Final Report for Assessment of PAH Content in Eggs of American White Pelicans Nesting in Minnesota

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Introduction

The American white pelican (*Pelecanus erythrorhynchos*) is a Species of Special Concern that breeds colonially in several Minnesota marshes and winters along the coast of the Gulf of Mexico. After wintering along the coast, American white pelicans arrive in Minnesota during March and early April. The Deepwater Horizon oil spill in the Gulf of Mexico began on April 20, 2010, and posed a potentially significant threat to pelican populations breeding in Minnesota. The 2011 breeding season represents the first breeding season following potential exposure to petroleum contaminants while wintering in the Gulf of Mexico for American white pelicans nesting in Minnesota. We examined the prevalence and levels of polycyclic aromatic hydrocarbons (PAH) and dioctyl sodium sulfosuccinate (DOSS; a surfactant used to disperse oil during the 2010 Deepwater Horizon spill) in American white pelican eggs and tissues collected from 2011-2015 from three pelican colonies in Minnesota.

Sample Collection

We collected eggs and rhynotheca (i.e., bill knobs) from American white pelican (*Pelecanus erythrorhynchos*) colonies located in Minnesota from 2011-2015.

In 2011 we collected a total of 96 eggs and 27 bill knobs from three colonies in Minnesota. We collected 70 eggs from the Marsh Lake colony (located on the Minnesota River in the Lac qui Parle Wildlife Management Area in Big Stone and Lac qui Parle counties in southwestern Minnesota), 14 eggs from the Swartout Lake colony (located in Wright county in south-central Minnesota) and 12 eggs from the Pigeon Lake colony (located in Meeker county in south-central Minnesota). Egg collections from Marsh Lake were made over a five-week period (with approximately equal numbers collected each week), and eggs were collected within approximately 24 hours from laying. Egg collections from Swartout and Pigeon Lakes occurred on a single date, and included only unincubated, abandoned eggs. We collected 20 bill knobs shed in 2011 from the Marsh Lake colony, two bill knobs shed in 2011 from the Swartout Lake colony and five bill knobs shed in 2011 from the Pigeon Lake colony. Mixed yolk and albumen samples from 27 eggs from the Marsh Lake colony, from all 14 eggs from the Swartout Lake colony and from all 12 eggs from the Pigeon Lake colony were prepared and sent to the University of Connecticut for analysis of PAH and DOSS (Tables 1 and 2). Samples from remaining eggs (i.e., those not sent for analysis) have been preserved in storage (-80 C). All bill knobs collected in 2011

(i.e., 20 from Marsh Lake, 2 from Swartout Lake and 5 from Pigeon Lake) plus 10 knobs shed in years before 2011 (all from the Marsh Lake colony) were sent for analysis of PAH and DOSS at the University of Connecticut (Table 3).

In 2012 we collected a total of 40 eggs and 15 bill knobs from the American white pelican colony nesting at Marsh Lake. Egg collections from Marsh Lake were made over a four-week period (with approximately equal numbers collected each week), and eggs were collected within approximately 24 hours of laying. We also collected eggs (within 24 hours of laying) from 10 Ring-billed gulls (*Larus delawarensis*) nesting at Marsh Lake for comparative analyses with the American white pelican eggs. Mixed yolk and albumen samples from 20 eggs (16 American white pelican eggs, 4 Ring-billed gull eggs) were prepared and sent to the University of Connecticut for analysis of PAH and DOSS (Tables 1 and 2). Samples from remaining eggs (i.e., those not sent for analysis) have been preserved in storage (-80 C). Bill knobs were preserved in storage (0 C) for analysis at a later date if needed.

In 2013 we collected a total of 15 pelican eggs from the Marsh Lake colony (Table 1). Egg collections from Marsh Lake were made in the third week of May only (and all eggs were collected within 24 hours of laying), because nesting was delayed more than three weeks from observations of previous years. Mixed yolk and albumen samples from 7 eggs were prepared and sent to the University of Connecticut for analysis of PAH and DOSS (Tables 1 and 2). Samples from remaining eggs (i.e., those not sent for analysis) have been preserved in storage (-80 C).

In 2014 we collected a total of 20 pelican eggs from the Marsh Lake colony (Table 1). Egg collections from Marsh Lake were made in the first week of May only (and all eggs were collected within 24 hours of laying). We also collected eggs (within 24 hours of laying) from five Ring-billed gulls nesting at Marsh Lake for comparative analyses with the American white pelican eggs. Mixed yolk and albumen samples from 20 eggs (15 American white pelican eggs, 5 Ring-billed gull eggs) were prepared and sent to the University of Connecticut for analysis of PAH and DOSS (Tables 1 and 2). Samples from remaining eggs (i.e., those not sent for analysis) have been preserved in storage (-80 C).

In 2015 we collected a total of 64 pelican eggs from the Marsh Lake colony (Table 1). Egg collections from Marsh Lake were made each week from the third week of April to the second week of May (and all eggs were collected within 24 hours of laying). We also collected eggs (within 24 hours of laying) from four Ring-billed gulls, two Great blue herons (*Ardea herodias*) and one Canada goose (*Branta canadensis*) nesting at Marsh Lake for comparative analyses with the American white pelican eggs. Mixed yolk and albumen samples from 24 eggs (19 American white pelican eggs, 3 Ring-billed gull eggs, 1 Great blue heron egg and 1 Canada goose egg) were prepared and sent to the University of Connecticut

for analysis of PAH and DOSS (Tables 1 and 2). Samples from remaining eggs (i.e., those not sent for analysis) have been preserved in storage (-80 C).

Preliminary Findings

Prevalence of PAH in American white pelican eggs differed among colonies and from the prevalence of PAH in Ring-billed gull eggs. Concentrations of PAH were detected in 56 of 124 egg samples analyzed (Table 1). Concentrations of PAH were present in American white pelican eggs from all locations (Table 1). In 2011, detectable concentrations of PAH were more likely to be found in the eggs of American white pelicans nesting at Marsh Lake ($\chi^2(2) = 9.7$, $p = 0.008$; Table 1). However when all years were combined detectability did not differ among locations ($\chi^2(2) = 1.0$, $p = 0.617$; Table 1). Among all locations, detection rates of PAH were significantly higher in pelican eggs from the 2011 and 2012 breeding seasons ($\chi^2(4) = 49.4$, $p < 0.001$; Table 1). For pelican eggs collected from nests at Marsh Lake, PAH was significantly more likely to be detected in eggs from 2011 and 2012 compared to eggs from 2013-2015 ($\chi^2(4) = 58.2$, $p < 0.001$; Table 1). However, detection rates of PAH in pelican eggs laid in 2011 and 2012 did not differ at Marsh Lake ($\chi^2(1) = 0.3$, $p = 0.600$; Table 1). Detection of PAH in pelican eggs collected from Marsh Lake was not significantly different from detection in Ring-billed gull eggs ($\chi^2(1) = 0.2$, $p = 0.698$; Table 1). However in 2012 detection of PAH was more likely in pelican eggs compared to Ring-billed gull eggs ($\chi^2(1) = 12.4$, $p < 0.001$; Table 1), but less likely in pelican eggs in 2014 ($\chi^2(2) = 7.7$, $p = 0.006$; Table 1) and 2015 ($\chi^2(1) = 4.3$, $p = 0.038$; Table 1). PAH was not detected in the eggs of Great blue herons or Canada geese nesting at Marsh Lake in 2015 (Table 1).

Concentrations of PAH found in American white pelican eggs were significantly higher at the Marsh Lake colony in 2012. American white pelican eggs from the Marsh Lake colony had higher PAH concentrations in 2012 (6.2 ± 0.2 log ng/g) than in 2011 (5.2 ± 0.2 log ng/g), 2013 (4.4 ± 0.5 log ng/g) and 2014 (4.1 ± 0.5 log ng/g) ($F_{3,36} = 9.3$, $p < 0.001$, $r^2 = 0.30$; Figure 1). For eggs collected in 2011, log-transformed concentrations of PAH in pelican eggs from Marsh Lake (mean ± standard error: 5.2 ± 0.2 log ng/g) were not significantly different than concentrations in eggs from Pigeon Lake (4.6 ± 0.4 log ng/g) or Swartout Lake (4.6 ± 0.3 log ng/g) ($F_{2,30} = 2.1$, $p = 0.142$, $r^2 = 0.12$; Figure 2). Recovery rates for the PAH assay were 89.6 ± 1.7%.

Prevalence of DOSS in American white pelican eggs did not differ among colony locations in 2011, but was significantly more prevalent in 2011 compared to 2012. Concentrations of dispersant (DOSS) were detected in 27 of 66 egg samples analyzed (Table 2). Detection rates for DOSS in American white pelicans did not differ among colony locations in 2011 ($\chi^2(2) = 4.2$, $p = 0.124$; Table 2). DOSS was significantly more likely to be detected in pelican eggs collected in 2011 from Marsh Lake compared to eggs collected from Marsh Lake

in 2012 ($\chi^2(1) = 20.2$, $p < 0.001$; Table 2). Dispersant was not detected in Ring-billed gull eggs collected from Marsh Lake in 2012. We are still awaiting results for the analysis of DOSS in the 2013-2015 samples.

Concentrations of PAH were detected in 29 of 37 bill knob samples analyzed. Concentrations of PAH were detected in bill knobs collected from each of the colonies at rates that were not significantly different ($\chi^2(2) = 5.6$, $p = 0.062$; Table 3). Concentrations of PAH were more likely to be detected in knobs collected before 2011 ($\chi^2(2) = 5.8$, $p = 0.016$; Table 3) when comparing all sites. However at the Marsh Lake colony, detection rates for PAH in knobs did not differ between knobs collected before 2011 and those collected in 2011 ($\chi^2(1) = 3.5$, $p = 0.060$; Table 3).

PAH concentrations in pelican bill knobs did not differ among colony locations, but were higher for knobs collected prior to 2011. Log-transformed concentrations of PAH in knobs from Marsh Lake (2.0 ± 0.2 log ng/g) were not significantly different from concentrations in knobs from Pigeon Lake (0.9 ± 0.6 log ng/g) or Swartout Lake (1.4 ± 0.8 log ng/g) ($F_{2,26} = 1.8$, $p = 0.183$, $r^2 = 0.12$). However knobs collected prior to 2011 had higher PAH concentrations (2.5 ± 0.2 log ng/g) than knobs collected in 2011 (1.5 ± 0.2 log ng/g) ($F_{1,27} = 12.7$, $p = 0.001$, $r^2 = 0.32$) across all locations. Likewise, knobs from the Marsh Lake colony collected prior to 2011 had higher PAH concentrations (2.5 ± 0.2 log ng/g) than knobs collected from Marsh Lake in 2011 (1.6 ± 0.1 log ng/g) ($F_{1,25} = 15.0$, $p < 0.001$, $r^2 = 0.39$). However, recovery rates for the PAH assay on knobs were below 50% (45.7 ± 6.7%).

Prevalence of DOSS in bill knobs from American white pelican eggs did not differ among colony locations or year collected. Concentrations of dispersant (DOSS) were detected in 14 of 37 knobs analyzed (Table 4). Detection rates for DOSS in American white pelican knobs did not differ among colony locations ($\chi^2(2) = 0.9$, $p = 0.630$; Table 4) or year collected ($\chi^2(1) = 0.03$, $p = 0.869$; Table 4). Neither did detection rates for DOSS in knobs collected only from Marsh Lake differ with year collected ($\chi^2(1) = 0.0$, $p = 1.000$; Table 4).

Summary and Future Plans

Based on the preliminary findings, both petroleum-based hydrocarbons (i.e., PAH) and dispersant (i.e., DOSS) occur in the eggs of American white pelicans nesting in Minnesota. More than half of the pelican eggs analyzed had detectable concentrations of both PAH and DOSS in 2011 and 2012 (Tables 1 & 2). However since 2012, prevalence of PAH in pelican eggs from Marsh Lake has declined, with no PAH detected in eggs collected in 2015 (Table 1). Thus, American white pelicans nesting in Minnesota have been exposed to PAH and DOSS, but exposure after 2012 is declining. Ring-billed gull eggs collected from Marsh Lake in 2012 (where pelicans also nest and have eggs with detectable levels of PAH) did not contain detectable concentrations of PAH, but PAH was

detected in Ring-billed gull eggs in 2014 and 2015. Based on these findings, it is unlikely that exposure to PAH occurs at Marsh Lake because prevalence in pelican eggs is declining while prevalence in Ring-billed gull eggs is increasing.

Concentrations of PAH found in eggs from the Marsh Lake colony in 2011 were not significantly higher than concentrations found in eggs from two other pelican colonies in Minnesota (Figure 2). However, eggs collected from the Pigeon Lake and Swartout Lake colonies were not collected within 24 hours of laying and some volatile compounds in those eggs may have evaporated prior to collection. Our subsequent collections have focused on the Marsh Lake colony, because this colony has been more closely monitored since 2004.

Concentrations of PAH in pelican eggs from Marsh Lake were higher in 2011 and 2012 compared to eggs collected in later years (Figure 1). This indicates that pelicans nesting in Minnesota have been exposed to PAH at some point in their life cycle, but continued exposure may be declining. Comparing concentrations between years may differ in part due to assay effects. We have stored samples from previous years, and future egg collection and analysis will enable us to quantify annual variation from variation associated with assays and therefore definitively characterize the temporal pattern in PAH concentrations in the Marsh Lake colony.

Determination of PAH in rhynotheca collected from American white pelicans has been problematic. Both PAH and DOSS were detected in pelican bill knobs. However, the assay recovery rates were below 50%, and therefore not reliable. We have discontinued bill knob analyses because of concerns related to the efficiency of the assay in extracting PAH from the keratin matrix of the bill knob.

We anticipate preparing our findings in a manuscript submitted for publication in a peer-reviewed journal. Manuscript preparation will begin in late 2016, and we expect to submit the manuscript for review in 2017.

Based on these findings, eggs from American white pelicans nesting in Minnesota have petrogenic polycyclic aromatic hydrocarbons as well as chemicals used as petroleum dispersants. Evidence indicates that prevalence of these compounds declined significantly after 2012. To our knowledge, this study represents the only multi-year investigation of petroleum contaminants in the eggs of colonial waterbirds.

Table 1: Numbers of eggs for which PAH was detected or not detected by location, year and species (AWPE = American white pelican, RBGU = Ring-billed gull, GBHE = Great blue heron, CAGO = Canada goose).

Location	Year	Species	PAH detected	PAH not detected
Marsh Lake	2011	AWPE	22	5
Marsh Lake	2012	AWPE	14	2
Marsh Lake	2013	AWPE	2	5

Marsh Lake	2014	AWPE	2	13
Marsh Lake	2015	AWPE	0	19
Pigeon Lake	2011	AWPE	4	8
Swartout Lake	2011	AWPE	7	7
Marsh Lake	2012	RBGU	0	4
Marsh Lake	2014	RBGU	4	1
Marsh Lake	2015	RBGU	1	2
Marsh Lake	2015	GBHE	0	1
Marsh Lake	2015	CAGO	0	1

Table 2: Numbers of eggs for which DOSS was detected or not detected by location, year and species (AWPE = American white pelican, RBGU = Ring-billed gull).

Location	Year	Species	DOSS detected	DOSS not detected
Marsh Lake	2011	AWPE	15	5
Marsh Lake	2012	AWPE	0	16
Marsh Lake	2012	RBGU	0	4
Pigeon Lake	2011	AWPE	5	7
Swartout Lake	2011	AWPE	7	7

Note: The analysis for DOSS in the 2013-15 samples has not been completed.

Table 3: Numbers of American white pelican rhynotheca (i.e., bill knobs) for which PAH was detected or not detected by location and year collected.

Location	Year	PAH detected	PAH not detected
Marsh Lake	Pre-2011	10	0
Marsh Lake	2011	16	4
Pigeon Lake	2011	2	3
Swartout Lake	2011	1	1

Table 4: Numbers of American white pelican rhynotheca (i.e., bill knobs) for which DOSS was detected or not detected by location and year

Location	Year	DOSS detected	DOSS not detected
Marsh Lake	Pre-2011	4	6
Marsh Lake	2011	8	12
Pigeon Lake	2011	1	4
Swartout Lake	2011	1	1

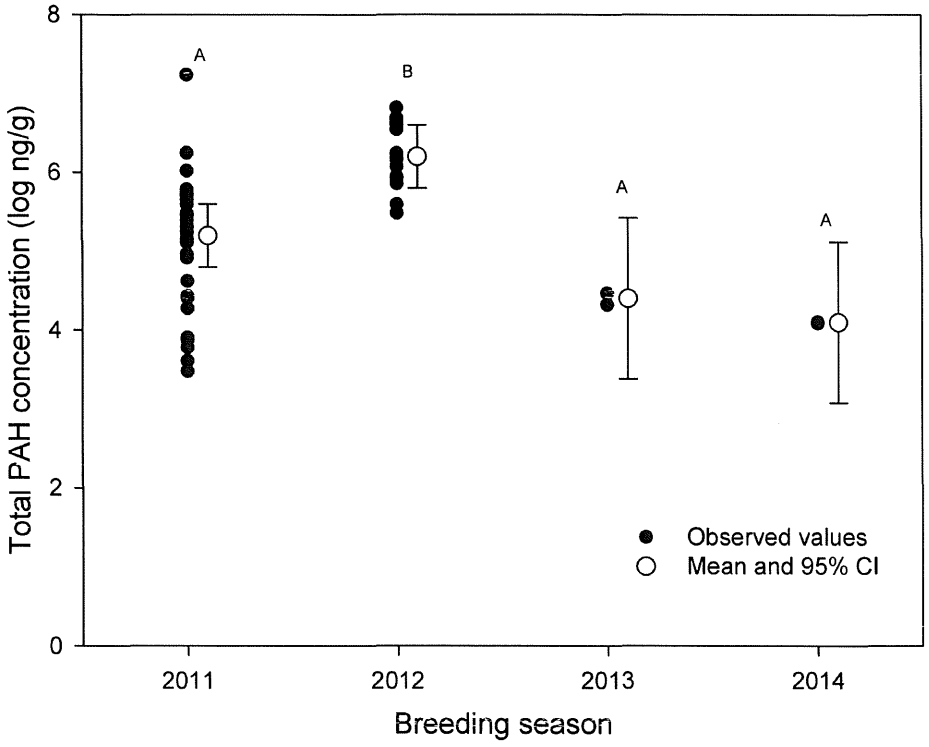

Figure 1: Log-transformed total polycyclic aromatic hydrocarbon concentrations present in the eggs of American white pelicans from Marsh Lake (blue circles) collected in 2011-2014 (blue-filled circles) along with mean concentrations (open circles, with bars indicating 95% confidence limits) for year of collection. Different letters indicate mean levels that are significantly different ($F_{3,36} = 9.3$, $p < 0.001$, $r^2 = 0.30$). Note that detectable levels of PAH were not found in eggs collected in 2015.

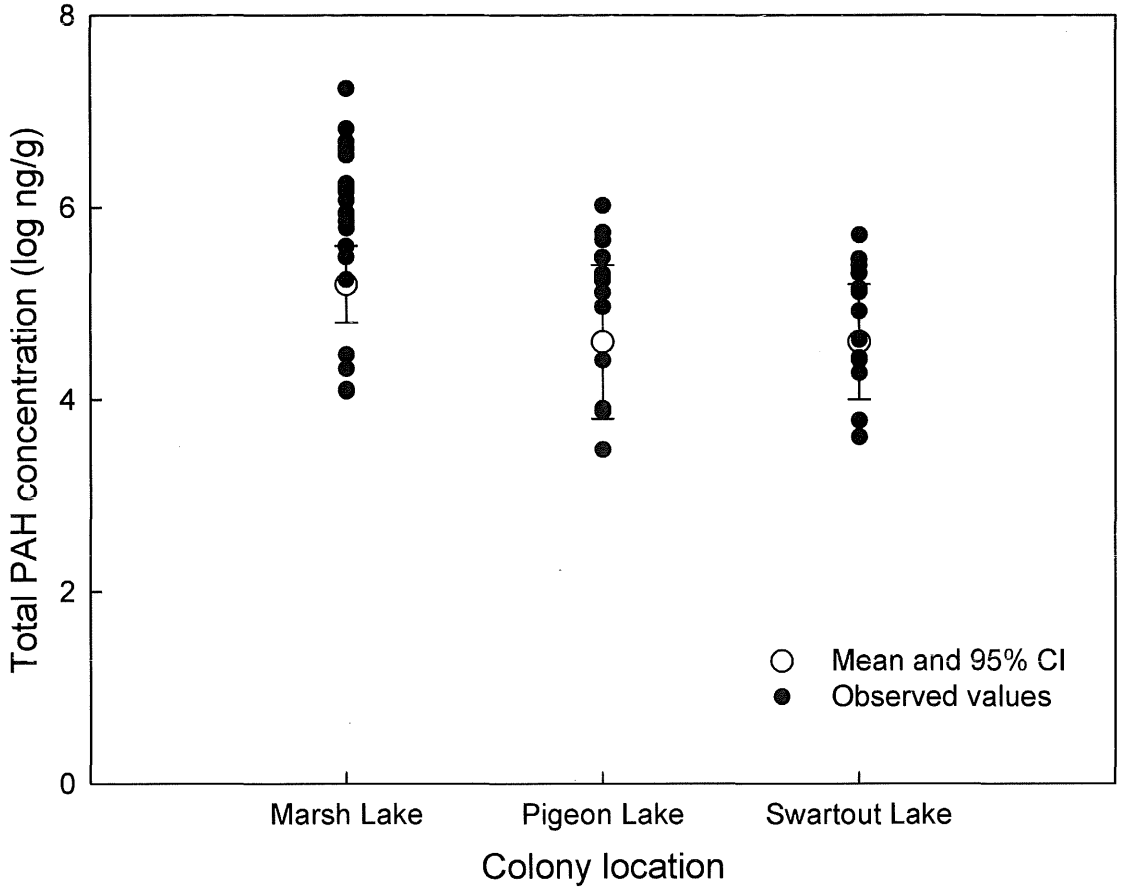

Figure 2: Log-transformed total polycyclic aromatic hydrocarbon concentrations present in the eggs of American white pelicans from Marsh Lake, Pigeon Lake and Swartout Lake in Minnesota (blue-filled circles), along with mean concentrations (open circles, with bars indicating 95% confidence limits) for the respective colonies collected in 2011. Concentrations did not differ among colonies ($F_{2,30} = 2.1$, $p = 0.142$, $r^2 = 0.12$).