

INVESTIGATING THE RELATIONSHIPS BETWEEN MATERNALLY-TRANSFERRED
MERCURY AND HATCHLING DEVELOPMENT, BEHAVIOR, AND SURVIVAL IN THE
AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*)

By

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(Under the Direction of Benjamin Parrott)

ABSTRACT

Mercury is a toxic, pervasive environmental contaminant that can be transferred from mother to offspring during development. We examined how maternally-transferred mercury and incubation temperature influence reproductive success, offspring behavior, and subsequent survival in American alligators. We collected alligator eggs from a mercury contaminated reservoir on the Savannah River Site, SC, and incubated them at female- and male-promoting temperatures. Mercury in egg yolk ranged from 0.248–0.554 ppm compared to 0.018–0.052 ppm for a low mercury site; levels in hatchling blood ranged from 0.090–0.490 ppm. We found mostly negligible correlations between life history traits and mercury but noted a positive relationship with egg mass. Incubation temperature exerted strong effects on hatchling phenotypes, with male-promoting temperatures producing larger, bolder hatchlings with increased survival. Overall survivorship over eight months was ~20%. Our study suggests that incubation temperature has a stronger effect on alligator neonate behavior and survival than maternally-transferred mercury.

INDEX WORDS: Crocodylian, Incubation temperature, Mercury, Ecotoxicology, Reproduction

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B.A., Colby College, 2019

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2022

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December 2022

ACKNOWLEDGEMENTS

I would like to thank the members of the Parrott lab: Samantha Bock, Emily Bertucci-Richter, Abby Bickle, Steven Gardner, Laura Kojima, Marilyn Mason, Benjamin Parrott, Ethan Shealy, Chris Smaga, Elise Webb, and Kristen Zemaitis, for their assistance with hatchling recaptures and suggestions with my study design. Thank you to Angela Lindell for her assistance with running the mercury analyses, and to Max Lambert for his assistance with statistical analyses. Thank you to Thomas Rainwater, Randeep Singh, Philip Wilkinson, and the staff at The Tom Yawkey Wildlife Center for additional field assistance and logistical support. I would like to thank my committee, Benjamin Parrott, Stacey Lance, and Max Lambert, for their advice and tutelage throughout this study. Special thanks to my family and friends (Daniel Johnson, Sylvia Johnson, Rachel Johnson, Matthew Johnson, Mansi Hitesh, and broader SREL family) for their support and encouragement throughout my time in graduate school.

Funding and support for this research was provided by the Savannah River Site Nuclear Solutions-Area Completion Projects, the Department of Energy Office of Environmental Management under award DE-EM0004391 to the University of Georgia Research Foundation. This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name,

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CHAPTER 1

INTRODUCTION

Anthropogenic pollution has widespread consequences, and nearly every ecosystem on the planet is impacted by human contamination (Häder et al. 2020, Vareda et al. 2019). However, certain taxa, namely herpetofauna, remain poorly represented in ecotoxicological studies (Chételat et al. 2020, Sparling et al. 2010), despite their potential utility as biological indicators and recognized population declines over the past several decades (Saha et al. 2018, Alroy 2015, Falaschi et al. 2019). For example, it is estimated that from 1970–2012, global reptile populations underwent an average decline of 54–55% (Saha et al. 2018). In addition to other major drivers of decline such as habitat loss, overharvesting, and disease, anthropogenic pollution has been identified as a threat to herpetofauna populations (Alford 2010, Todd et al. 2010, Gibbons et al. 2000, Böhm et al. 2013). Greater focus on ecotoxicological processes in herpetofauna is therefore needed to fully characterize the risks posed to these underrepresented taxa. One pollutant of particular concern is mercury (Chételat et al. 2020, Schneider et al. 2013, Hopkins et al. 2013a, Campbell 2003), a highly toxic and globally pervasive contaminant, with major anthropogenic sources including fossil fuel combustion, artisanal small scale gold mining, metals manufacturing, cement production, and waste incineration (Streets et al. 2017, Pirrone et al. 2010). Mercury (Hg), and particularly its organic form, methylmercury, has been associated with a wide range of reproductive, behavioral, and neurological effects in wildlife (Weis et al. 2009, Scheuhammer et al. 2007, Wolfe 1998). Due to their long lifespans and high trophic positions, reptiles such as snakes (Haskins et al. 2021a, 2021b, Campbell and Campbell 2001),

turtles (dos Santos 2021, Eggins et al. 2015, Hopkins et al. 2013a, 2013b), and crocodylians (dos Santos 2021, Nilsen et al. 2017a, Eggins et al. 2015, Campbell 2003, Jagoe et al. 1998, Brisbin et al. 1998, Heaton-Jones et al. 1997) have the potential to serve as informative models for monitoring the organismal- and population-level impacts of mercury pollution.

Mercury, like many other contaminants, bioaccumulates with organismal size and/or age (Haskins et al. 2021a, Lawson et al. 2020, Eggins et al. 2015, Rainwater et al. 2005), and maternal mercury burdens have been shown to, at least in part, be deposited in eggs or otherwise transferred to offspring (Nilsen et al. 2020, Scheuhammer et al. 2007). Although much of our current understanding of this phenomenon and its consequences comes from research conducted on birds (Ackerman et al. 2020, 2017, Yu et al. 2015, Scheuhammer et al. 2007), maternal transfer of mercury has been demonstrated in numerous herpetofauna as well (Nilsen et al. 2020, Lemaire et al. 2021a, Hopkins et al. 2013a, 2013b, Chin et al. 2012, Bergeron et al. 2010, Guirlet et al. 2008), with many of the same associated sublethal impacts seen in their avian relatives (Figure 1; Hopkins et al. 2013b, Scheuhammer et al. 2007). For example, Lemaire et al. (2021a) found that hatchling size was negatively correlated with claw and scute mercury concentrations in smooth-fronted caimans (*Paleosuchus trigonatus*), and Bergeron et al. (2011a, 2011b) reported numerous consequences of maternally-transferred mercury in American toads (*Anaxyrus americanus*), including reduced clutch viability, altered larval behavior such as slower average swimming speeds and responsiveness, and reduced metamorph size. Chin et al. (2013) found that neonatal northern watersnakes (*Nerodia sipedon*) born to mothers with high body burdens of mercury displayed reduced strike efficiency and willingness to feed; decreased motivation to feed has also been observed in northern two-lined salamanders (*Eurycea bislineata*) from the same mercury contaminated river (Burke et al. 2010). Early growth is

critically tied to survival in certain species, so limitations to feeding, especially if combined with reduced body size at hatch, are likely to convey severe consequences for early survival and susceptibility to predation (McGovern et al. 2020, Haskell et al. 1996, Wilbur and Collins 1973).

Although the sublethal impacts of maternally-transferred mercury are thought to collectively reduce survival under natural conditions, this assumption has seldom been tested. When subjected to both maternally-transferred mercury and high mercury diets, mortality of American toad tadpoles increased 125% during metamorphosis and 50% overall compared to reference groups (Bergeron et al. 2011b). However, Todd et al. (2012), using a similar factorial design with the same dosing concentrations of mercury, did not find significant differences in mean survival of American toads held in pens for one year after metamorphosis, though size differences between maternal mercury and reference groups persisted. Alvarez et al. (2006) used regression trees and individual-based models to predict that delayed response to a vibratory stimulus observed in larval Atlantic croaker (*Micropogonias undulatus*) from mothers fed methylmercury enriched diets would cause an 86-93% reduction in survival. While these studies provide an indication of how maternally-transferred mercury might affect survival in the wild, the consequences of certain sublethal impacts, such as reduced body size and altered predator avoidance behavior, can only be fully realized *in situ* under real environmental pressures.

Here, we investigate the relationships between maternally-transferred mercury and offspring morphology, behavior, and subsequent survival in the American alligator (*Alligator mississippiensis*). The American alligator is a long-lived, apex predator that inhabits aquatic environments, making it especially susceptible to mercury bioaccumulation (Nilsen et al. 2017a, Brisbin et al. 1998). Nilsen et al. (2020) reported that mercury concentrations in egg yolk represented up to 12.8% of total mercury concentrations detected in corresponding maternal

alligator blood, and incidental monitoring has reported high levels of mercury in crocodilian eggs (American alligator: $\bar{x} = 0.54 \pm 0.06$ ppm [Ogden et al. 1974]; American crocodile [*Crocodylus acutus*]: $\bar{x} = 0.66 \pm 0.11$ ppm [dry weight; Stoneburner and Kushlan 1984]; Morelet's crocodile [*C. moreletii*]: $\bar{x} = 0.07 \pm 0.01$ ppm [Rainwater et al. 2002]). The majority of these concentrations are above the low magnitude effect threshold of 0.2 ppm Hg in fish muscle (Canham et al. 2020) while just reaching into the lower bound for bird eggs (0.6–2.7 ppm Hg, reviewed in Fuchsman et al. [2017]), suggesting a potential for deleterious effects in crocodilians.

We coupled data from laboratory-incubated hatchlings and behavioral trial assays with an *in situ* survival-recapture study to holistically examine the potential impacts of maternally-transferred mercury from initial development through the first eight months of life. Our study aimed to answer three main questions: 1.) What is the relationship between maternally-transferred mercury and clutch success and neonate morphology? 2.) What are the links between maternally-transferred mercury and neonate behavior? and 3.) What is the potential impact of maternally-transferred mercury and its sublethal effects on neonate survival? Alligators exhibit temperature-dependent sex determination (Lang and Andrews 1994), and it has previously been shown that incubation temperature can have a strong impact on hatchling morphology and behavior (Bock et al. 2021, While et al. 2018, Noble et al. 2017, McCoy et al. 2016), so we additionally incubated eggs at both female- and male-promoting temperatures (FPT and MPT, respectively) to determine how incubation temperature might interact with maternally-transferred mercury to affect hatchling traits and survival outcomes.

CHAPTER 2

METHODS

Egg collection and incubation

We collected alligator eggs from two sites in South Carolina, USA, with different contamination profiles: Par Pond, located on the United States Department of Energy's Savannah River Site, and The Tom Yawkey Wildlife Center (hereafter Yawkey) located on the coast. Par Pond is a 993-ha reservoir constructed in 1958 to serve as a source of coolant for multiple, now decommissioned reactors (Brisbin et al. 2008), and is contaminated with mercury due to water sourced from the Savannah River, which received mercury effluent from a chlor-alkali plant from 1965–1970 (Kuhne et al. 2015, Smith et al. 2007). Radionuclide inputs from associated reactors have contributed to the contamination profile of Par Pond (Carlton et al. 1992) but are thought to have minimal consequences for the resident alligator population (Brisbin et al. 1989, 1996, 1998). Yawkey is a remote, 6033-ha wildlife management area consisting of three main islands surrounded by brackish and marine habitats (Lawson et al. 2020), and previous research has detected relatively low concentrations of mercury in alligators and nest material from this site (Nilsen et al. 2020).

During the summer of 2021, visual surveys for alligator nests were conducted at both sites. Eggs were collected within 48 hours of oviposition and transported to the University of Georgia Savannah River Ecology Laboratory in natal nest material, where they were individually weighed and measured for length and width. One egg was randomly selected for embryo staging according to Ferguson (1985), while the remaining eggs were placed in bus pans with dampened sphagnum moss in programmable incubation chambers (model I36NLC, Percival Scientific,

Perry, IA, USA) at a temperature known to produce mixed sex ratios (32 °C; Lang and Andrews 1994). HOBO TidbiT® v2 Temp Loggers (Onset, Bourne, MA, USA) were placed in each incubator and checked daily to ensure the desired temperature was maintained. Egg bins were rotated between shelves daily to reduce effects of temperature gradients within incubators and were misted with water twice daily to prevent desiccation. Eggs were collected under South Carolina Department of Natural Resources Scientific Collection Permit #SC-08-2021, and all research was conducted in accordance with University of Georgia IACUC-approved protocols (AUP# A2019 06-001-Y3-A1).

Eggs were maintained at 32 °C until Stage 15, which marks the beginning of the thermosensitive period of sex determination (McCoy et al. 2015) and were then evenly divided into FPT (29.5 °C) and MPT (33.5 °C) incubation groups. At stage 20, four eggs per clutch were removed from the FPT group for yolk collection; these eggs were not counted when initially dividing clutches between FPT and MPT, such that their removal did not impact the balance between the two temperature groups. Eggshells were cut open with sterile, stainless steel dissecting scissors and peeled back to reveal the yolk. Approximately 8 mL of yolk was collected using 2 mL VWR® Disposable Graduated Transfer Pipets (VWR, Radnor, PA, USA) and placed in Cryovial® tubes (Simport, Beloeil, QC, Canada) in 4 mL aliquots. Yolk samples were frozen at -20 °C until analysis.

All remaining eggs were incubated until hatching. Upon the initiation of hatching, eggs were placed in mason jars to keep track of individual hatching times. Hatchlings were given 48 hours to complete hatching, after which they were manually assisted. Clutch viability was determined as the proportion of eggs from a clutch that successfully hatched, excluding eggs that were sampled during incubation. Hatchling morphometrics were measured as follows: mass was

measured using a digital balance (± 0.01 g); snout-vent length (SVL), total length (TL), and tail girth (TG) were measured using a flexible measuring tape (± 0.1 cm); and head length (HL) and head width (HW) were measured with calipers (± 1 mm). Hatchling SVL and TL were measured ventrally, and TG was measured at the posterior opening of the cloaca. Hatchlings were individually marked by clipping a unique combination of dorsal tail scutes using stainless steel dissection scissors. Hatchlings were separated by site but otherwise housed together at the Savannah River Ecology Laboratory aquatic animal facility. The aquatic animal facility is a semi climate-controlled building constructed with a translucent fiberglass roof and upper walls to allow natural light exposure (Tuberville et al. 2016); ambient temperatures in the facility ranged from 21–29 °C throughout the study period. Hatchlings from Par Pond were housed in 210 x 55 x 56 cm living stream tanks (Frigid Units Inc., Toledo, OH, USA); each tank was provided with two 39 x 39 cm concrete basking platforms and filled with 12 cm of water. Hatchlings from Yawkey were housed in 171 x 82 cm custom fiberglass tanks (Johnson’s Custom Fiberglass, Ocala, FL, USA) consisting of a deeper portion filled with 10 cm of water and an elevated basking area. Water was changed daily, and alligators were kept at densities of no more than 1 alligator per 154 cm². Blood was drawn from the Par Pond hatchlings 7–10 days post-hatch from the post-occipital sinus using sterile 25G PrecisionGlide™ Needles (BD, Franklin Lakes, NJ, USA) and disposable 1 cc luer slip syringes (Resway, Miami, FL, USA). Approximately 800 μ L was collected from each individual and placed in lithium heparin Vacutainer® tubes (BD, Franklin Lakes, NJ, USA). Blood samples were stored at -20 °C until analysis. Hatchlings from Yawkey were incorporated into a separate study and behavioral and survival analyses are not reported.

Behavior trials

The first four individuals to hatch from each incubation group per clutch from Par Pond ($n = 64$) were selected to undergo behavior trials 3–7 days post-hatch to assess movement, basking, and predator avoidance. Trial arenas consisted of 227.3-liter (50-gallon) cattle tanks that were marked with grid points 10 cm apart to delineate different regions of the arena. Four 22.5 x 15 x 5 cm basking platforms were placed in the arena, which was filled with 5 cm of water. A 75-watt basking lamp was suspended ~62 cm over one side of the arena to provide a thermal gradient, which was monitored via HOBO TidbiT® v2 Temp Loggers (Onset, Bourne, MA, USA) located on the basking platforms at opposite ends of the arena (Figure 2A). Temperatures during the behavior trials averaged 25.2 °C (range: 20.8–28.0 °C) on the warmer half of the arena (beneath the basking lamp) and 23.5 °C (range: 20.8–25.6 °C) on the cooler half of the arena, with an average differential of 1.7 °C. Trials ran for 120 minutes. The first 20 minutes consisted of an acclimation period where the hatchling was allowed to roam freely about the arena. After 20 minutes had elapsed, a decoy heron (simulated predator) was remotely raised via a string into view of the hatchling and remained in view for the remainder of the trial (Figure 2B). All trials were started between 7:57–8:17 am Eastern Standard Time (EST) to prevent inconsistencies related to potential behavioral differences at different times of day (Deitz 1979). Water in the trial arenas was changed daily in between trials. All trials were recorded in their entirety using KODAK PIXPRO SP1 Digital Action Cameras suspended above the middle of the trial arena.

Video recordings were manually assessed in their entirety to score hatchling position and activity levels. All behavior scoring was performed by a single observer who was blind to treatment group when scoring. The first and last minute of the trial were removed from the

analysis as behavior during these times may have been affected by the researcher entering/exiting the facility. For positional analysis, the trial arena was divided into eight zones that corresponded with different behaviors: warm peripheral, warm interior, cool interior, cool peripheral, basking platform #1, basking platform #2, basking platform #3, and basking platform #4 (Figure 6F). Whenever the hatchling moved to a different zone of the arena, the time stamp and new zone were recorded. Hatchlings were defined as having moved to a new zone based on the midpoint of their back; if this midpoint was directly on the edge between two zones, then the hatchling was said to have not transitioned yet. The total amount of time spent in each zone was calculated based on the recorded time stamps, from which we extracted the proportion of time spent basking, the proportion of time spent on the warm half of the arena, and the proportion of time spent in peripheral zones of the arena. Time stamps were separately recorded whenever the hatchling initiated and ended activity, from which we calculated the proportion of activity spent in each zone of the arena. Activity was defined as the hatchling's intent to move somewhere other than its current position. This included scenarios when the hatchling was trying to move but was not actually changing location (e.g., swimming into a wall or attempting to climb onto a basking platform). Head and tail movements, or slight repositioning limited to one step or less were not counted as activity. Position and activity data were divided into four blocks based on the presence/absence of the decoy heron: the first block was prior to the heron's introduction (Absent), with the remaining blocks divided into 20-minute periods based on how long the heron had been present (Heron – early, Heron – mid, Heron – late). Freeze time, a form of antipredator behavior reliant upon remaining still to reduce detection (Takada et al. 2018), was recorded as the length of time the hatchling remained motionless after its initial reaction to the introduction of the decoy heron.

Survival study

Hatchlings were released back onto Par Pond after 10–15 days post-hatch in ten pods of 9–24 ($\bar{x} = 16.1 \pm 4.6$). On the day of release, hatchling mass, SVL, TL, TG, HW, and HL were re-measured. Hatchlings were released at three locations which were selected based on the presence of an alligator nest from which we had collected eggs, availability of cover for hatchlings, and distance from other release sites (>2 km shoreline distance). Presumptive males and females were evenly divided between the three sites, and all hatchlings were released in the mid-afternoon between 3–5 pm EST. The first pod was released on 31 August 2021 and the last pod was released on 23 September 2021. Recapture surveys commenced three weeks after the last pod was released. Surveys occurred just after sunset so that individuals could be located via eyeshine by researchers using headlamps. Surveys occurred over two consecutive nights and were conducted until an area was exhausted (i.e., until no more individuals could be detected/captured). Hatchling alligators were captured by hand either from a canoe or by wading through shallow water. All captured individuals had their mass, SVL, TL, TG, HW, and HL measured, and any injuries were noted. Alligators were then released at their respective capture sites.

Mercury analysis

Yolk and blood samples were thawed at room temperature and vortexed for ~10 seconds to homogenize prior to analysis. Approximately 0.100 g of sample was placed into individual metal weigh boats and analyzed on a DMA-80 *evo* Direct Mercury Analyzer (Milestone, Shelton, CT, USA) according to U.S. EPA method 7473. The DMA-80 was calibrated each day prior to use using standard reference materials (TORT-3 and PACS-3, National Research

Council of Canada). For quality assurance, each batch of 10 samples was run with a sample replicate, blank, and either two different standard reference materials (TORT-3 and PACS-3) or two doses of the same standard reference material (TORT-3). Egg yolk samples were run as a single batch and used both standard reference materials during quality assurance checks. The method detection limit (calculated as threefold the standard deviation of procedural blanks) was 0.0646 ng/g of sample, and all samples exceeded this limit. Average relative percent differences between replicate samples were $4.88 \pm 0.98\%$ ($n = 7$). Mean percent recoveries of total mercury (THg) for the certified reference materials were $92.48 \pm 0.38\%$ ($n = 6$) for TORT-3 and $120.77 \pm 13.65\%$ ($n = 6$) for PACS-3. Blood samples were run in two separate batches and used two different doses of TORT-3 as standard reference material during quality assurance checks. The method detection limit was 0.124 ng/g of sample for the first batch and 0.202 ng/g of sample for the second batch, and all samples exceeded this limit. Average relative percent differences between replicate samples were $1.86 \pm 0.39\%$ ($n = 7$) and $1.16 \pm 0.41\%$ ($n = 9$), and mean percent recoveries of THg for TORT-3 were $98.67 \pm 0.77\%$ ($n = 12$) and $96.16 \pm 1.07\%$ ($n = 18$) for the first and second batches, respectively.

Statistical analyses

Since we obtained egg mass for the individual eggs sacrificed for yolk collection, we were able to model egg yolk THg concentrations directly against egg mass using a linear regression model. For all other analyses of THg levels in yolk, concentrations were averaged by clutch and modelled against corresponding clutch-averaged values. We assessed the relationships between THg concentrations in yolk and clutch size and clutch viability using generalized linear models with poisson and binomial distributions, respectively. Site was included as a covariate in all of the models, clutch was included as a covariate in the model for egg mass, and incubation

temperature was included as a covariate in the model for clutch viability. Clutch-averaged mean egg mass was additionally included as a covariate in the models for clutch size and clutch viability as a proxy to control for the potential effects of maternal provisioning. Body mass index ($BMI = \text{mass}/[2*SVL]$; modified from Nilsen et al. [2017a]) was calculated and included as an additional variable in data analyses. Hatchling morphometric data (mass, SVL, TL, TG, HL, HW, BMI) were averaged by clutch and modelled against THg concentrations in yolk using linear regression with site and incubation temperature as covariates. Egg mass was additionally included as a covariate in the model for hatchling mass to control for differences in maternal provisioning, and SVL was included as a covariate in the models for TG, HL, and HW as a scaling factor.

The same analyses for hatchling morphometrics were repeated for only the Par Pond individuals from which we obtained blood samples for each individual, using hatchling blood THg concentrations in place of yolk THg concentrations. Since we did not need to use clutch-averaged values for this set of analyses, we took a generalized linear mixed model (glmm) approach using the R package “lme4” (Bates et al. 2015) with THg concentrations in blood, incubation temperature, and the interaction between THg concentration in blood and incubation temperature as covariates and clutch as a random effect. Goodness-of-fit and overdispersion were diagnosed using the “simulateResiduals” function in the R package “DHARMA” (Hartig 2022). Much of the variation in THg levels was observed between clutches as opposed to within a clutch, so we applied the same models using linear regression with clutch included as a covariate for comparison.

This dual modelling approach was repeated to analyze our behavior data. We modelled each of our behavior metrics using a generalized linear mixed model approach with blood THg

levels; incubation temperature; presence/absence of the heron; and the interaction between blood THg levels and incubation temperature, blood THg levels and the presence/absence of the heron, and incubation temperature and the presence/absence of the heron as covariates; and clutch as a random effect. We supplemented glmm's with linear regression models where clutch was included as an additional covariate. Raw data were log or square-root transformed where appropriate to improve model fit.

To examine potential effects on survival, we ran three different sets of survivorship models in Program MARK (White and Burnham 1999) using the Cormack-Jolly-Seber modelling approach. To remove potential issues stemming from multicollinearity, we grouped independent variables into three candidate model sets. The first set examined early environmental influences on survival and included THg concentration in blood, incubation temperature, and mass at hatching. The second set assessed morphological characteristics (measured the day of release) and included hatchling mass, SVL, TL, TG, HL, HW, and BMI, which we collapsed to three principal components using principal component analysis. The third set looked at behavioral characteristics and only included individuals which underwent the aforementioned behavior trials. For this set, the presence/absence of the decoy heron was excluded, and behavior metrics were calculated as a proportion of time spent in each behavior over the entirety of the trial; freeze time was left as a nominal value in seconds. Each candidate model set additionally included pod and clutch as independent covariates, and models were coded such that survivorship and recapture probability were assumed to vary between recapture events to account for differential survival/recapture probability due to seasonality. We applied a logit-link function and allowed MARK to standardize individual covariates to permit numerical optimization of parameters in order to calculate recapture probability and weekly survival

probability. Due to low recapture rates at Sites 1 and 2, survivorship modelling was restricted to Site 3. Survivorship modelling in MARK was supplemented with dual glmm's and generalized linear models following a binomial distribution using post-winter survival as a binary dependent variable. Individuals captured during any of the last three surveys (occurring in early spring) were scored as surviving post-winter.

We determined the most supported models for each analysis using AICc model selection using the “dredge” function in the package “MuMIn” (Barton 2020). Models with a ΔAICc value >2 were considered to be supported. We report results for top models with corresponding beta values ($\pm\text{SE}$) for quantitative variables (e.g., THg in egg yolk and blood), and for the binary variable incubation temperature (reported as the change in MPT relative to FPT). Unless otherwise noted, all statistical analyses were conducted in R version 4.1.2 (R Core Team 2021).

CHAPTER 3

RESULTS

We collected a total of nine clutches from Par Pond (clutch size: $\bar{x} = 38$, range = 24–50) and eight clutches from Yawkey (clutch size: $\bar{x} = 52$, range = 38–81). Clutch viability at Par Pond ranged from 0.25–1.00 ($\bar{x} = 0.78$) for MPT and 0.38–0.84 ($\bar{x} = 0.60$) for FPT; clutch viability at Yawkey ranged from 0.31–1.00 ($\bar{x} = 0.75$) for MPT and 0.17–0.80 ($\bar{x} = 0.51$) for FPT. Clutch-averaged THg in egg yolk ranged from 0.248–0.554 ppm ($\bar{x} = 0.368$ ppm, $n = 33$) at Par Pond, compared to 0.018–0.052 ppm ($\bar{x} = 0.027$ ppm, $n = 30$) for eggs from Yawkey (Figure 3A). THg levels in blood from Par Pond hatchlings ranged from 0.090–0.490 ppm ($\bar{x} = 0.240$ ppm, $n = 158$; Figure 3B). Mercury concentrations were significantly associated with clutch for both egg yolk ($F_{16, 46} = 961.5$, $p < 0.001$) and hatchling blood ($F_{8, 149} = 16.04$, $p < 0.001$).

Reproductive output and hatchling morphometrics

Three models were supported for egg mass, of which the two top models had equal AICc values (Table 1). Both top models incorporated clutch and THg concentration in yolk ($\beta = 0.114 \pm 0.046$), and one additionally included site (both models: log-likelihood = -161.7, AICc = 379.2, Akaike weight = 0.315; Figure 4A). Interestingly, THg concentration in yolk was positively correlated with egg mass in the top models. The top model for clutch size incorporated site, THg concentration in yolk ($\beta = 0.0007 \pm 0.0006$), and a site by THg concentration in yolk interaction term (log-likelihood = -62.4, AICc = 136.1, Akaike weight = 0.414; Figure 4B). Similar to the relationships observed for egg mass, THg concentration in yolk was positively correlated with clutch size in the top model. The top model explaining clutch viability incorporated site; THg

concentration in yolk ($\beta = 0.0011 \pm 0.0020$); incubation temperature ($\beta = -4.08 \pm 1.65$); and site by THg concentration in yolk, THg concentration in yolk by incubation temperature, and site by incubation temperature interaction terms (log-likelihood = -67.1, AICc = 152.6, Akaike weight = 0.651; Figure 4C). THg concentration in yolk and incubation at MPT were positively associated with clutch viability. Clutches from Yawkey had larger average egg mass, larger clutch size, and lower clutch viability in their respective top models. A summary of model statistics for reproductive output is shown in Table 1.

The top model for hatchling mass incorporated site, yolk THg concentration ($\beta = -0.137 \pm 0.050$), incubation temperature ($\beta = 3.48 \pm 0.77$), a site by yolk THg concentration interaction term, and clutch mean mass (log-likelihood = -69.5, AICc = 157.6, Akaike weight = 0.330; Figure 5A). In the top model, yolk THg concentration was positively correlated with hatchling mass, and individuals from Yawkey and those incubated at MPT had larger hatchling mass. Only one model was supported for hatchling SVL, which incorporated site and incubation temperature ($\beta = 0.336 \pm 0.137$; log-likelihood = -14.4, AICc = 38.3, Akaike weight = 0.322; Figure 5B). The top model for hatchling TL incorporated only incubation temperature ($\beta = 0.621 \pm 0.273$; log-likelihood = -37.8, AICc = 82.4, Akaike weight = 0.363; Figure 5C). Individuals from Yawkey (SVL only) and those incubated at MPT had larger SVL and TL in each of their respective top models. The top model for hatchling TG incorporated THg concentration in yolk ($\beta = -7.8E4 \pm 2.9E4$), incubation treatment ($\beta = -0.223 \pm 0.056$), site, a site by incubation treatment interaction term, and SVL ($\beta = 0.477 \pm 0.051$; log-likelihood = 29.9, AICc = -41.3, Akaike weight = 0.411; Figure 5D). In the top model, TG was negatively correlated with THg concentration in yolk, and individuals incubated at MPT had smaller TG. Only one model was supported for hatchling HL, which incorporated site, yolk THg concentration ($\beta = 0.020 \pm 0.007$), incubation temperature (β

= 0.530 ± 0.172), site by yolk THg concentration and yolk THg concentration by incubation temperature interaction terms, and SVL ($\beta = 2.120 \pm 0.152$; log-likelihood = -4.4, AICc = 30.7, Akaike weight = 0.610; Figure 5E). Only one model was supported for hatchling HW, which incorporated site, incubation temperature ($\beta = -0.311 \pm 0.127$), and SVL ($\beta = 1.114 \pm 0.154$; log-likelihood = -8.3, AICc = 28.8, Akaike weight = 0.490; Figure 5F). For both head measurements, individuals from Yawkey had decreased HL and HW whereas individuals incubated at MPT had larger head measurements. The top model for hatchling BMI incorporated only THg concentration in yolk ($\beta = -2.9E4 \pm 1.6E4$; log-likelihood = 14.2, AICc = -21.5, Akaike weight = 0.181; Figure 5G), wherein there was a negative correlation with BMI. A summary of model statistics for the hatchling morphometrics is shown in Table 1.

Results for the analysis of hatchling morphometrics using THg levels in blood for the individuals from Par Pond revealed that THg levels in blood are closely linked to clutch, and that when clutch is set as a random effect in the glmm's, it also removes any correlation with THg levels in blood (Table 1; we elaborate further on this approach in the discussion). Otherwise, incubation temperature was the only variable included in the top models of both approaches, wherein individuals incubated at MPT were larger across all morphometrics with the exception of TG.

Behavior

A summary of model statistics for the behavioral data is shown in Table 2. Data for the proportion of time spent basking were square-root transformed, and there was a single supported model under the glmm approach, which incorporated incubation temperature ($\beta = 0.189 \pm 0.036$) and the categorical presence/absence of the heron decoy (log-likelihood = 61.1, AICc = -105.7, Akaike weight = 0.982; Figure 6A). Individuals incubated at MPT spent more time basking than

their FPT counterparts, and individuals spent more time basking in the presence of the heron decoy. We calculated post-hoc least square means using the “emmeans” function in the R package “emmeans” (Lenth 2022), which revealed a significant increase in the time spent basking over the course of the trial with some exceptions between consecutive time blocks. Additionally, there were significant differences between the incubation temperature groups at each time block. The top model under the linear regression approach incorporated THg levels in blood ($\beta = -0.00086 \pm 0.00026$), clutch, presence/absence of the heron decoy, and incubation temperature, with THg levels in blood negatively correlated to the proportion of time spent basking ($\beta = 0.143 \pm 0.027$; log-likelihood = 66.5, AICc = -103.3, Akaike weight = 0.480).

Data for the proportion of time spent on the warm side of the behavior arena were log-transformed, and the top model only incorporated incubation temperature ($\beta = -0.069 \pm 0.024$; log-likelihood = 109.4, AICc = -208.5, Akaike weight = 0.527; Figure 6B). However, the null model was also supported (log-likelihood = 108.2, AICc = -208.3, Δ AIC = 0.22, Akaike weight = 0.472). There was only one supported model under the linear regression approach, which incorporated incubation temperature ($\beta = -0.071 \pm 0.019$) and clutch (log-likelihood = 120.0, AICc = -219.1, Akaike weight = 0.491). For both the top models under the glmm and linear regression analyses, individuals incubated at MPT spent less time on the warm side of the arena.

When examining the proportion of time spent on the periphery of the arena, the top model under the glmm approach only incorporated the presence/absence of the decoy heron (log-likelihood = 54.1, AICc = -93.7, Akaike weight = 0.521), and post-hoc analysis using least square means revealed that hatchlings spent progressively less time on the periphery as the trial progressed (Figure 6C). A second supported model was the null model (log-likelihood = 50.5, AICc = -92.9, Δ AIC = 0.85, Akaike weight = 0.340). The top model under the linear regression

approach incorporated THg levels in blood ($\beta = -0.0007 \pm 0.0002$), the presence/absence of the decoy heron, incubation temperature ($\beta = -0.117 \pm 0.090$), and a blood THg concentration by incubation temperature interaction term ($\beta = 0.0007 \pm 0.0004$; log-likelihood = 42.3, AICc = -68.0, Akaike weight = 0.338), wherein individuals incubated at MPT spent less time in the periphery, which was also negatively correlated with THg concentration in blood.

Activity data were square-root transformed, and both the glmm and linear regression approaches included the same variables within the top model, which incorporated incubation temperature ($\beta = -0.045 \pm 0.008$) and an Interior/Periphery binary variable (glmm: log-likelihood = 601.1, AICc = -1190.1, Akaike weight = 0.521, Figure 6D; lm: log-likelihood = 613.4, AICc = -1218.7, Akaike weight = 0.264), wherein there was higher activity in peripheral zones and individuals incubated at MPT displayed lower activity. Among the other supported models using the linear regression approach, one incorporated THg concentrations in blood, incubation temperature, and an Interior/Periphery binary variable (log-likelihood = 613.8, AICc = -1217.6, Δ AIC = 1.11, Akaike weight = 0.152), with individuals with higher THg blood concentrations displaying elevated activity.

The length of time hatchlings froze upon the presentation of the heron decoy was log-transformed, and top models from both the glmm and linear regression approaches only incorporated incubation temperature ($\beta = -0.966 \pm 0.390$; glmm: log-likelihood = -112.0, AICc = 232.7, Akaike weight = 0.855, Figure 6E; lm: log-likelihood = -111.3, AICc = 228.9, Akaike weight = 0.573), wherein individuals incubated at FPT tended to freeze for longer than individuals incubated at MPT ($\bar{x} = 425$ seconds versus $\bar{x} = 141$ seconds, respectively).

Survival

We conducted six recapture surveys over the course of the study. Three surveys occurred in the fall (October-December) and three in the spring (March-April) each 3–4 weeks apart and punctuated by a 15-week interval during the winter when hatchlings were assumed to enter brumation. Due to low recapture rates in the fall at Sites 1 and 2, we restricted our survivorship modelling to Site 3. This reduced the number of individuals in the behavior model set ($n = 10$) and precluded robust analysis, so we only proceeded with our environmental and morphology model sets. For the environmental model set, our top model incorporated THg levels in blood ($\beta = 0.531 \pm 0.216$) and incubation temperature ($\beta = 0.522 \pm 0.193$; log-likelihood = 287.3, AICc = 316.0, Akaike weight = 0.247), indicating a negative effect of incubation at FPT and a positive correlation with THg levels in blood (Figure 7A). For the morphology model set, we included three principal components, which reflected 93.46% of the variance. The first principal component was negatively associated with all morphometrics (-0.28– -0.42); the second principal component was positively associated with mass (0.33), TG (0.36), and BMI (0.56) and negatively associated with SVL (-0.31), TL (-0.34), HL (-0.29), and HW (-0.37); and the third principal component was positively associated with SVL (0.33), TL (0.31), and HL (0.14) and negatively associated with HW (-0.88) and BMI (-0.12; see Table 3 for full breakdown of components). Our top model incorporated only the second principal component ($\beta = 0.326 \pm 0.182$; log-likelihood = 289.0, AICc = 315.3, Akaike weight = 0.095), indicating a positive correlation with mass, TG, and BMI (Figure 7B; see Table S39 for full model output with AICc model comparisons). Apparent survivorship ranged from 0.88–0.97 for both the environment and morphology model set (Table 4).

In order to incorporate data from all three release locations, we took a conservative approach by applying dual glmm and generalized linear models with post-winter survival as a binary dependent variable. For the environmental model set, the top models under both modelling approaches incorporated mass at hatch (both models: $\beta = 0.096 \pm 0.047$), release location, and incubation temperature (glmm: $\beta = 1.79 \pm 0.45$, log-likelihood = -80.8, AICc = 176.3, Akaike weight = 0.471; glm: $\beta = 1.79 \pm 0.45$, log-likelihood = -80.8, AICc = 171.9, Akaike weight = 0.327; Table 5). For the morphology model set, the top model under the glmm approach incorporated the first principal component ($\beta = -0.276 \pm 0.105$), and release location (log-likelihood = -89.9, AICc = 192.2, Akaike weight = 0.312). The top model under the generalized linear model approach only incorporated pod and site (log-likelihood = -87.5, AICc = 183.3, Akaike weight = 0.274). For the behavior model set, the top model under both modelling approaches incorporated the proportion of time spent on the warm half of the arena (glmm: $\beta = -3.47 \pm 1.76$, log-likelihood = -37.7, AICc = 84.1, Akaike weight = 0.173; glm: $\beta = -3.33 \pm 1.85$, log-likelihood = -33.7, AICc = 73.8, Akaike weight = 0.094); the top model under the generalized linear model approach also incorporated pod, which was a random effect under the glmm.

CHAPTER 4

DISCUSSION

Despite high levels of maternally-transferred mercury, we found little evidence that mercury substantially impaired the behavior and survival of alligator hatchlings. This is surprising given that alligators are apex predators and bioaccumulation of mercury is expected to disproportionately impair the fitness of high trophic level organisms (Nilsen et al. 2017a, Brisbin et al. 1998). In contrast to the correlations observed with mercury, we observed dominant impacts of incubation temperature. Our study highlights the complexity of discerning impacts of chemical pollution in wild animal populations, particularly when those effects can be overshadowed by other environmental variables.

The levels of mercury in egg yolks collected from Par Pond (0.248–0.554 ppm) were elevated compared to levels observed in other crocodylian eggs. Nilsen et al. (2020) reported mean concentrations ranging from 0.0088–0.0263 ppm in alligator egg yolk from three alligator populations in the southeastern United States, Lemaire et al. (2021a) reported mercury concentrations in egg membranes from smooth-fronted caimans in French Guiana ranging from 0.020–0.040 ppm, and Rainwater et al. (2002) reported mean mercury concentrations of 0.070 ± 0.010 ppm in Morelet's crocodile eggs in Belize. The only comparable levels that exceed our values were reported for alligator eggs (0.54 ± 0.06 ppm) in the Everglades, an area which is also subject to high mercury contamination (Ogden et al. 1974). Mercury concentrations in hatchling blood were expectedly lower than that in egg yolk, since the yolk sac has not been fully absorbed at this stage and mercury may be distributed throughout multiple tissues (Allsteadt and Lang

1995, Nilsen et al. 2017b). Despite seemingly high mercury levels, these concentrations are on the low end for those reported in birds. For example, Fuchsman et al. (2017) reviewed numerous studies focused on mercury's impact in birds and found that typical effect thresholds for mercury ranged from 0.6–2.7 ppm weight wet in eggs. Homeotherms such as birds have lower growth efficiencies than heterotherms such as alligators due to the need to allocate energy towards body temperature regulation, resulting in higher biomagnification factors (Debruyn and Gobas 2006); as a result, alligators may bioaccumulate mercury at slower rates than birds (Weir et al. 2010). Our comparatively lower levels compared to birds may explain why we found relatively weak and disparate correlations with maternally-transferred mercury.

While previous studies have reported negative (Hopkins et al. 2013b, Bergeron et al. 2011a, Yu et al. 2015) or no relationships (Rendón-Valencia et al. 2014, Chin et al. 2012) between clutch viability and egg mercury levels, we observed the opposite trend. Additionally, egg mass and clutch size were also positively correlated with THg concentration in yolk (although the effect size of the latter was biologically irrelevant [i.e., one additional egg correlates with an ~1.5 ppm increase in egg yolk THg]). Reproductive metrics are strongly tied with maternal effects (Rendón-Valencia et al. 2014), and we postulate that the observed relationships between THg levels in yolk and clutch viability are reflective of general maternal provisioning rather than a direct effect of THg. Egg mass is known to scale allometrically with female size in crocodylians (Murray et al. 2013, Larriera et al. 2004, Verdade 2001, Thorbjarnarson 1996, Hall 1991, but see Deitz and Hines 1980), and larger females (and larger individuals generally) accumulate more mercury (Haskins et al. 2021a, Horai et al. 2014, Hopkins et al. 2013b, Rainwater et al. 2005, Heaton-Jones et al. 1997, Yanochko et al. 1997), which in some cases might lead to increased transfer of mercury to eggs (Bergeron et al. 2011a).

If mercury is indeed serving as a proxy for maternal provisioning, then the benefit of having larger, better provisioned eggs may outweigh, or at least mask, any detriment associated with simultaneously increasing mercury inputs.

When analyzing hatchling morphology, we initially used clutch-averaged egg yolk THg, as we did not have blood samples for hatchlings from Yawkey. Although we found positive relationships between THg levels in egg yolk and both hatchling mass and HL, effect sizes for these relationships were small (e.g., 0.39 g of mass gained per 0.100 ppm THg). There was a slight negative relationship between THg in yolk and hatchling TG and BMI, although beta values for these relationships were likewise very small. When we analyzed relationships between THg levels in blood and morphology and behavior of Par Pond individuals, we initially employed generalized linear mixed models with clutch as a random effect, with the intent of parsing potential effects of mercury external from other clutch effects (e.g., maternal provisioning). However, variation in blood THg concentrations was primarily observed across clutches, rather than within, and we found that this approach removed any correlation with the former. We therefore supplemented our analyses using linear regressions with clutch incorporated as an additional covariate. Although this broader approach would allow for better connecting mercury levels to hatchling traits, it lessened confidence that these connections were not due to other factors associated with clutch. Models incorporating THg in blood mirrored those incorporating THg in yolk, with similarly small relationships, except weak, positive relationships between THg levels in blood and hatchling TG and BMI. The slight negative correlation between THg concentration in blood and both the proportion of time spent basking and in the periphery suggests maternally-transferred mercury might have an indiscriminatory, depressive effect on these seemingly opposite behaviors (i.e., desire to openly bask versus desire

to seek out peripheral areas). Different behaviors can have dissimilar responses to maternally-transferred mercury (Alvarez et al. 2006, Chin et al. 2013, Burke et al. 2010), however, making broad generalizations difficult.

Survivorship was negatively associated with individuals incubated at FPT but positively correlated with THg concentration in blood. Decreased survivorship amongst FPT individuals was anecdotally confirmed during our recapture surveys, wherein the proportion of recaptured to released FPT individuals was far less than that for individuals incubated at MPT. The positive relationship between mercury and survival probabilities may be due to indirect effects, as noted above, although clutch did not appear in any of the supported models. Our models also showed a general positive correlation between survivorship and various metrics of hatchling size. This is unsurprising, since size is often correlated with neonate survival (McGovern et al. 2020, Haskell et al. 1996, Wilbur and Collins 1973), although the effect of body condition specifically appears to be more mixed and species-specific (Bertolero et al. 2018, Hare et al. 2012, Schmutz and Ely 1999, Cope et al. 2022). Although we only observed weak correlations between mercury and hatchling size, this relationship can still be of functional importance, especially for other organisms where reductions in body size due to mercury have been observed (Lemaire et al. 2021a, Todd et al. 2012, Bergeron et al. 2011b). The only relationship with hatchling behavior came from our supplemental survivorship models, wherein there was a negative relationship with the proportion of time spent on the warm side of the behavioral arena. Time spent thermoregulating is important for physiological functioning and growth in ectotherms, but this may come at the cost of increased visibility and predation (Kashon and Carlson 2017, Carter et al. 2010, Smith and Blumstein 2008, Stamps 2007).

There are a couple potential reasons why we did not observe strong, negative relationships between THg concentrations in alligator egg yolk/blood and hatchling survivorship. Juvenile organisms can decrease mercury concentrations by effectively outstripping any additional bioaccumulation through rapid growth (Chételat et al. 2020). Rendón-Valencia et al. (2014) report that all but three Colombian slider hatchlings (*Trachemys callirostris*; n = 22) from a mercury contaminated area had mercury body burdens below detection limits five months post-hatch despite elevated mercury concentrations in embryos from the same clutch, and Todd et al. (2012) noted a 12.3–13.6 fold decrease in body burdens of mercury in juvenile American toads fed mercury laden diets as tadpoles. A similar biodilution effect could be occurring with our hatchlings, though the effect would be less drastic as reported in Rendón-Valencia et al. (2014) and Todd et al. (2012), which both held individuals in mercury free environments post-hatch. Additionally, Bergeron et al. (2011a) postulated that there may be a counterbalancing effect between hatching success and early life survival, wherein an early environmental pressure such as maternally-transferred mercury removes less fit individuals from the population prior to hatching, leaving only the most fit individuals with resultant higher survivorship in early life compared to controls that do not go through this early contaminant pressure. This scenario has been observed in American toads (Bergeron et al. 2011a) and to a moderate degree in zebra finches (*Taeniopygia guttata*), which experience sharp reductions in hatch rate correlated with levels of mercury dosing, but no post-hatch effects (Yu et al. 2015). However, this hypothesis does not fully explain our results, as we saw positive relationships between maternally-transferred mercury and both clutch viability and survivorship.

Overall survivorship ranged from 0.88–0.97 per week, and 0.185–0.208 for the whole six-month study period. Other reports of survival in hatchling alligators vary depending on

location and habitat. Deitz (1979) reported first-year survivorship of 0.30 in lake habitats and 0.17 for shallow marsh habitats. Woodward et al. (1987) reported relatively high survivorship (0.76) for the first six months that decreased during the second six months (0.54; 0.41 survivorship for the whole year). Wood et al. (2017) noted that a small cohort ($n = 9$) of one year old alligators released in southeast Oklahoma only had a 22% survival rate through the winter. A previous study conducted at Par Pond using clutches from 1985 and 1987 reported survival rates of 0.524 for the first year (Brandt 1989). With the exception of Wood et al. (2017), our survivorship estimates are lower than previously reported values. Tamsiripong et al. (2006) noted that the presence of an actively guarding female alligator significantly increased survival in hatchling alligators. One of our criteria for choosing release locations was the presence of an active alligator nest from which we had collected that season. We did this in hopes that the mother alligator would still be around to protect the released hatchlings, though we were unable to verify this, which may explain our lower survivorship if mothers were not present.

An effect of incubation temperature was included in many of our top models. Incubation temperature was present in nearly all of the top models for hatchling morphology, in which case individuals incubated at MPT were generally larger. This is not surprising given previous results that have shown a positive impact of warmer incubation temperature on size in alligators (Bock et al. 2021, Allsteadt and Lang 1995). The exception was with hatchling TG, where we found a negative relationship with individuals incubated at MPT. Incubation temperature also featured heavily in our behavior trial analyses and was present in all but one of our top models.

Individuals incubated at MPT spent significantly more time on basking platforms. Basking behavior is indicative of 'boldness,' since, although important for maintaining physiological function and growth in ectotherms, it may leave individuals more exposed to predation (Kashon

and Carlson 2017, Carter et al. 2010, Smith and Blumstein 2008, Stamps 2007). Anti-predator behavior can similarly be viewed as a trade-off, with evidence showing that increased habituation to low level threats enhances body condition (Rodríguez-Prieto et al. 2010). However, relationships between bold behavior and survivorship are tenuous at best: Bremner-Harrison et al. (2004) found that captive-raised swift foxes (*Vulpes velox*) categorized as bold exhibited higher mortality when released, whereas Allard et al. (2019) did not find any relationship between boldness and survivorship in Blanding's turtles (*Emydoidea blandingii*). We found that proportion of time spent in warm areas was negatively correlated with survivorship, as opposed to basking or freeze time. Additionally, individuals incubated at FPT spent proportionally more time in warm areas, potentially explaining their lower survival rates. Future studies should explore this linkage further as well as incorporating repeated trials to examine the consistency of the behaviors we analyzed and possibility of personality (Waters et al. 2017, MacKay and Haskell 2015) within hatchling alligators.

To our knowledge, our study is the first to employ a mark-recapture analysis to examine the effects of maternally-transferred mercury and its sublethal effects on hatchling survival in herpetofauna. Our intent was observational in design, opting to observe effects in a naturally exposed population rather than employ artificial experimental exposures. Although this design prevented us from disentangling certain variables (e.g., maternally-transferred mercury vs. general maternal provisioning), it establishes a baseline for expected effects in an ecologically relevant context. Despite mercury exposure in these individuals being relatively high for crocodylians, we only observed weak relationships with mercury that were largely overshadowed by prominent relationships with incubation temperature. The concentration of mercury we detected in egg yolk from Par Pond ($\bar{x} = 0.368$ ppm) was above the low magnitude effect

threshold in fish muscle (0.200 ppm Hg; Canham et al. 2020) but below that for bird eggs (0.600–2.700 ppm; Fuchsman et al. 2017), so it is quite possible we are approaching a similar threshold for alligators. However, no such threshold has been developed, and there are a number of other processes such as feeding efficiency, metabolism, and physiological function (Lemaire et al. 2021b, Cusaac et al. 2016, Hopkins et al. 1999) that we did not measure. Incubation temperature has been found to influence neonatal morphology, behavior, and/or survival in a variety of species (While et al. 2018, Noble et al. 2017), and here we document correlations with all three of these aspects within the same group of individuals, highlighting the significance of incubation temperature in the American alligator. Environmental mercury contamination is a widespread issue, posing a potential hazard to threatened crocodylian species that live in affected areas (e.g., American crocodile, Orinoco crocodile [*C. intermedius*]; Balaguera-Reina et al. 2017, Rendón-Valencia et al. 2014, Stoneburner and Kushlan 1984). Future research should therefore focus on establishing threshold effect levels in alligators, as well as expanding the breadth of focus to incorporate other crocodylian species.

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Table 1. Summary of model statistics for measures of reproductive output and hatchling morphometrics in relation to THg concentrations in egg yolk (Yawkey and Par Pond) and in hatchling blood (Par Pond). Abbreviations used for certain independent variables are as follows: YOLK_THg: total mercury concentration in egg yolk; BLOOD_THg: total mercury concentration in hatchling blood.

	Statistical approach	Dependent variable	Independent variables in top model	Random effect	AICc	Akaike weight	p-value^a	R²
YOLK_THg	Linear model	Egg mass	Clutch + YOLK_THg + Site	NA	379.2	0.315	> 0.001	0.821
	Generalized linear model	Clutch size	YOLK_THg + Site + YOLK_THg:Site	NA	136.1	0.414	> 0.001	0.763 ^b
	Generalized linear model	Clutch viability	YOLK_THg + Incubation_temp + Site + YOLK_THg:Site + YOLK_THg:Incubation_temp + Incubation_temp:Site	NA	152.6	0.651	> 0.001	0.926 ^b
	Linear model	Hatchling mass	YOLK_THg + Incubation_temp + Site + YOLK_THg:Site	NA	-69.5	0.330	> 0.001	0.835
	Linear model	Hatchling SVL	Incubation_temp + Site	NA	38.3	0.322	0.004	0.264
	Linear model	Hatchling TL	Site	NA	82.4	0.363	0.030	0.115
	Linear model	Hatchling BMI	YOLK_THg + Incubation_temp + Site + Incubation_temp:Site	NA	-248.7	0.348	> 0.001	0.539
	Linear model	Hatchling HL	YOLK_THg + Incubation_temp + SVL + Site + YOLK_THg:Incubation_temp + YOLK_THg:Site	NA	30.7	0.610	> 0.001	0.890
	Linear model	Hatchling HW	Incubation_temp + SVL + Site	NA	28.8	0.490	> 0.001	0.657
BLOOD_THg	Generalized linear mixed model	Hatchling mass	Egg_mass + Incubation_temp	Clutch	190.3	0.999	> 0.001	0.414 ^c
	Generalized linear mixed model	Hatchling SVL	Incubation_temp	Clutch	183.1	0.973	> 0.001	0.331 ^c
	Generalized linear mixed model	Hatchling TL	Incubation_temp	Clutch	401.0	0.965	> 0.001	0.342 ^c
	Generalized linear mixed model	Hatchling TG	Incubation_temp + SVL	Clutch	-88.6	0.942	> 0.001	0.442 ^c
	Generalized linear mixed model	Hatchling HL	Incubation_temp + SVL	Clutch	325.6	0.906	> 0.001	0.587 ^c

Generalized linear mixed model	Hatchling HW	Incubation_temp + SVL	Clutch	301.1	0.975	> 0.001	0.341 ^c
Generalized linear mixed model	Hatchling BMI	Incubation_temp	Clutch	-257.3	0.937	> 0.001	0.691 ^c
Linear model	Hatchling mass	Clutch + Egg_mass + BLOOD_THg + Incubation_temp	NA	740.4	0.632	> 0.001	0.749
Linear model	Hatchling SVL	Clutch + Incubation_temp	NA	169.8	0.649	> 0.001	0.332
Linear model	Hatchling TL	Clutch + Incubation_temp	NA	384.2	0.466	> 0.001	0.254
Linear model	Hatchling TG	Clutch + BLOOD_THg + Incubation_temp + SVL	NA	-120.4	0.433	> 0.001	0.513
Linear model	Hatchling HL	Clutch + BLOOD_Hg + Incubation_temp + BLOOD_THg:Incubation_temp + SVL	NA	310.0	0.548	> 0.001	0.338
Linear model	Hatchling HW	Clutch + BLOOD_THg + Incubation_temp + BLOOD_THg:Incubation_temp + SVL	NA	289.2	0.412	> 0.001	0.349
Linear model	Hatchling BMI	Clutch + BLOOD_THg + Incubation_temp + BLOOD_THg:Incubation_temp	NA	-306.3	0.806	> 0.001	0.694

^ap-values for generalized linear mixed model calculated by comparing top model vs. model with just random effects in ANOVA

^bNagelkerke pseudo-R²

^cConditional R²

Table 2. Summary of model statistics for hatchling behavior. Abbreviations used for certain independent variables are as follows: BLOOD_THg: total mercury concentration in hatchling blood; Heron: categorical variable indicating presence or absence of the decoy heron; Interior/Exterior: binary variable indicating zone where activity occurred.

Statistical approach	Dependent variable	Independent variables in top model	Random effect	AICc	Akaike weight	p-value ^a	R ^{2(b)}
Generalized linear mixed model	Proportion of time spent basking	Incubation_temp + Heron	Clutch, Subject	-105.7	0.982	> 0.001	0.519
Generalized linear mixed model	Proportion of time spent on warm side	Incubation_temp	Clutch, Subject	-208.5	0.527	0.005	0.237
Generalized linear mixed model	Proportion of time spent on periphery	Heron	Clutch, Subject	-93.7	0.521	> 0.001	0.486
Generalized linear mixed model	Proportion of time spent active	Incubation_temp + Interior/Exterior	Clutch, Subject	-1190.1	0.990	> 0.001	0.046
Generalized linear mixed model	Freeze time	Incubation_temp	Clutch	232.7	0.855	0.014	0.093
Linear model	Proportion of time spent basking	Clutch + Incubation_temp + Heron + BLOOD_THg	NA	-103.2	0.480	> 0.001	0.271
Linear model	Proportion of time spent on warm side	Clutch + Incubation_temp	NA	-219.1	0.491	> 0.001	0.094
Linear model	Proportion of time spent on periphery	BLOOD_THg + Incubation_temp + Heron + BLOOD_THg:Incubation_temp	NA	-68.0	0.338	> 0.001	0.086
Linear model	Proportion of time spent active	Incubation_temp + Interior/Exterior	NA	-1218.7	0.264	> 0.001	0.045
Linear model	Freeze time	Incubation_temp	NA	228.9	0.573	0.016	0.078

^ap-values for generalized linear mixed model calculated by comparing top model vs. model with just random effects in ANOVA

^bConditional R² for generalized linear mixed model

Table 3. Full breakdown of PCA components used to collapse hatchling morphometrics collected the day of release (10–15 days post-hatch).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Mass	-0.4165	0.3328	0.0196	0.3036	-0.0023	0.2146	0.7597
SVL	-0.3963	-0.3150	0.3254	-0.0338	0.2413	0.7096	-0.2739
TL	-0.3891	-0.3445	0.3051	0.2530	0.4121	-0.6347	0.0092
TG	-0.3773	0.3601	-0.0543	-0.8009	0.2479	-0.1486	-0.0004
HL	-0.4046	-0.2859	0.1362	-0.1775	-0.8277	-0.1394	0.0077
HW	-0.2838	-0.3720	-0.8750	0.0502	0.1051	0.0436	-0.0021
BMI	-0.3626	0.5671	-0.1163	0.4088	-0.1200	-0.0648	-0.5896
Standard deviation	2.2450	0.9430	0.7827	0.4934	0.3444	0.3091	0.0231
Proportion of Variance	0.7200	0.1270	0.0875	0.0348	0.0170	0.0137	0.0001
Cumulative Proportion	0.7200	0.8470	0.9346	0.9693	0.9863	0.9999	1.0000

Table 4. Summary of apparent survivorship (ϕ) and recapture probability (p) for Site 3 under the environmental and morphology model sets. We additionally calculated the number of individuals presumed alive at each recapture event based on the apparent survivorship calculated by Program MARK.

Recapture #		1	2	3	4	5	6
Environment (n = 54)	ϕ	0.96	0.88	0.97	0.97	0.94	0.88
	p	0.51	0.69	0.89	0.86	0.74	0.99
	# alive	46	31	28	18	15	10
Morphology (n = 53)	ϕ	0.96	0.88	0.97	0.97	0.95	0.89
	p	0.52	0.69	0.89	0.87	0.75	1.00
	# alive	46	31	28	18	15	11

Table 5. Summary of model statistics for post-winter survival under three different model sets analyzing relationships with early environmental influences, morphology, and behavior. The following abbreviations are used: PC1: principal component 1 (see Table 3); Prop_warm: proportion of time spent on the warm side of the behavior trial arena.

Statistical approach	Model set	Independent variables in top model	Random effect	AICc	Akaike weight	p-value	R²
Generalized linear mixed model	Environmental	Mass + Release_site + Incubation_temp	Clutch, Pod	176.3	0.471	> 0.001 ^a	0.321 ^b
Generalized linear mixed model	Morphology	PC1 + Release_site	Clutch, Pod	192.2	0.312	0.012 ^a	0.276 ^b
Generalized linear mixed model	Behavior	Prop_warm	Clutch, Pod	84.1	0.173	0.039 ^a	0.211 ^b
Generalized linear model	Environmental	Mass + Release_site + Incubation_temp	NA	171.9	0.327	> 0.001	0.284 ^c
Generalized linear model	Morphology	Pod + Release_site	NA	183.3	0.274	> 0.001	0.229 ^c
Generalized linear model	Behavior	Pod + Prop_warm	NA	73.8	0.094	> 0.001	0.306 ^c

^ap-values for generalized linear mixed model calculated by comparing top model vs. model with just random effects in ANOVA

^bConditional R²

^cNagelkerke pseudo-R²

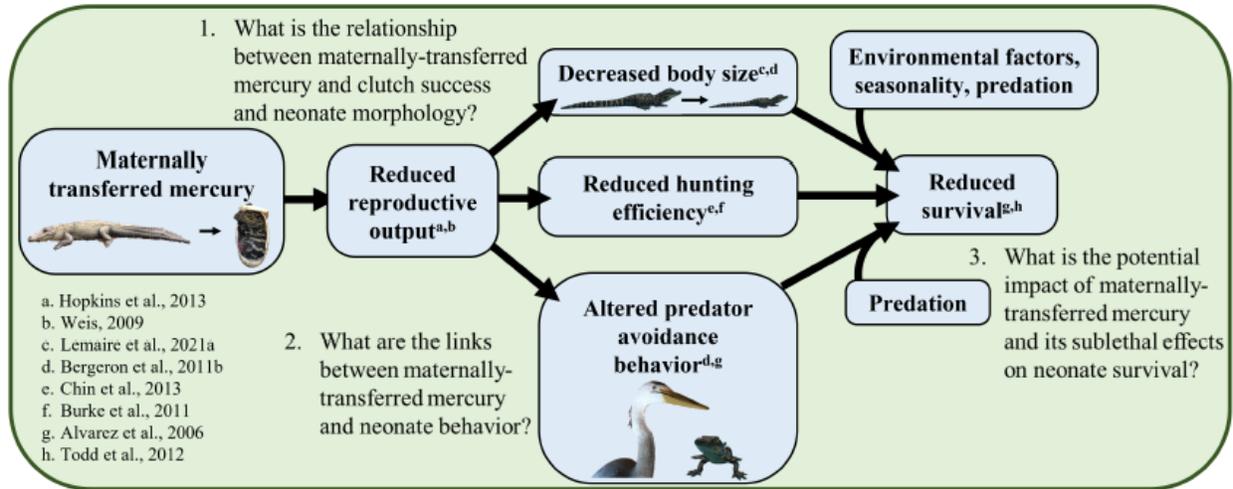


Figure 1. Conceptual model of documented sublethal effects of maternally-derived mercury, showcasing in particular the three main objectives of our study (numbered).

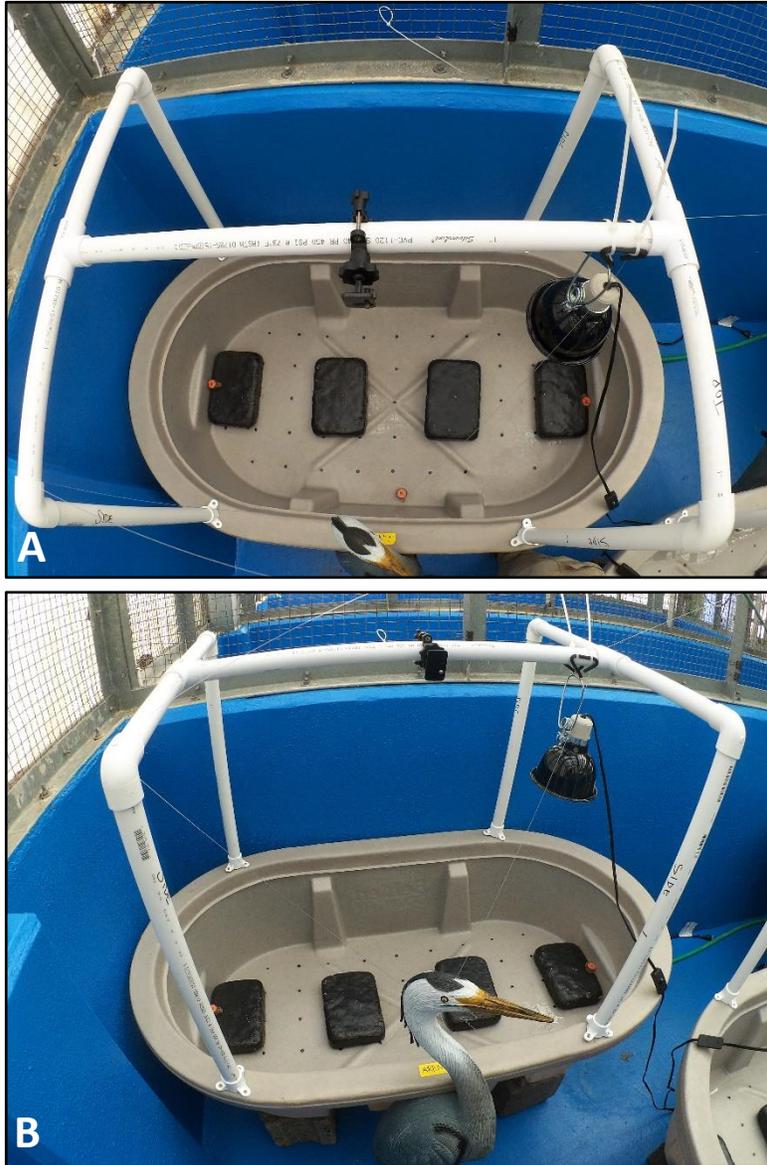


Figure 2. Images of our behavioral trial arena setup highlighting the suspended basking lamp and camera fixture (A) and the decoy heron (B).

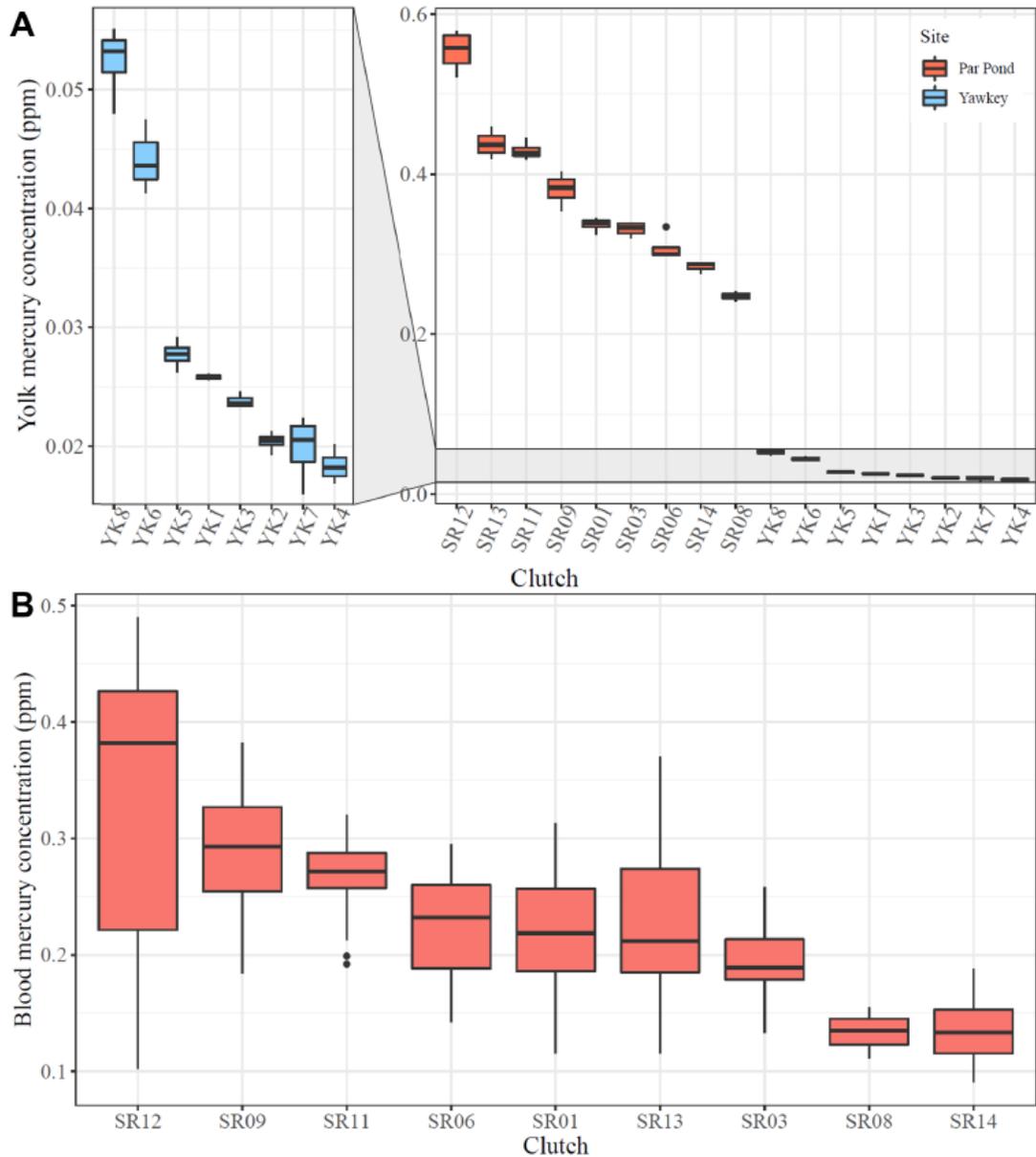


Figure 3. Comparison of THg concentrations found in egg yolk (A) from both Yawkey and Par Pond, and THg concentrations found in blood (B) from Par Pond hatchlings. Boxes show the median and first and third quartiles (inter-quartile range); whiskers extend up to 1.5 times the inter-quartile range, and any outliers beyond are shown as individual points.

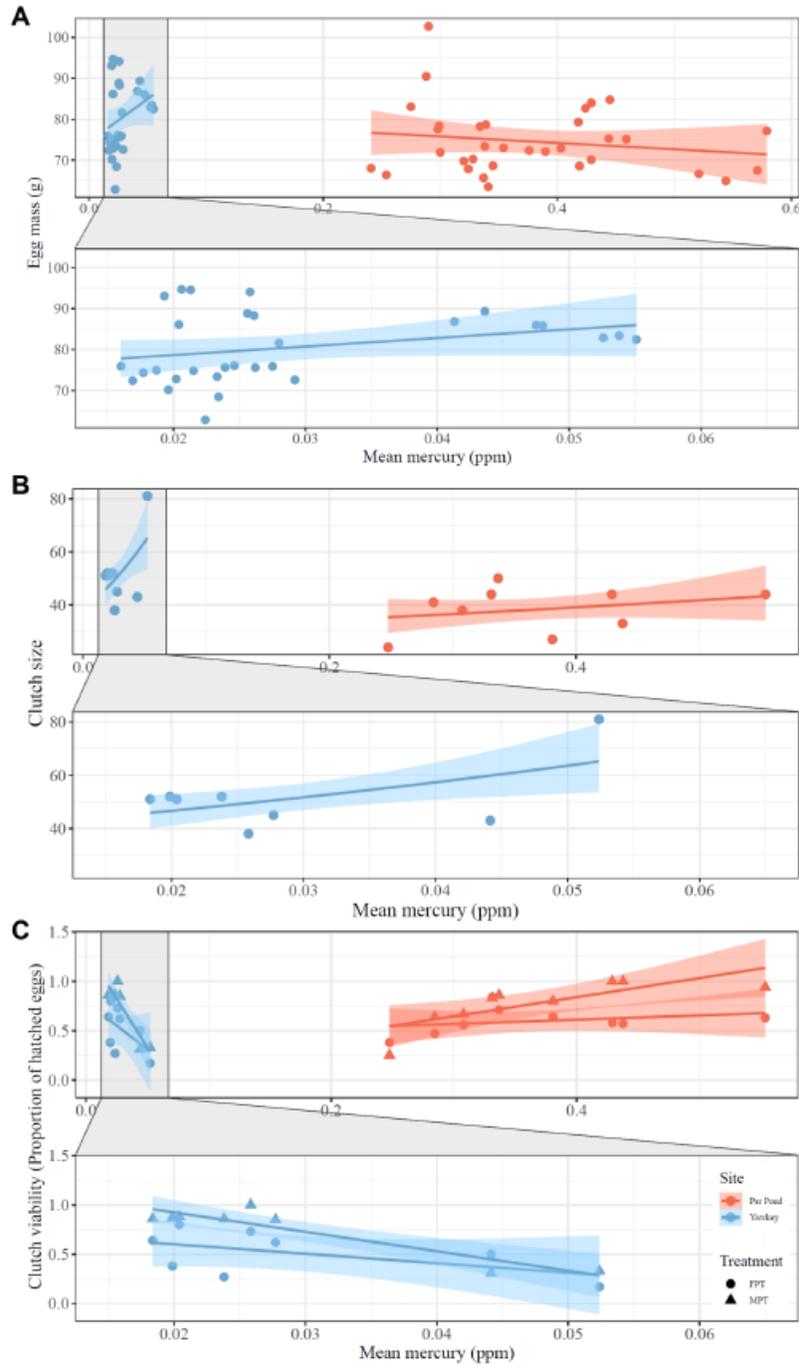


Figure 4. Reproductive output metrics for clutches from Yawkey and Par Pond. For both egg mass (A) and clutch size (B), metrics were larger at Yawkey and positively correlated with THg concentration in egg yolk. Clutch viability (C) was higher at Par Pond and in eggs incubated at MPT, and was positively correlated with THg concentration in egg yolk. Metrics are separated by site in the plots (Yawkey – blue, Par Pond – red) but are included in single models as reported in the Results. Shaded portions represent standard error.

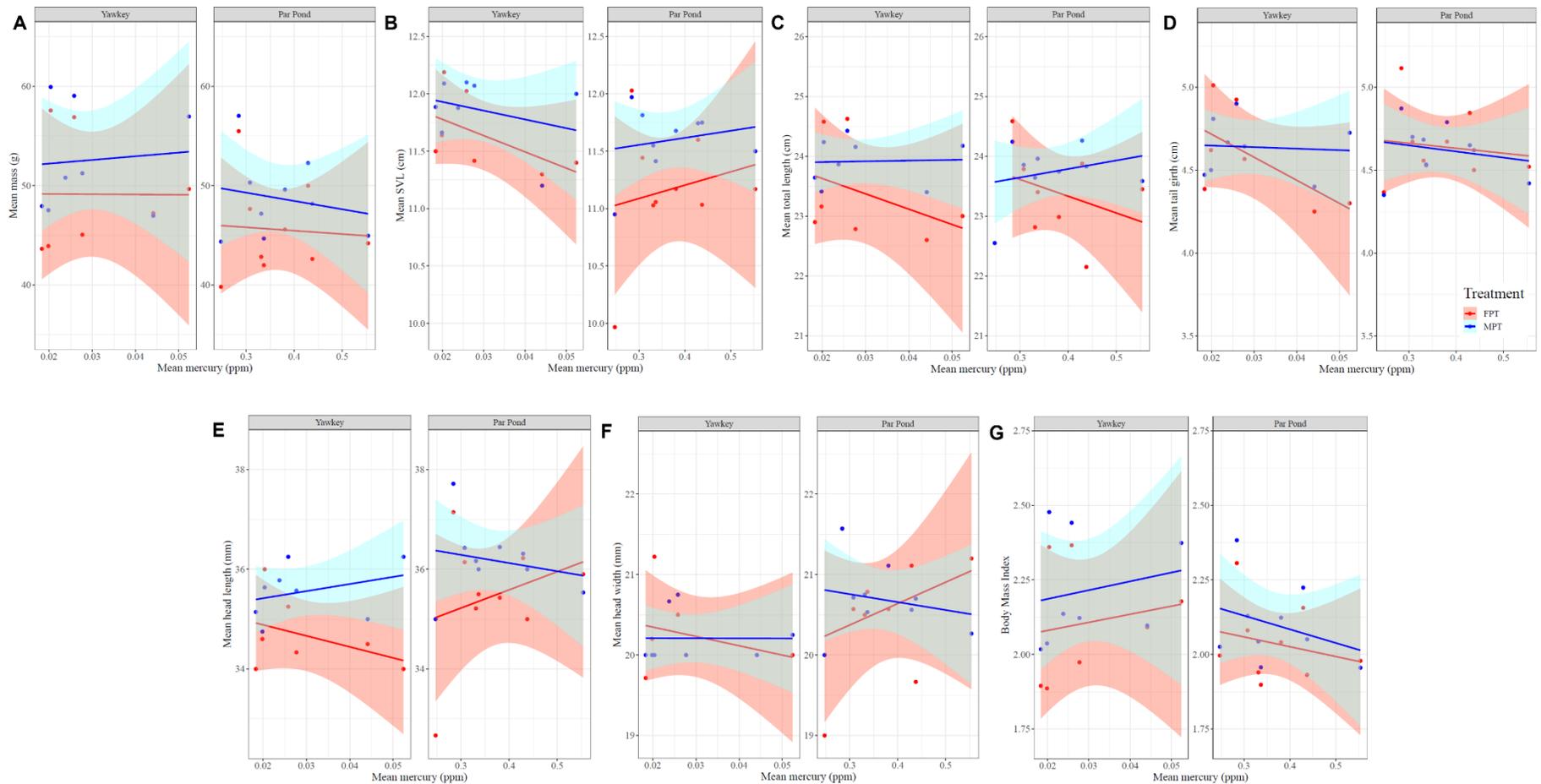


Figure 5. Clutch-averaged hatchling morphometrics. Hatchling mass (A), SVL (B), and TL (C) were greater in individuals from Yawkey and those incubated at MPT. Hatchling TG (D) was smaller in individuals from Yawkey and those incubated at MPT. Hatchling HL (E) and HW (F) were smaller in individuals from Yawkey but larger in those incubated at MPT. Across all metrics, THg concentration in egg yolk was positively correlated with hatchling mass and HL but negatively correlated with hatchling TG and BMI (G). Metrics are separated by site and incubation temperature treatment in the plots (MPT – blue, FPT – red) but are included in single models as reported in the Results. Shaded portions represent standard error.

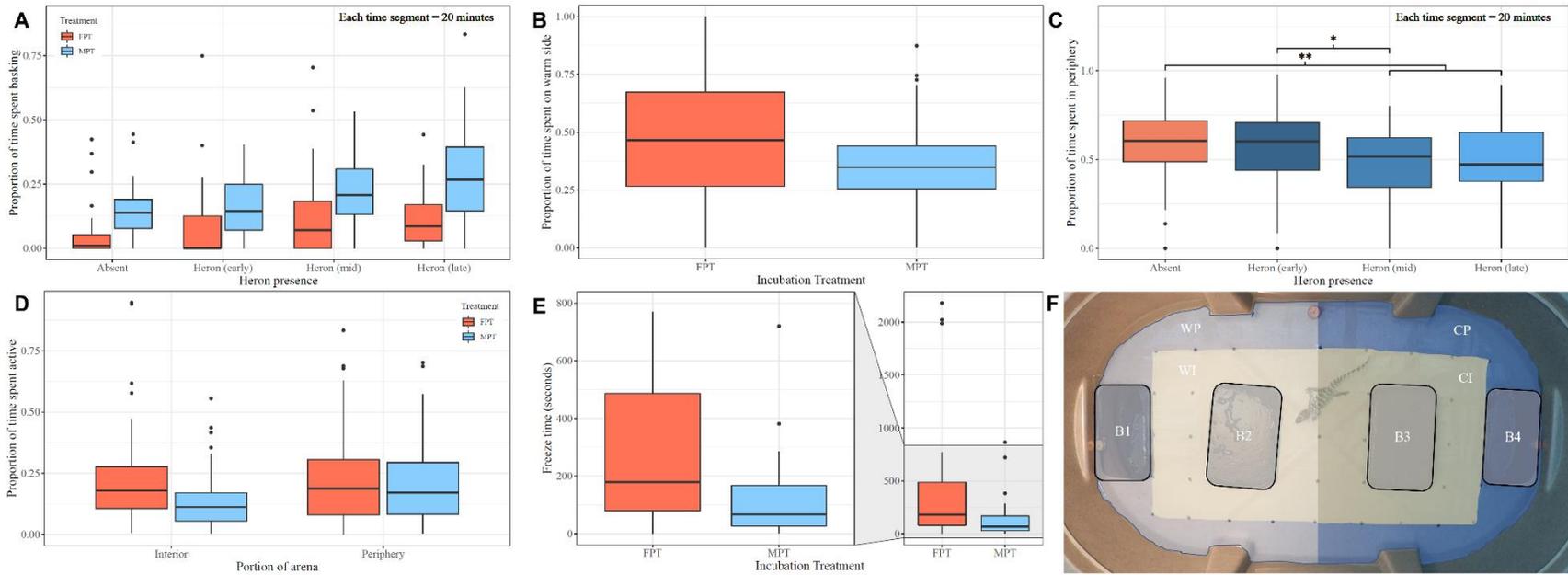


Figure 6. Behavior trial metrics, showing only the variables present in the top models under the generalized linear mixed model approach. For the proportion of time spent basking (A), significant differences were found between successive time blocks and between presumptive sexes within each time block. Individuals incubated at FPT spent a greater proportion of time on the warm side of the arena (B), and the proportion of time spent in the periphery (C) declined throughout the behavior trial. Colors shown in (C) delineate the various time blocks of the trial. Individuals incubated at FPT spent a greater proportion of time active (D), and there was marginally more activity in peripheral areas. Freeze time (E) was greater in individuals incubated at FPT. A schematic of the behavior trial arena, with a hatchling alligator for scale, is shown in (F). Regions of interest were divided into the following eight zones: WP = Warm side, periphery; WI = Warm side, interior; CI = Cool side, interior; CP = Cool side, periphery; B1 = Basking platform #1; B2 = Basking platform #2; B3 = Basking platform #3; B4 = Basking platform #4.

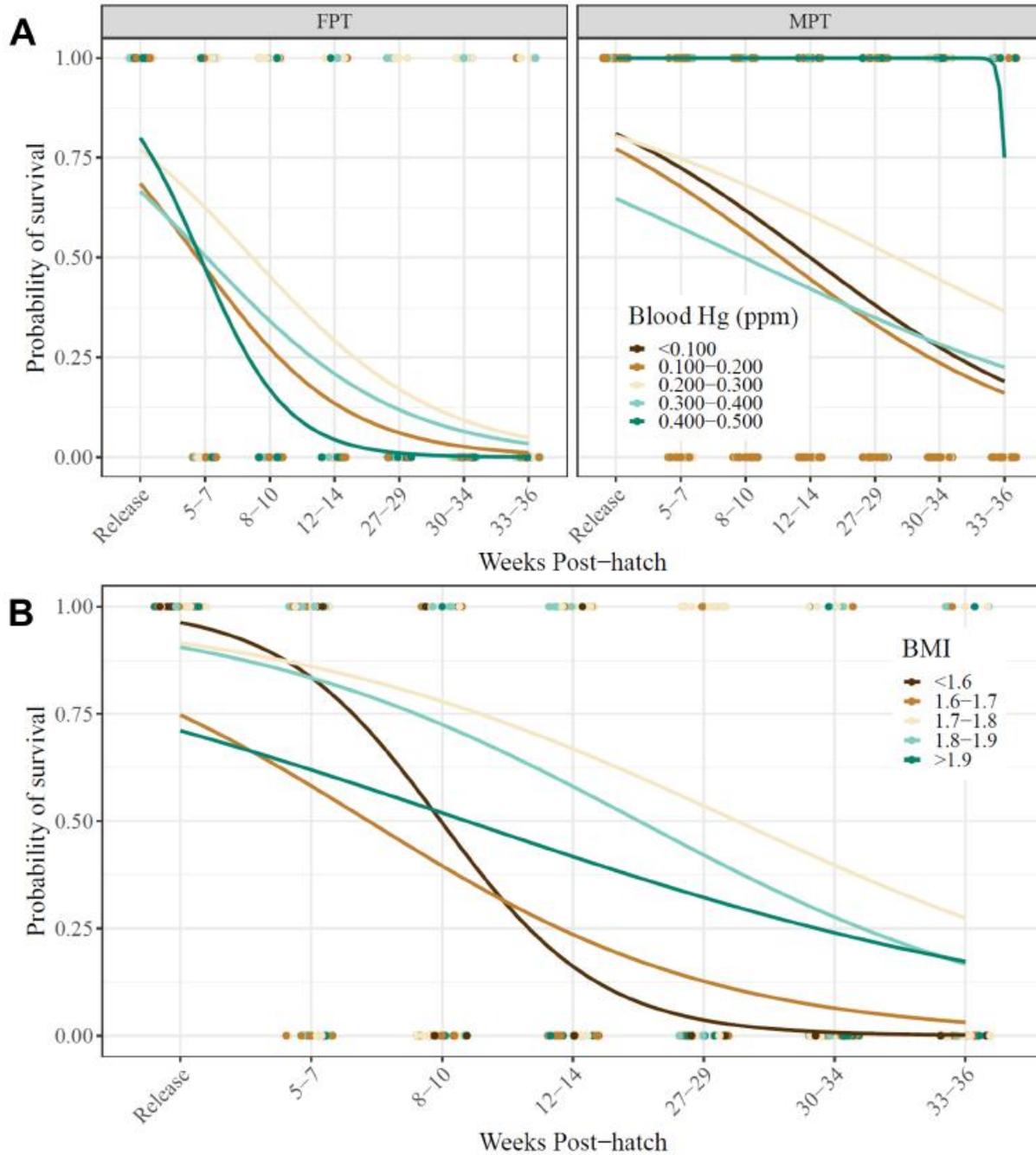


Figure 7. Survivorship of hatchlings based off the environmental (A) and morphology (B) model sets as calculated in Program MARK. For both plots, THg concentration in blood and BMI are grouped into classes for better visualization but are kept as continuous variables in their respective model sets. Points represent individual hatchlings, with trendlines showing the overall probability of survival over the given time period.