

# Blood Lead Exposure Concentrations in Mottled Ducks (*Anas fulvigula*) on the Upper Texas Coast

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**Abstract:** The mottled duck (*Anas fulvigula*) is a non-migratory waterfowl species dependent upon coastal marsh systems, including those on the Texas Chenier Plain National Wildlife Refuge (NWR) Complex, and considered a regional indicator species of marsh habitat quality. Research from the early 1970s, 1990s, and early-2000s indicated that mottled ducks continued to exhibit elevated wing-bone lead (Pb) concentrations, decades after implementation of non-toxic shot regulations. However, wing-bone concentrations reflect lifetime accumulation of Pb, whereas blood Pb concentrations reflect more recent exposure. To identify current potentially relevant temporal windows of Pb exposure, we collected 260 blood samples from mottled ducks during summer (n = 124) and winter (n = 136) from 2010–2012 on the Texas Chenier Plain NWR Complex. We quantified baseline blood Pb concentrations for all ages of mottled ducks, and hypothesized that blood lead concentrations would remain elevated above background levels (200  $\mu\text{g L}^{-1}$ ) despite the 1983 and 1991 lead shot bans. Blood Pb concentrations ranged from below detection limits to  $>12,000 \mu\text{g L}^{-1}$ , where  $>200 \mu\text{g L}^{-1}$  was associated with exposure levels above background concentrations. Male mottled ducks had the greatest blood Pb concentrations (30 times greater than females) with concentrations greater during winter than summer. Likewise, the proportion of exposed ( $>200 \mu\text{g L}^{-1}$ ) females increased from 14%–47% from summer to winter, respectively. Regardless of sex, adult mottled duck blood Pb concentrations were five times greater than juveniles, particularly during winter. We identified five plausible models that influenced blood Pb levels where year, site, and interactions among age\*sex\*season and between age\*season were included in the top-ranked models. Frequency of exposure was greatest during winter, increasing from 12% in summer to 55% in winter, indicating that a temporal exposure window to environmental Pb exists between nesting and hunting seasons. Blood Pb concentrations remain elevated in mottled ducks despite Pb shot bans enacted  $>25$  years prior to this study. If Pb levels in mottled ducks becomes a conservation concern, regional monitoring of blood Pb concentrations would be appropriate with a focus upon elucidating potential reasons for the variation among age and sex groups. Finally, identifying potentially available sources of environmental Pb may be key to minimizing this apparently persistent threat to mottled ducks on the upper Texas coast.

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**Key words:** *Anas fulvigula*, blood, lead, mottled duck, Pb, Texas coast

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Mottled duck (*Anas fulvigula*) populations on the upper Texas coast are experiencing long-term declines, where recent estimates suggest that  $<30,000$  mottled ducks exist regionally (Rigby 2008, Rigby and Haukos 2012, Kruse 2013, Moon 2014). Loss of wetlands due to anthropogenic alteration and development are thought to be primary causes of regional declines, but other factors such as drought, salt water intrusion, increased predator populations, exotic invasive plants, and lead (hereafter Pb) exposure may also play significant roles (Stutzenbaker 1988, Merchant et al. 1991, Merendino et al. 2005, Rigby 2008). Mottled ducks have long been known to be susceptible to Pb exposure (Stendell et al.

1979, Anderson et al. 1987, Merendino et al. 2005) with reported wing-bone Pb concentrations the greatest among seven focal species (Stendell et al. 1979) prior to the national ban on Pb shot in 1991. Lead toxicosis has been identified as a source of mortality in waterfowl for  $>100$  years (Bowles 1908, McAtee 1908, Zwank et al. 1985), primarily caused by ingestion of spent Pb shot deposited into waterfowl habitat as a result of sport hunting (Anderson and Havera 1985). By the early 2000s, average wing-bone Pb concentrations in mottled ducks had decreased to  $18.8 \pm 3.5$  and  $11.6 \pm 1.5$  ppm from 88.9 and 41.5 ppm in the late 1980s for Anahuac and McFaddin National Wildlife Refuges (NWR), respectively (Mer-

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chant et al. 1991, Merchant et al. unpublished data). Although recent measures of mottled duck wing-bone Pb concentrations have declined, they remain near concentrations reported in mallards (*A. platyrhynchos*) and greater than levels reported in American black ducks (*A. rubripes*) and Northern pintails (*A. acuta*) in the early 1970s prior to non-toxic shot requirements (Stendell et al. 1979, Merendino et al. 2005).

Prior to non-toxic shot requirements, approximately 33% of mottled ducks were reported to have ingested Pb shot as measured by presence of lead shot in gizzards (apparent ingestion rate; Merendino et al. 2005). From 1980–1983, years during which non-toxic shot was phased-in on coastal Texas NWRs and public lands, apparent ingestion rates fell to 17%, and by 2002, apparent ingestion rates had declined to 9% (Merendino et al. 2005). However, apparent ingestion rates of spent shot currently continue to exceed those of other waterfowl species prior to the national Pb shot ban in 1991 (Anderson et al. 1987, Moulton et al. 1988, Merendino et al. 2005) with 2011–2012 estimates for the upper Texas coast ranging from 5%–8%, nearly double those of other waterfowl species (USFWS/TPWD, unpublished data). While it is known that waterfowl may either inadvertently or intentionally ingest shot while foraging to aid digestion (Moore et al. 1998, Mateo et al. 2000), when and where mottled ducks ingest Pb shot remains unclear.

The upper Texas coast consistently has the greatest mottled duck population concentrations within the state (USFWS/TPWD, unpublished data) along with the greatest mottled duck wing-bone Pb concentrations of any coastal NWR in Texas (Merchant et al. 1991). However, blood Pb concentration data for mottled ducks are lacking. Although wing-bone concentrations indicate long-term (presumably lifetime accumulation) Pb exposure, Pb in bone has a half-life of 5–19 years, which precludes specific delineation of timing of exposure (Jordan and Bellrose 1951, Pain 1996). Similarly, gizzard analyses used to estimate Pb shot ingestion (Merendino et al. 2005) rates may be unreliable because birds with ingested Pb shot may be more susceptible to harvest as they may be in generally poor physical condition such that shot ingestion rates and corresponding Pb levels may be overestimated (Jordan and Bellrose 1951, Anderson and Havera 1985, Samuel and Bowers 2000). Conversely, frequency of spent Pb shot detected in gizzard surveys may also be underestimated, as gizzard retention time of Pb pellets has been reported to ~20 days (Sanderson and Bellrose 1986), but certainly <30 days in experimentally dosed *Anas* congeners (Chasko et al. 1984, Rodriguez et al. 2010). Regardless of detectability of Pb pellets in gizzards, dissolved Pb may be mobilized physiologically and transported and deposited in various soft tissues as well as bone (Pain 1996; Martinez-Haro et al. 2009, 2011).

Blood Pb concentration may be a more sensitive estimate of

near real-time Pb exposure in waterfowl (Anderson and Havera 1985, Havera et al. 1992, Binkowski and Meissner 2013) and potentially provide a better indicator of recent Pb exposure and absorption than bones, feathers, or organs. For example, Pb can be detected in blood up to approximately 5–7 weeks after exposure (Franson et al. 1986, Havera et al. 1992). In contrast to wing-bone analyses, which reflect longer-term Pb accumulation, or gizzard pellet count analyses, which may over or underestimate potential Pb risk, examining blood Pb may be more useful to identify temporal windows of recent exposure (see Ely and Franson 2014), as well as Pb exposure in young, flightless birds (Samuel and Bowers 2000, Newth et al. 2012).

Finally, changes in blood Pb concentrations between summer and winter coincide with changes in mottled duck foraging behavior and diet selectivity (Moon 2014). Although diet studies are limited (Stutzenbaker 1988), mottled ducks switch from a late-summer protein-rich diet dominated by invertebrates (for brood-rearing and remigal molt) to a fall-winter diet dominated by plant matter, including seeds (Stutzenbaker 1988, Moon 2014), which would require more grit for digestion (Gionfriddo and Best 1996). During this study, we quantified baseline blood Pb concentrations for all ages of mottled ducks on the Texas Chenier Plain NWR Complex. Given the established history of comparatively greater frequencies of spent Pb shot in gizzards coupled with consistently elevated wing-bone Pb concentrations, we hypothesized that blood Pb concentrations would remain elevated above background levels (200  $\mu\text{g L}^{-1}$ ) despite the 1983 and 1991 Pb shot bans. More specifically, using blood Pb analyses, we compared the frequency of mottled ducks exposed to Pb from samples collected during summer and winter to identify potentially relevant temporal windows of exposure.

## Methods

### Study Area

This research was conducted on the Texas Chenier Plains NWR Complex, where mottled ducks were captured on McFaddin and Anahuac NWRs, which consisted of >30,000 ha of fresh, intermediate, and saline marsh; coastal prairie; and cultivated rice fields within Chambers, Galveston, and Jefferson counties, Texas. Mottled ducks were captured via nightlighting during 10–15 day windows centered on the new moon phases between 1 June and 31 August 2010 and 2011. Specific focal capture periods were 8–19 June 2010; 6–17 July 2010; 3–14 August 2010 and 1–13 June 2011; 23 June–8 July 2011; and 21 August–4 September 2011. All captured mottled ducks were aged, sexed, and had a U.S. Geological Survey numbered aluminum leg band attached. Cloacal examination was used to determine sex (Hochbaum 1942) and each duck was clas-

sified into one of three age classes; (1) local (L): birds too young to fly, (2) hatch-year (HY): those born that year and capable of limited escape flight, and (3) after-hatch-year (AHY): adults capable of full flight (Stutzenbaker 1988, Carney 1992). Body plumage, retrix inspection, and cloacal examination were used to age each bird (Stutzenbaker 1988, Carney 1992).

Blood samples ( $\leq 3.0$  ml) were collected from live captured mottled ducks in 2010 and 2011 by brachial venipuncture using a 25-gauge needle fitted to a 3.0-ml syringe (Spears et al. 2007). Samples were injected into uniquely numbered (according to leg band number) 3.0-ml Vacutainers coated with ethylenediaminetetraacetic acid (EDTA) (Anderson and Havera 1985, Spears et al. 2007) and stored at  $-20$  C. Blood samples were also collected from hunter-harvested mottled ducks during the 2010–2011 and 2011–2012 waterfowl seasons at hunter-check stations on Anahuac and McFaddin NWRs. We attended and assisted operating hunter-check stations where age, gender, mass (g), and flattened wing chord (cm) (Carney 1992) were recorded for each mottled duck delivered by refuge hunters. At that same time, mottled duck blood samples (up to 3.0 ml) were collected from the thoracic or abdominal cavity of mottled ducks using a 25-gauge needle fitted to a 3.0-ml syringe. Special care and attention was taken to collect uncoagulated blood, at the time of sample collection. All blood samples were collected at the time hunters attended check stations, prior to departing each refuge, such that no blood samples were removed from frozen carcasses. Blood samples were injected into uniquely numbered (by check station bird number) 3.0-ml Vacutainers coated with EDTA, and stored at  $-20$  C.

### Blood Pb Analysis

Blood Pb analyses were conducted following a protocol developed by Perkin-Elmer specifically for AAnalyst 600 and 800 Atomic Absorption Systems and covering ranges specified by the Center for Disease Control and the Occupational Safety and Health Administration. For waterfowl, the following reference concentrations were used to define exposure levels:  $< 200 \mu\text{g L}^{-1}$  was considered background,  $\geq 200 \mu\text{g L}^{-1}$  was “exposed” above background levels;  $\geq 500 \mu\text{g L}^{-1}$  was toxic, but sub-lethal; and  $\geq 1000 \mu\text{g L}^{-1}$  was considered to be lethal (Anderson and Havera 1985, Friend 1985, Samuel and Bowers 2000, Binkowski and Meissner 2013). Three stock standards of 200, 500, and  $1000 \mu\text{g L}^{-1}$  were created to match waterfowl-centric Pb exposure concentrations and standardize and calibrate the analytical equipment.

Blood samples were allowed to warm to room temperature, and once thawed, a  $100\text{-}\mu\text{L}$  aliquot was removed from each sample and placed in individual autosampler cups and mixed with  $900 \mu\text{L}$  of

previously prepared diluent. Standards were prepared in the same manner. If a sample was  $> 1000\text{-}\mu\text{g L}^{-1}$  standard, it was diluted by 10X and recalibrated, where resulting data were then multiplied by 10 to quantify sample concentration. All samples were analyzed using a Perkin Elmer AAnalyst 700 graphite furnace coupled with a Perkin Elmer AS 800 Autosampler and data were recorded in  $\mu\text{g L}^{-1}$ , where the lower detection limit was set at  $100 \mu\text{g L}^{-1}$ . Stock standards were inserted, along with a blank, at the 20th sample, for quality control purposes to ensure that instrument drift had not occurred. Data are reported in geometric means ( $\pm$  geometric SD) so that no range dominated the weighting and percent changes had the same effect on the geometric mean.

### Data Analysis

Blood Pb concentration data analyses were conducted using the Nondetects and Data Analysis (NADA; Lee 2012) package in program R (R Core Team 2012), which accounts for both discrete and continuous data. Thirty-two *a priori* linear regression models were identified where age (L, HY, AHY), sex (M, F), season (live-capture vs hunter harvest), site (Anahuac NWR, McFaddin NWR), and year (2010–2011, 2011–2012) were used as covariates (independent variables). For all analyses, we assumed that seasonal variability (i.e., live-captures vs hunter harvest) in blood Pb concentration were not artifacts of differences in blood sampling procedures from live and harvested ducks, which is supported by work on similarly sampled mallards that were similar enzymatically, haematologically, and in Pb concentrations (see Binkowski and Meissner 2013). The model set also included the following covariate interactions: age\*sex\*season, age\*sex, age\*season, and sex\*season. Models were ranked based on Akaike’s Information Criterion corrected for small sample sizes ( $\text{AIC}_c$ ). Models with  $\Delta\text{AIC}_c$  values  $< 2$  had empirical support and models with  $\Delta\text{AIC}_c$  values between 2 and 7 had less support (Burnham and Anderson 2002). Parameter likelihoods were determined using model averaging (Burnham and Anderson 2004). Covariate influence was considered to be different from zero when variables were present in top-ranked models and confidence intervals did not include zero. Parameter estimates, standard errors, and 95% confidence intervals are presented for the two top-ranked models.

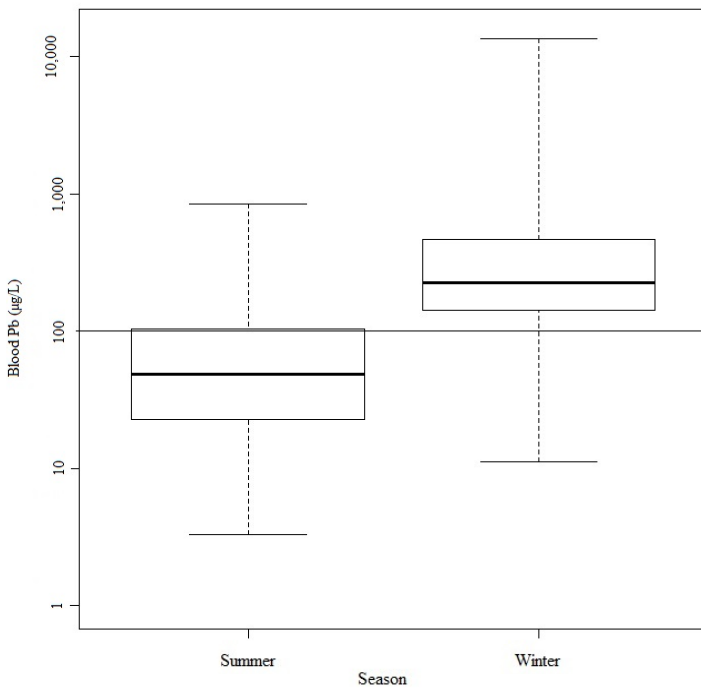
### Results

A total of 260 whole blood samples (live [summer]:  $n = 124$ ; hunter harvested [winter]:  $n = 136$ ) were collected from 2010 to 2012 and used to quantify total blood Pb concentration data. Blood Pb concentrations ranged from below detection limits ( $< 100 \mu\text{g L}^{-1}$ ) to  $13,470 \mu\text{g L}^{-1}$  (Figure 1, Table 1). Of these, 90 (35%) samples

**Table 1.** Blood lead concentrations ( $\mu\text{g L}^{-1}$ ) in total and exposed ( $>200 \mu\text{g L}^{-1}$ ) summer and winter mottled ducks from Anahuac and McFaddin National Wildlife Refuges, 2010–2012.

		n	% of Total	Low	High	Geometric mean	Standard deviation	Median
Total		260	—	< DL	13470	21	127	111
Sex	Male	165	63%	< DL	13470	30	107	141
	Female	95	37%	< DL	4210	1	169	7
Age	L	19	7%	< DL	109	0	360	4
	HY	150	58%	< DL	841	0	157	5
	AHY	85	33%	< DL	371	5	35	52
Age/Sex cohorts	L/F	12	5%	< DL	109	0	426	4
	L/M	7	3%	< DL	10	0	345	1
	HY/F	52	20%	< DL	1610	18	108	85
	HY/M	98	38%	< DL	13470	21	193	144
	AHY/F	27	10%	< DL	4210	18	197	105
	AHY/M	58	22%	< DL	2224	109	9	154
$>200 \mu\text{g L}^{-1}$		90	35%	200	13470	499	2	360
Sex	Male	63	70%	205	13470	520	3	353
	Female	27	30%	200	4210	452	2	371
Age	HY	56	62%	203	13470	533	3	388
	AHY	30	33%	200	4210	433	2	339
Age/Sex cohorts	HY/F	15	17%	203	1610	446	2	412
	HY/M	41	46%	205	13470	569	3	388
	AHY/F	10	11%	200	4210	467	3	363
	AHY/M	20	22%	207	2224	412	2	320

L = Local  
 HY = Hatch-year  
 AHY = After-Hatch-year  
 M = Male  
 F = Female  
 DL = Detection Limit



**Figure 1.** Censored box plot, including 95% confidence interval, of blood lead concentrations between seasons for mottled ducks ( $n = 241$ ) sampled during summer (live) and winter (hunter harvested) on the upper Texas coast, 2010–2012. Horizontal line indicates minimum threshold of detection ( $100 \mu\text{g/L}$ ).

(live:  $n = 15$  [12%]; hunter harvested:  $n = 75$  [55%]) were “exposed” ( $>200 \mu\text{g L}^{-1}$ ) to Pb (Figure 1). For those “exposed,” blood Pb concentrations ranged from  $200$ – $13,470 \mu\text{g L}^{-1}$  with a geometric mean of  $499 \pm 2 \mu\text{g L}^{-1}$  (Table 1), nearly 25 times greater than the geometric mean of all samples combined (Table 1). Sixty-three (38%) male mottled ducks were exposed to Pb, while 27 (28%) females were exposed. Likewise, 56 (33%) juvenile mottled ducks (L:  $n = 0$ , HY:  $n = 56$ ) were exposed to Pb as compared to 30 (35%) AHY mottled ducks (Table 1).

**Summer Samples**

A total of 124 (Anahuac NWR:  $n = 73$ ; McFaddin NWR:  $n = 39$ ; Unknown:  $n = 12$ ) blood samples were collected from live captured mottled ducks during summers 2010 and 2011 (2010:  $n = 59$ ; 2011:  $n = 65$ ). Blood concentrations ranged from below detection limits to  $841 \mu\text{g L}^{-1}$  with a geometric mean of  $1 \pm 325 \mu\text{g L}^{-1}$  (Table 2). Fifteen (12%) birds were exposed ( $>200 \mu\text{g L}^{-1}$ ) to Pb, where concentrations ranged from  $200$ – $841 \mu\text{g L}^{-1}$  with a geometric mean of  $300 \pm 1 \mu\text{g L}^{-1}$  (Table 2). Eight (11%) male mottled ducks were exposed to Pb, while 7 (14%) females were exposed. Likewise, 2 (3%) juvenile mottled ducks (L:  $n = 0$ , HY:  $n = 56$ ) were exposed to Pb as compared to 13 (23%) AHY mottled ducks (Table 2).

**Table 2.** Blood lead concentrations ( $\mu\text{g L}^{-1}$ ) in total and exposed ( $>200 \mu\text{g L}^{-1}$ ) live captured summer mottled ducks from Anahuac and McFaddin National Wildlife Refuges, 2010–2012.

		n	% of Total	Low	High	Geometric mean	Standard deviation	Median
Total		124	48%	< DL	841	1	325	18
Sex	Male	73	59%	< DL	384	2	315	21
	Female	51	41%	< DL	841	1	354	11
Age	L	19	15%	< DL	109	0	360	4
	HY	49	40%	< DL	841	0	377	4
	AHY	56	45%	< DL	384	27	57	89
Age/Sex cohorts	L/F	12	10%	< DL	109	0	345	4
	L/M	7	6%	< DL	10	0	426	1
	HY/F	20	16%	< DL	841	0	336	6
	HY/M	29	23%	< DL	28	0	373	4
	AHY/F	19	15%	< DL	371	5	323	52
	AHY/M	37	30%	< DL	384	65	11	101
$>200 \mu\text{g L}^{-1}$ Total		15	12%	200	841	300	1	283
Sex	Male	8	53%	231	384	286	1	284
	Female	7	47%	200	841	316	2	253
Age	HY	2	13%	412	841	588	2	626
	AHY	13	87%	200	384	270	1	255
Age/Sex cohorts	HY/F	2	13%	412	841	588	2	626
	AHY/F	5	33%	200	371	246	1	234
	AHY/M	8	53%	231	384	286	1	284

L = Local  
 HY = Hatch-year  
 AHY = After-Hatch-year  
 M = Male  
 F = Female  
 DL = Detection Limit



Winter Samples

A total of 136 (Anahuac NWR: n = 79, McFaddin NWR: n = 57) blood samples were collected from hunter harvested mottled ducks during the 2010–2011 (n = 116) and 2011–2012 (n = 20) waterfowl seasons. Blood Pb concentrations ranged from below detection limits to 13,470 µg L<sup>-1</sup> with a geometric mean of 259 ± 3 µg L<sup>-1</sup> (Table 3). Seventy five (55%) birds were exposed (>200 µg L<sup>-1</sup>) to Pb with concentrations ranging from 203–13,470 µg L<sup>-1</sup> and a geometric mean of 552 ± 3 µg L<sup>-1</sup> (Table 3). Fifty-five (60%) male mottled ducks were exposed to Pb, while 20 (47%) females were exposed. Likewise, 54 (53%) juvenile mottled ducks were exposed to Pb as compared to 18 (62%) AHY mottled ducks. Blood Pb concentrations were greatest in AHY/F mottled ducks (Table 3). Of the 75 mottled ducks exposed (>200 µg L<sup>-1</sup>) to Pb, 42 had blood Pb concentrations between 200–500 µg L<sup>-1</sup>, 16 were between 500–1,000 µg L<sup>-1</sup>, and 14 were >1,000 µg L<sup>-1</sup>. Geometric means were 295 ± 1, 701 ± 1, and 2,599 ± 2 µg L<sup>-1</sup> for 200–500, 500–1,000, and >1,000 µg L<sup>-1</sup> for each group, respectively.

Because outliers can influence these data, particularly with concentration data, the top 5% (n = 13) of the dataset were removed to clarify and refine model performance. Geometric means were not recalculated for the live blood Pb concentration samples as none of them were included the top 5% that were removed. Blood Pb concentrations for the remaining dataset (n = 247) ranged from non-detection to 1,108 µg L<sup>-1</sup> with a geometric mean of 16 ± 125 µg L<sup>-1</sup>. For hunter-harvested birds (n = 62), the geometric mean decreased to 388 ± 2 µg L<sup>-1</sup> over a range from 203–1,108 µg L<sup>-1</sup>.

Five of 32 candidate models were plausible (ΔAIC<sub>c</sub> < 2) and indicated that blood Pb concentrations were influenced by age, sex, season, site, and year. Parameter likelihoods indicate age\*sex\*season (AIC<sub>w</sub> = 0.64), year (AIC<sub>w</sub> = 0.46), age\*season (AIC<sub>w</sub> = 0.36), and site (AIC<sub>w</sub> = 0.28) were the most important variables in the top-ranked models (Table 4). For the top-ranked model, blood Pb concentrations were positively influenced by sex (M), season (Winter), age (HY)\*season (Winter), age (HY)\*sex (M)\*season (Winter). Age (HY) and age (HY)\*sex (M), and sex (M) and season (Winter) were negatively related with blood Pb concentrations (Table 4). However, the 95% confidence interval for all coefficients in the model with the exception of season overlapped zero. For the second-ranked model, blood Pb concentrations were positively influenced by sex (M), season (Winter), year (2011–2012), the age (HY)\*season (Winter), and age (HY)\*sex (M)\*season (Winter) even though the 95% confidence interval contained zero for all covariates except season (Table 4).

**Table 3.** Blood lead concentrations (µg L<sup>-1</sup>) in total and exposed (>200 µg L<sup>-1</sup>) winter hunter harvested mottled ducks from Anahuac and McFaddin National Wildlife Refuges, 2010–2011 and 2011–2012.

		n	% of Total	Low	High	Geometric mean	Standard deviation	Median
Total		136	52%	< DL	13470	259	3	222
Sex	Male	92	68%	< DL	13470	288	3	247
	Female	43	32%	< DL	4210	213	3	179
Age	HY	101	74%	< DL	13470	248	3	209
	AHY	29	21%	< DL	4210	306	3	286
Age/Sex cohorts	HY/F	32	24%	< DL	1610	181	2	167
	HY/M	69	51%	< DL	13470	288	3	238
	AHY/F	8	6%	102	4210	413	4	364
	AHY/M	21	15%	< DL	2224	273	3	260
>200 µg L <sup>-1</sup>								
Total		75	55%	203	13470	552	3	389
Sex	Male	55	73%	205	13470	568	3	389
	Female	20	27%	203	4210	513	2	412
Age	HY	54	72%	203	13470	531	3	375
	AHY	18	24%	207	4210	591	2	496
Age/Sex cohorts	HY/F	13	17%	203	1610	428	2	352
	HY/M	41	55%	205	13470	569	3	388
	AHY/F	5	7%	356	4210	921	3	672
	AHY/M	13	17%	207	2224	498	2	477

HY = Hatch-year  
 AHY = After-Hatch-year  
 M = Male  
 F = Female  
 DL = Detection Limit

**Table 4.** Maximum likelihood parameter estimates from censored linear regression for plausible models (AIC<sub>c</sub> < 2.0) of blood lead concentrations without the top 5% of mottled ducks sampled on the upper Texas coast, 2010–2012.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
<b>Top-ranked model</b>				
β0	2.004	0.275	1.454	2.554
Age (HY)	-0.700	0.403	-1.506	0.106
Sex (M)	0.285	0.330	-0.375	0.945
Season (Winter)	1.073	0.492	0.089	2.057
Age (HY): Sex (M)	-5.162	699.520	-1404.202	1393.878
Age (HY): Season (Winter)	0.354	0.605	-0.856	1.564
Sex (M): Season (Winter)	-0.126	0.574	-1.274	1.022
Age (HY): Sex (M): Season (Winter)	5.357	699.520	-1393.683	1404.397
<b>Second-ranked model</b>				
β0	1.822	0.311	1.200	2.444
Age (HY)	-0.606	0.409	-1.424	0.212
Sex (M)	0.115	0.349	-0.583	0.813
Season (Winter)	1.078	0.494	0.090	2.066
Year (2011–2012)	0.353	0.268	-0.183	0.889
Age (HY): Sex (M)	-5.123	698.225	-1401.573	1391.327
Age (HY): Season (Winter)	0.435	0.609	-0.783	1.653
Sex (M): Season (Winter)	-0.054	0.578	-1.210	1.103
Age (HY): Sex (M): Season (Winter)	5.383	698.225	-1391.067	1401.833

## Discussion

The proportion of mottled ducks exposed to Pb in this study (35%) was greater than historic mallard (15%) and American black duck (11%) exposure frequencies prior to the Pb shot ban (Stendell et al. 1979), and greater than all studies performed on mottled duck wing-bones alone (Stendell et al. 1979, Merchant et al. 2001, Merendino et al. 2005). The proportion of mottled ducks that were exposed ( $>200 \mu\text{g L}^{-1}$ ) in this study were comparable to Bewick's (*Cygnus bewickii*) and whooper swan (*C. cygnus*) in Britain (Newth et al. 2012) and canvasback (*Aythya valisineria*), lesser scaup (*A. affinis*), and ring-necked duck in Louisiana (*A. collaris*; Peters and Afton 1993), but tended to be greater than blood Pb levels reported for breeding tundra swans (*C. columbianus*) from Alaska and urban mallards in Poland (Binkowski and Meissner 2013). However, many mottled ducks in the current study exhibited exposure levels comparable to waterfowl (Spears et al. 2007) and various non-game species (Chapa-Vargas et al. 2010) living in wetlands contaminated by historic mining and ore processing activities.

Blood Pb concentrations in mottled ducks were related to age, sex, and season, which tended to be greater in males than females and in AHY than HY and L birds, which corroborate previous Pb concentration studies (Merchant et al. 1991, Merendino et al. 2005). Interestingly, Merchant et al. (1991) noted that sex-differences in wing-bone Pb concentrations had not been previously reported, but speculated that it may be due to greater longevity of males. Coupled with the hypothesis that females may "purge" Pb into eggshells via mobilization of Ca and Pb from bone to eggs may also partially explain lower Pb concentrations in females (Finley et al. 1976, Merchant et al. 1991). However, the greatest blood Pb concentrations in L mottled ducks in this study were barely above the DL, indicating although females may eliminate some Pb to eggshells, Pb may not necessarily impact developing embryos. However, further work needs to examine these relationships more closely.

Sex-related differences in Pb concentrations, particularly during winter is likely a reflection of a poorly described change in mottled duck foraging behavior and diet selectivity (Moon 2014). Although diet studies are limited (Stutzenbaker 1988), mottled ducks switch from a late-summer protein-rich diet dominated by invertebrates (for brood-rearing and remigal molt) to a fall-winter diet dominated by plant matter, including seeds (Stutzenbaker 1988, Moon 2014), which would require more grit for digestion (Gionfriddo and Best 1996). Males, theoretically should switch to this plant-dominated diet sooner than females, as they are emancipated from incubation and brood caring duties, such that their

elevated blood Pb levels in winter may reflect a longer temporal window to Pb in the environment (encountered as grit). This same pattern may also hold for AHY bird versus HY or L birds, where AHY mottled ducks may be attributed to an increased exposure window or increased frequency of exposure as compared to HY and L mottled ducks, as AHY mottled ducks are capable of extended flight throughout most of the summer whereas HY and L birds are not. This increased mobility may allow AHY birds access to potential sources of Pb not available to HY and L mottled ducks. While only 3% (2) of summer, juvenile (L and HY) mottled ducks were exposed to Pb, exposure rates increased to 53% during winter. Likewise, 23% of summer AHY mottled ducks were exposed to Pb, but this increased to 62% during winter.

The significant increase in blood Pb concentrations from summer to winter clearly indicate a temporal window of exposure in late fall and winter for mottled ducks, which coincides with when mottled ducks increase their use of agricultural fields (Stutzenbaker 1988, Moon 2014). Lead deposition in these fields may occur through multiple routes and may be a persistent source of Pb exposure among mottled ducks; however, the most direct route of Pb contamination in these fields may come from Pb shot deposition during fall and winter mourning dove (*Zenaidura macroura*) seasons. While Pb shot was banned for waterfowl hunting on the Texas coast by 1985 and nationwide by 1991, it remains legal for harvest of upland game birds and webless migratory game birds on private land. Dove season in the south zone of Texas extends from the third weekend of September to the third weekend of October and from the third weekend of December to the third weekend of January with the first split falling between summer and winter blood collection windows used in this study. Some dove harvest occurs over rice fields adjacent to the NWR Complex that are also used as foraging habitat by mottled ducks (Stutzenbaker 1988, Moon 2014) and may be a current source of Pb for mottled ducks (Merendino et al. 2005). A shot ingestion study in Jefferson and Chambers counties, Texas, during the implementation of steel shot found that by 1984–85, 50% of ingested shot were steel pellets, suggesting that any shot ingested by waterfowl was probably recently deposited (Moulton et al. 1988).

There are no known biological requirements for Pb and prolonged exposure causes loss of body mass, suppression of the immune system, nervous and digestive system dysfunction, and death (Friend 1987). With mottled ducks facing multiple factors influencing population decline, the percentage of birds with exposed blood Pb concentrations ( $\geq 200 \mu\text{g L}^{-1}$ ) is a relevant conservation concern. Poor nest success in mottled ducks (Durham and Afton 2003) raised concern among habitat managers, and it was corroborated by Finger et al. (2003) and Rigby and Haukos

(2012) who found that nesting propensity was far lower (77% and 63%, respectively) than expected even during years with excellent habitat conditions. This is significant as it is generally assumed that nesting propensity of waterfowl reaches 100% in years with excellent habitat conditions (Mauser and Jarvis 1994). Rigby (2008) also noted that nest success studies were generally conducted in conjunction with nest searches, which ignore non-nesting females. Therefore, realized nest success will be lower if nesting propensity is significantly <100% in most years. Prolonged, sub-lethal Pb toxicosis is known to cause decreased nest success in multiple waterfowl species. If female mottled ducks are obtaining Pb during fall and winter, sub-lethal Pb concentrations may negatively impact female nesting potential, egg survival, subsequent hatching, and even brood rearing success. Combined with an already decreased nest propensity, long-term, sub-lethal exposure may be playing a significant role in current mottled duck declines.

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