 2 and 4, Toxic Metals in Caprine Blood, were used as a control materials, with reference values for total Hg at 4.95 ng/g ± 0.76 ng/g and 33.9 ng/g ± 2.1 ng/g, respectively. NIST SRM 955c was run concurrently with blood samples to ensure replicable measurements from the instrument. Blood samples were analyzed randomly, using field codes rather than metadata, to remove bias during analysis. Procedural and field blanks were analyzed concurrently, by use of an empty sample vessel and field-matched whole blood sampling supplies prepared with Milli-Q water. If blanks were above the detection limit, the samples were blank corrected. The relative standard deviation (RSD) for SRM 955c level 2 and level 4 were 0.10 and 0.08, respectively, and the expanded uncertainty values were 0.05 ng/g and 0.03 ng/g, respectively.

2.4. Statistical analysis

Kolmogorov's D goodness of fit test and Levene's Test were used to test the assumptions of normality and homoscedasticity. The data failed both tests, but under a log10 transformation, the data met the assumptions of parametric statistics. Outliers were removed from the following analyses based on the standard 1.5 × interquartile range (IQR) guidelines of the transformed data, but the measured values are presented in Table S1 (Navidi, 2006). A Generalized Linear Model (GLM) using the maximum likelihood estimation method was used to examine the variation of Hg across MINWR based on all available variables (BMI, year, season, sex, SVL, and sub-location within MINWR) following the guidelines provided by Carey (2013). It should be noted that the seasonal periods of winter 2011, summer 2013, spring 2013 and fall 2013 were not represented in this analysis, as the sample integrity (i.e., number of freeze/thaw cycles, low vial integrity (cracked or broken)) was questionable. Seasons are defined as winter (December to February), spring (March to May), summer (June to August), and fall (September to November).

The interaction between variables that were biologically appropriate were included in the GLM: sex × SVL, year × season, sex × season, MINWRsub-location × year, MINWRsub-location × sex and sex × season × SVL. The Akaike Information Criterion, corrected for finite sample sizes, was used to determine the quality of the GLM (AICc) (Bartón, 2013). The altered BMI status of alligators in this population is a predictor for changes in Hg concentration, as the mobilization of fat and muscle lead to increased contaminant concentrations in the blood (Jepson et al., 2005; Keller et al., 2014). The initial model resulted in BMI status being highly significant ($p < 0.001$), so the data were separated based on BMI, to determine if other variables were predictive of Hg concentration.

When BMI was separated, the GLM over fit the low BMI data, and did not provide useful information (Table S3). To further investigate the BMI relationship that was observed in the initial GLM analysis, an ANOVA was used to compare the 18 alligators that were captured with a low BMI and 18 alligators of normal BMI (value >0.18), of the same sex, capture season, and SVL (Table S1). The exploratory analysis conducted on the recaptured alligators used the non-parametric matched-pair Wilcoxon signed rank analysis, as the smaller sample size lead to a non-normal distribution.

2.5. ArcGIS methods

ArcGIS ArcMap 10.3 (ESRI, Redlands, CA) was used to interpolate the spatial variation in alligator Hg concentration using capture locations and measured blood Hg concentrations, to assess the potential for Hg point sources and to examine temporal and spatial dynamics of alligator Hg concentration at MINWR. MINWR was divided into three separate sub-locations using physical barriers of the roads within MINWR and the west bank of the Banana River to assist with the location of a point source and determine if elevated Hg concentrations are localized within

MINWR. The three sub-locations were the Indian River area (IR), Vehicle Assembly Building area (VAB), and the Banana River area (BR). The shape file for the MINWR base map was downloaded from http://geodata.myfwc.com/ at ocean.floridamarine.org/mrgis/Description_Layers_ Marine.htm and trimmed to display only the results from the study site. All GPS points for alligator capture locations were plotted as positive or negative decimal degrees using the World Geodetic Survey (WGS) 1984 coordinate plotting system, which plots coordinates on a 3-dimensional globe for accurate representation of the location. Blood Hg concentrations were interpolated on the map using the spline with barriers spatial analysis tool, based on the shape file boundaries. Data were re-sampled for display using the nearest neighbor method for discrete data and manually classified into four groups based on increments of 50 ng/g of Hg. Seasonal average rainfall (SAR) data was collected from the two closest monitoring stations (Daytona Beach, FL (north of MINWR) and Melbourne, FL (south of MINWR)) and averaged them to estimate MINWR's rainfall.

3. Results and discussion

3.1. BMI

Within the seven-year sampling period, 18 animals of the 174 unique alligators captured were identified as having a low BMI (Fig. S1, Table S1 and S2). The cutoff of 0.18 was assigned based on the condition noted in the field by the MINWR biologists upon capture. The mathematical metric is combined with the field notes, as there is great value in the institutional knowledge of scientists that have worked with the alligators at MINWR for decades. A BMI score of 0.18 is where the mathematical equation and field notes coincided, indicating a precipice value for health status of alligators at MINWR. This equation was developed rather than using a known metric, such as Fulton's condition factor (K), as the weight of the animals captured and sampled for this study was not recorded (Charles and Alan, 2003). In addition, Fulton's K was developed for salmonid fishes and uses the total length of the individual, which is an inconsistent measurement in alligators as they can lose the tips of their tails (Charles and Alan, 2003; Wilkinson et al., 2016). The use of total length and weight for Fulton's K make it inappropriate for the alligator. By using twice the SVL measurement, total length is estimated without the inconstancy of measuring the tail, and by using TG body condition is estimated as these animals cannot be weighed in the field. Our BMI calculation is quite similar to Fulton's K, but is modified for a larger, different class of animal. Using this equation to distinguish the two BMI groups, the low BMI alligators had an average BMI of 0.16 ± 0.01, and the normal BMI alligators had an average BMI of 0.22 ± 0.01 (Table S1).

3.2. Effect of hg burden on BMI status

Across MINWR, Hg concentrations in blood of adult alligators with normal BMI ranged from 39 to 568 ng/g, with the average being 174 ± 86 ng/g. Blood Hg concentrations in adult alligators with a low BMI ranged from 112 to 510 ng/g, with the average being 260 ± 115 ng/g (Table S1). For comparison of Hg concentration between the two BMI states, 18 alligators of normal BMI of matched sex, capture season, and SVL were paired to the low BMI alligators. The 18 normal BMI alligators had Hg concentrations ranging from 39 to 421 ng/g, with an average concentration of 165 ± 78 ng/g, differing slightly from the entire normal population, but provided a more accurate comparison as the morphometrics were similar to the low BMI alligators. The alligators with low BMI exhibited significantly higher Hg concentrations in blood ($p = 0.0087$, ANOVA) than alligators with normal BMI (Fig. S2).

These values as well as previously reported liver concentrations, place MINWR as a moderate site for Hg contamination (Burger et al., 2000; Delany et al., 1988; Heaton-Jones et al., 1997; Horai et al., 2014; Yanochko et al., 1997). Hg concentrations observed at MINWR are well below the average values reported in the Everglades (Table 1, Fig. 1 left) (Nilsen et al., 2016; Rumbold et al., 2002). While these values are below the threshold that has been observed to cause reproductive behavior changes in water birds, the values are approaching the lower limit of the threshold of Hg concentrations that correlate to a sub-lethal epigenetic modification in alligators (Frederick and Jayasena, 2010; Nilsen et al., 2016).

3.2.1. Recaptured alligators

Within the 193 total alligators sampled, there were 174 unique individuals, and 19 recaptured throughout the sampling period. Of these 19 recaptured individuals, 13 had normal BMI upon recapture while the remaining five had a low BMI upon recapture (Fig. S1, Table S2). One animal had low BMI on both capture dates (Table S2). The 13 alligators with normal BMI had Hg concentrations ranging from 55 to 450 ng/g at first capture and from 98 to 568 ng/g at second capture. Between the first and second captures for this group, the average Hg concentration increased from 199 to 220 ng/g as well as the standard deviation, which went from 117 to 134 ng/g. The five alligators re-categorized as low BMI had Hg concentrations ranging from 145 to 286 ng/g at their first capture and from 152 to 252 ng/g at their second capture. The average Hg concentration for this group also increased between the two captures, going from 179 ng/g to 216 ng/g; however, the standard deviation dropped from 60 ng/g to 39 ng/g. To elucidate the changes in Hg concentration, the percent change in BMI was calculated. The five alligators that changed BMI status, had an average BMI change of -4.7% (range -2.1% to -6.4%), while the alligators that remained at a normal BMI demonstrated average positive increase in BMI that was $+0.31\%$ (range -1.3% to 2.6%). Using a matched-pair Wilcoxon sign rank analysis, a significant difference ($p = 0.04$) was observed in the change in Hg concentrations between recaptured normal and recaptured low BMI alligators that initially had a normal BMI.

Though the etiology of low BMI in the alligators sampled is unknown, it appears that the elevated Hg burden may be at the least a secondary contributing factor, as the bioavailable Hg concentration in blood rises with decreasing body condition due to increased metabolism of muscle and fat. However, there is the possibility that the increased concentrations of Hg caused neurological impairment that affected foraging, resulting in the decreased body condition which is documented in other species with high Hg concentrations (Axelrad et al., 2009, Basu et al., 2009; Keller et al., 2014; Rustam and Hamdi, 1974; Weihe et al., 2002).

3.3. Mercury in MINWR alligators

Previous studies have demonstrated that a correlation can exist between the health status of a wildlife population and their respective contaminant burdens (Jepson et al., 2005; Keller et al., 2014). To analyze this population of alligators for factors that could lead to changes in Hg concentration, a GLM model was employed. Initially, BMI status emerged as the most highly significant factor related to Hg concentration ($p < 0.001$), as the mobilization of fat and muscle leads to an increase in blood concentrations of contaminants (Jepson et al., 2005; Keller et al., 2014). The two BMI groups were separated to determine if any other factors influenced Hg concentration. The GLM over fit the low BMI data, and did not provide useful information (Table S3). However, using the normal BMI data in the GLM, the AICc = 34.5, indicating a well-fit model and justified our further analysis of only alligators with a normal BMI. In this regard, several interacting variables that significantly effect, and could lead to the prediction of the Hg concentration in an alligator at MINWR, were observed. The interaction of sex × SVL, year × season, sex × season × SVL, and MINWRsub-location × sex × season were significantly predictive of Hg concentration in the normal BMI population (Table 2, Table S4).

The most significant predictor was SVL × sex ($p = 0.001$), specifically the relationship between SVL and females ($p = 0.001$, Table 2 and

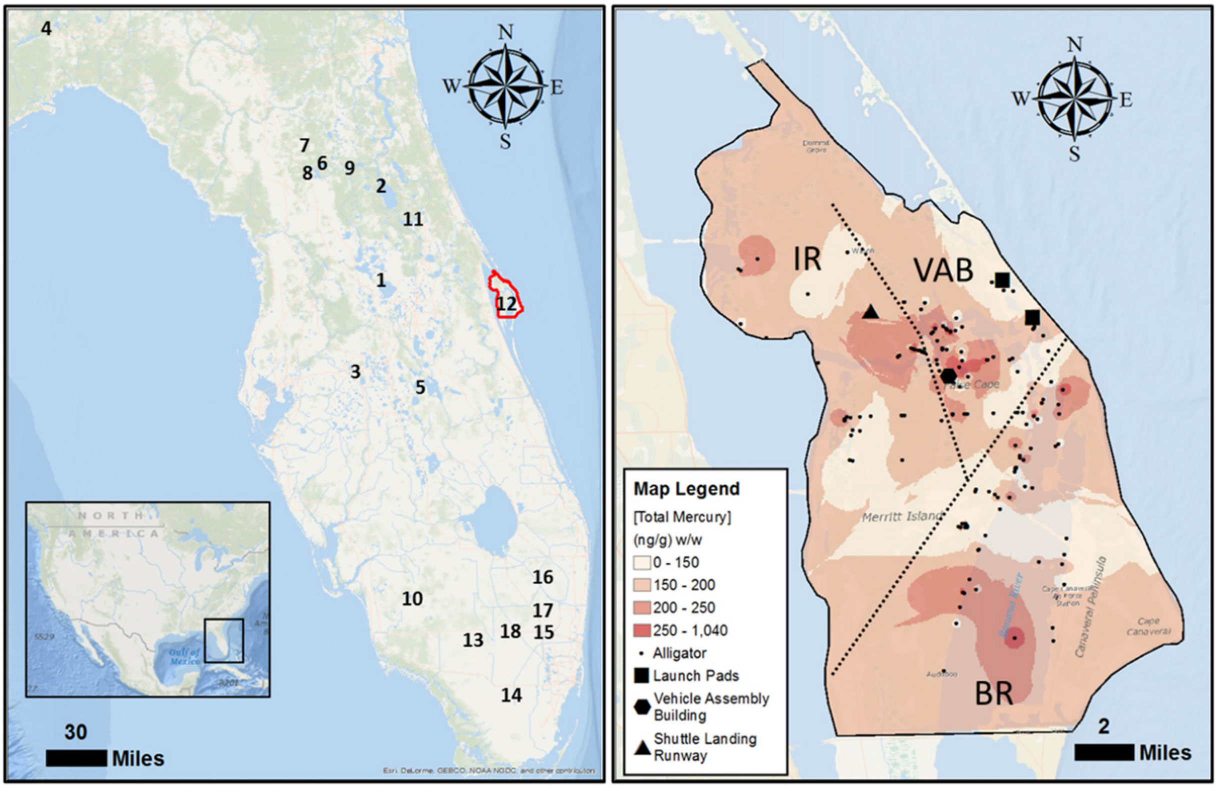

Fig. 1. A map of the state of Florida with Merritt Island National Wildlife Refuge (MINWR), FL outlined in red and previous Hg studies denoted with numbers corresponding to Table 1 (left). Spatial interpolation of the total mercury concentration found in whole blood of American alligators at MINWR, including anthropogenic points of interest (potential point sources of Hg), the dotted lines indicate the sub-locations within MINWR, specifically the Indian River area (IR), Vehicle Assembly Building area (VAB) and the Banana River area (BR) (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

S4). When the entire population was compared, no relationship was observed; however, the separation of the sex illustrates that there is a significant relationship between SVL and Hg concentration for females (Fig. S3 and S4). This relationship may be significant for the females at MINWR, since alligators are sexually dimorphic for size (Wilkinson et al., 2016). The females do not grow as large and therefore reach their determinant size at a lesser SVL and older age than males (Wilkinson et al., 2016). This appears to lead to the significant accumulation of Hg over a smaller range of SVL (Fig. S4). The remaining interactions of MINWR sub-location, sex, season, and year are specific to MINWR and are discussed below.

3.4. Temporal variation in Hg at MINWR

3.4.1. Annual assessment

Annual variation did not have much influence on the Hg concentration in the alligators at MINWR. The only year × season relationship that was significant was the summer of 2010 ($p = 0.03$) (Table 2 and S3). When Hg data was plotted annually, 2010 alligator Hg measurements were the approximate mean of all other summer seasons (Fig. 2, top). However, when the data was separated based on season and year, there was no observable relationship (Fig. S5). The average Hg concentration for the summer of 2010 was 171 ng/g ± 57, and the overall Hg concentration for all summers analyzed was 165 ng/g ± 80. The significance in the GLM is likely due to the 2010 proximity to the mean for all summer seasons, thus suggesting a spurious statistical relationship in summer 2010 alligators (Fig. 2, top). The differences observed in the plotted data do not indicate a temporal relationship as many other contaminant studies do, but show a more varied pattern that may be reflective of the different individuals sampled each year (Lauenstein and Daskalakis, 1998; Smithwick et al., 2006; Van Metre and Mahler, 2005; Fig. 2, top). Recapturing individuals for a temporal assessment was not possible and may be a contributing factor in the degree of biological variability per season and year.

3.4.2. Seasonal assessment

The annual data show variation within each season when plotted, and when all years are combined, there does not appear to be a difference between seasons (Fig. 2, top and bottom). The GLM model showed seasonal interactions that have an effect on Hg concentration (Table 2).

Table 2
A summary of the significant variables in the Generalized Linear Model (GLM) for alligators at Merritt Island National Wildlife Refuge (MINWR), FL with normal body mass index (BMI). The full table is presented in the supplementary information (Table S4). A significant value is noted by $p \leq 0.05$.

Effect summary		Parameter estimates		
Source	*p* Value	Term	Estimate	Prob > ChiSq
SEX × SVL (cm)	0.001	SEX[FEMALE] × (SVL (cm)-131.489)	0.003	0.001
YEAR × Season	0.033	(YEAR-2010.01) × Season[Summer]	0.034	0.010
SEX × Season × SVL (cm)	0.025	SEX[FEMALE] × Season[Spring] × (SVL (cm)-131.489)	−0.004	0.015
		SEX[FEMALE] × Season[Summer] × (SVL (cm)-131.489)	0.004	0.029
LOCATION × SEX × season	0.044	LOCATION[*BR*] × SEX[FEMALE] × Season[Summer]	−0.066	0.041

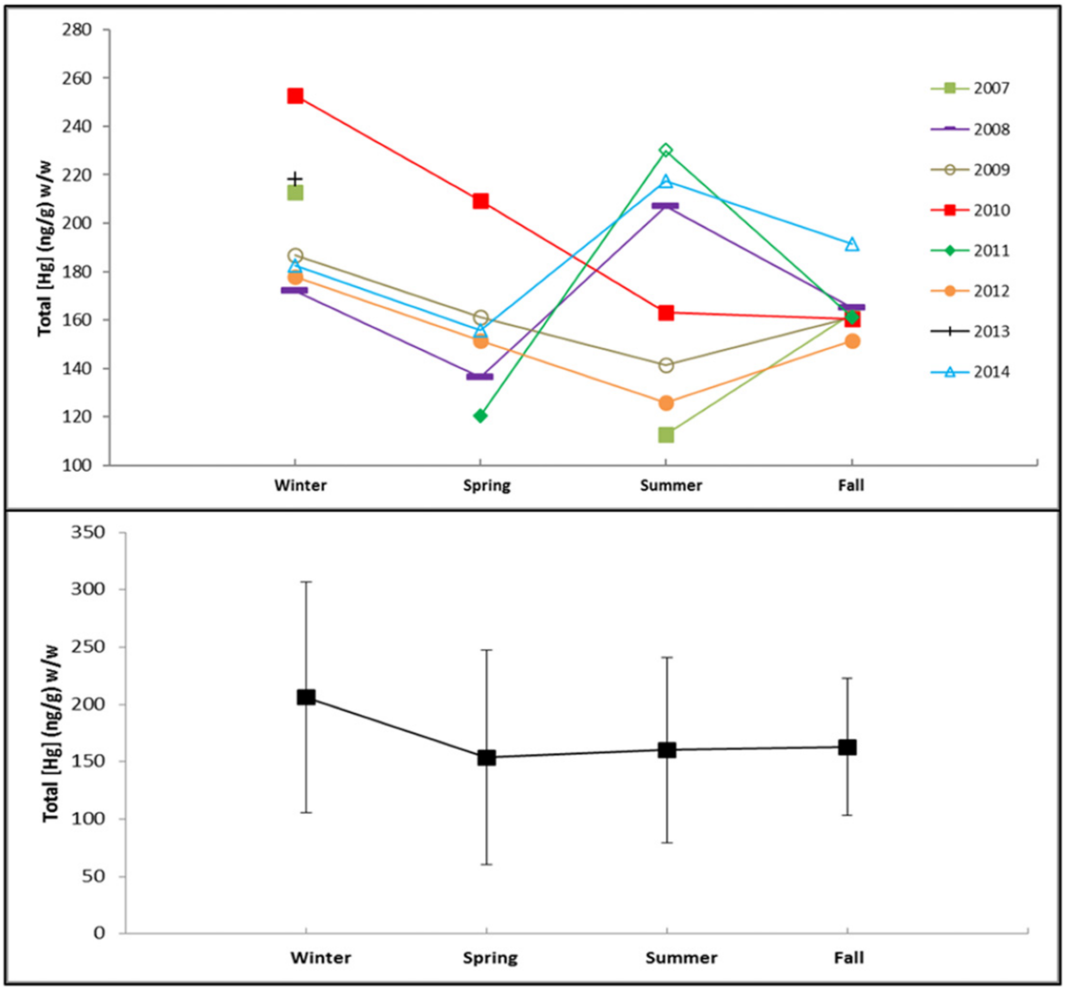

Fig. 2. The average mercury (Hg) concentration for all alligators collected in each season from 2007 to 2014 at Merritt Island National Wildlife Refuge (MINWR). Each season is separated annually (top) and all seasons from 2007 to 2014 are averaged together (bottom).

The interaction between females, of SVL up to 130 cm, in spring and summer have higher Hg concentrations than males at that size, in those seasons (Table 2, Fig. S6). These relationships, while significant (spring $p = 0.02$, summer $p = 0.03$) are likely due to the age discrepancy in alligators of this size. Females mature slower than males, and do not grow as large as males (Wilkinson et al., 2016). The females driving this relationship are likely older and have therefore accumulated more Hg than the males of the same size. Females nest in the spring and summer, while the rest of the population forages (Wilkinson et al., 2016). When females are nesting, they stay close to their nest, facilitating capture and sampling. This could account for the larger sample sizes of females in this season. Uneven sample sizes may be responsible for the

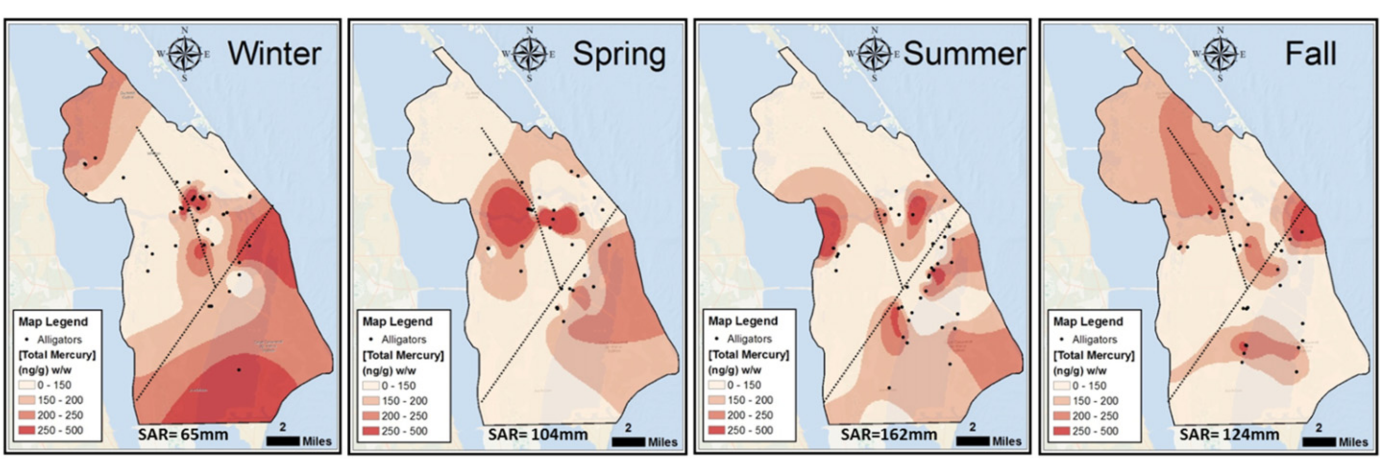

Fig. 3. The Geographic Information Systems (GIS) interpolation of the total mercury measurements taken at Merritt Island National Wildlife Refuge (MINWR), FL from 2007 to 2014 by season (Winter = December–February, $n = 49$; Spring = March–May, $n = 37$; Summer = June–August, $n = 44$; Fall = September − November, $n = 43$). The seasonal average rainfall (SAR) is provided within the map of each season. The dotted lines indicate the three sub-locations within MINWR.

statistical relationship for this interaction (Fig. S6). However, as the seasons coincide with very different behaviors, seasonal changes in behavior are likely driving the interaction effects on Hg concentration.

To illustrate the cumulative seasonal variation of Hg concentrations present at MINWR and the spatial component to the seasons, ArcMap10.3 was used to create maps displaying the varying seasonal concentrations of Hg (Fig. 3). The projected interpolations visually demonstrate the variation in Hg concentration across MINWR through the seasons.

In the spring, alligators emerge from brumation and begin breeding activity. During this season, foraging and resource utilization extend from very limited to nearly maximal in a short period, to ensure there is adequate fuel for this energetically expensive behavior. The Hg concentration of females during the spring at MINWR suggests that they may increase foraging to prepare for nesting and egg laying since the females average Hg concentration is 150 ng/g $\pm$ 68, and the males average Hg concentration is 110 ng/g $\pm$ 90 ($p = 0.01$, Table 2, Fig. S6). Mercury is transferred vertically to the eggs from the nesting female; however, the effect of maternal transfer of Hg on the adult female blood Hg concentration is unclear (Rainwater et al., 2002; Stoneburner and Kushlan, 1984). Higher Hg concentrations may be problematic for their eggs, for example, mother: fetus Hg concentration ratios reflect weak offloading in beluga whales (*Delphinapterus leucas*), and Hg concentrations of beluga mothers are lower than the males of the population, suggesting that even the weak offloading via maternal transfer can elicit changes in adult female circulation blood concentrations (Hoguet et al., 2013).

During the summer, increased activity, such as foraging, mating, nesting, and rearing hatchlings may result in variable Hg concentrations among individuals (Fig. 2, top), as not all individuals participate in breeding every year - <50% of sexually mature females breed (Lance, 2003). The GLM showed that the females Hg concentrations in the summer are related to their SVL, with the average being 157 ng/g $\pm$ 96, compared to males of the same size in summer with an average Hg concentration of 198 ng/g $\pm$ 91 ($p = 0.03$, Table 2 and S4). This relationship suggests some of the elevated Hg in the spring may be transferred to eggs in the early summer, resulting in lower Hg concentrations that are still related to SVL. During the fall, the population comes back to synchronous behavior as temperatures begin to decrease and preparations for brumation begin that could explain the decrease in Hg concentration variation in the fall from the wider range observed in the summer (Fig. 2, top).

In the winter, alligators enter a brumation period, where body temperature drops and foraging decreases from the warmer months (Lance, 2003). This leads to higher Hg concentrations in the blood as the animals become dehydrated and metabolize both muscle and fat for energy (Jepson et al., 2005; Keller et al. 2014). Though winter temperatures at MINWR are warm (15 to 16 °C), resulting in a short brumation period (taking place below 16 °C), a decrease in foraging activity resulting in metabolism of tissues and mobilization of Hg could be responsible for changes observed in this season (Fig. 2) (Jepson et al., 2005; Lance, 2003).

In addition to the seasonal behavior effect on Hg concentration in alligator blood, the possibility of seasonal effects related to changes in Hg transformation by bacteria was considered, which is dependent on deposition and fluctuating environmental parameters (Baldi et al., 2012; Julian, 2013; Selin and Jacob, 2008). During the winter in Florida, dry Hg deposition is dominant since there is less seasonal rainfall, thus, this type of deposition can lead to a greater accumulation of Hg in the environment than in seasons dominated by wet deposition (Fig. 3) (Guentzel et al., 1995; Guentzel et al., 2001; Sexauer Gustin et al., 2012). The increased amounts of rainfall in the spring and summer in Florida lead to a greater amount of wet Hg deposition, bringing less Hg from the atmosphere into the wetland ecosystem (Fig. 3) (Guentzel et al., 1995; Guentzel et al., 2001; Sexauer Gustin et al., 2012). This change in the amount of rainfall and the subsequent switch in wet and dry depositions in these seasons could be related to the variability observed in alligator Hg concentration, as less Hg is present in the environment in the spring and summer while much of the population is foraging. However, for this to have an effect on the Hg concentrations found in large predators, Hg must cycle quickly through the food web in this environment and have high bioavailability, which has not been assessed to date. The toxicokinetics of Hg in reptiles is also not currently described, making a definitive connection tenuous. In mammals, there is a fast assimilation of Hg from digestion to being detectable in blood, and the subsequent elimination half–life in blood averages seven weeks (range 4.3 to 7.5 weeks) (Carrier et al., 2001; Lieske et al., 2011). There could be similarly fast Hg assimilation in reptiles, which would make the changing environmental parameters incredibly relevant to the Hg accumulation discussion, but further research is needed to better understand this relationship.

3.5. Spatial variation in Hg at MINWR

In an effort to determine if there are local point sources for Hg contamination within MINWR, and to interpret the spatial interaction provided by the GLM (MINWRsub-location $\times$ sex $\times$ season), a visual tool was needed. The 174 normal BMI alligators Hg blood concentrations collected from 2007 to 2014 were plotted using ArcMap10.3 (Table S1, Figs. 3, right and 5). The three sub-location boundaries were overlaid onto the results, to aide in the observation of point sources (Fig. 3).

The projected interpolation of Hg concentrations highlights certain areas of elevated Hg concentrations in alligators within MINWR, but moderate in comparison to the Everglades (200–500 ng/g at MINWR, and 1300 ng/g is a mean value for Everglades adult alligator blood samples) (Nilsen et al., 2016). Moderate concentrations of Hg in alligators are in close proximity to the Vehicle Assembly Building (Fig. 1, right). Activities near this location may be creating a more favorable environment for alligators and Hg accumulation in the surrounding water bodies (i.e. warmer water, altered pH, greater amount and variety of prey items because of incidental human interaction).

Moderate concentrations of Hg in alligators were also observed in the Banana River area of MINWR (Fig. 2, right and 4). The pattern is sporadic throughout the seasons, but GLM showed a significant relationship for females at the Banana River in the summer ($p = 0.04$), despite the uneven sample sizes across the locations in summer (Fig. S7). In the summer, the females in this area have an average Hg concentration of 150 $\pm$ 69 ng/g, and the males have an average Hg concentration of 187 ng/g $\pm$ 38. The statistical relationship between females that have a lower average Hg concentration than males at this location may indicate that this area is used for nesting and foraging, and results in lower blood concentrations in females relative to males, because they have recently transferred some of their Hg burden to their eggs (Fig. S7, Table 2). However, the home range of the specific alligators sampled is not known and how this affects dietary preferences would affect the spatial distribution compared to the pattern measured here. To better observe the spatial distribution of Hg in alligators at MINWR, animals need to be recaptured every season, which is a difficult task even with a routinely monitored population.

4. Conclusion

This project was designed to assess the variation of Hg concentration within the MINWR alligator population that is a moderate site of Hg contamination in comparison to the rest of Florida. A metric was developed using BMI for assessing the health status of individual alligators and Hg concentration of alligators with a low BMI at MINWR was significantly elevated compared to alligators with normal BMI. Results indicate that the alligator population displays small seasonal variation in Hg concentration corresponding to seasonal behaviors, although this warrants further investigation with recaptured individuals. The American alligator population at MINWR is a model system for assessing long-term Hg exposure trends, especially as global concentrations of Hg continue to rise.

Disclaimer

Certain commercial equipment, instruments, or materials identified in this paper specify new technological advancements in the field. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology; nor does it imply that the materials or equipment identified is necessarily the best for the purpose.

Acknowledgements

We would like to thank the members of the Florida Fish and Wildlife Conservation Commission, Integrated Mission Support Systems and the Guillette laboratory for assistance in sample collection, as well as NASA and Kennedy Space Center for granting access to MINWR and permitting sample collections on their property, without their support, this project would not have been possible. We would also like to thank Jared Ragland (NIST) for his statistical expertise and guidance in data analysis.

We dedicate this work to the memory of Louis J. Guillette Jr., a mentor, colleague, and friend, who believed that through evidence and innovation we could create new ways to view the world.

The National Institute of Standards and Technology, Integrated Mission Support Systems and the Florida Fish and Wildlife Conservation Commission funded this research. Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.07.073.

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