



Original Article

Critically Assessing the Utility of Portable Lead Analyzers for Wildlife Conservation

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ABSTRACT Lead (Pb) exposure in wildlife is a widespread management and conservation concern. Quantitative determination of Pb concentrations in wildlife tissues is the foundation for estimating exposure and risk. Development of low-cost, portable instruments has improved access and cost-effectiveness of determining Pb concentrations in blood samples, while also facilitating the ability for wildlife researchers to conduct near real-time Pb testing. However, these instruments, which use anodic stripping voltammetry (ASV) methodology, may produce an analytical bias in wildlife-blood Pb concentrations. Additionally, their simplicity invites use without appropriate quality-assurance–quality-control measures. Together, these factors can reduce data quality and hamper the ability to evaluate it, raising concerns about use of these instruments to inform important conservation issues. We document the extent to which this bias is addressed in the wildlife toxicology literature, develop quantitative approaches for correcting the bias, and provide recommendations to ensure robust data quality when using these instruments. Of the 25 studies we reviewed that referenced ASV use for determining Pb exposure in wildlife, only 32% acknowledged the existence of bias from the instrument. Importantly, another 20% of the studies actually reported ASV and spectroscopic-based results together without acknowledging their lack of equivalence. Using a multispecies data set of avian blood Pb concentrations, we found that ASV-based estimates of paired blood Pb concentrations were 30–38% lower than those from standard spectrometric-based methods. We provide regression equations based on this analysis of 453 blood samples to allow users of ASV instruments to adjust Pb concentrations to spectrometric-equivalent values, and propose a series of guidelines to follow when using these instruments to improve data validity. Published 2018. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS anodic stripping voltammetry, blood lead, graphite-furnace atomic absorption spectrometry, inductively-coupled mass spectrometry, LeadCare[®], portable lead analyzer.

Lead (Pb) exposure in wildlife is a widespread conservation threat that has been documented for decades and recognized in >120 bird species (Goddard et al. 2008, Haig et al. 2014, Golden et al. 2016), as well as a number of mammalian species (Burco et al. 2012, Rogers et al. 2012). Wildlife exposure to Pb is generally determined by measuring Pb concentrations in wildlife tissues such as blood, liver, and kidney (Franson and Pain 2011, Haig et al. 2014, Golden et al. 2016), or their prey (Herring et al. 2016, McTee et al.

2017). However, blood is the most commonly used nonlethal sampled indicator of short-term Pb exposure (Redig and Arent 2008, Franson and Pain 2011).

The most common analytical procedures for determining Pb concentrations in avian and mammalian blood are standard, well-described methods for environmental chemistry, and include graphite-furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS). These methods produce accurate results with a high degree of precision (Andersen 1996, Zhang et al. 1997, Sobin et al. 2011). Concentrations are highly correlated between the 2 methods (Zhang et al. 1997), but they involve laborious digestion procedures and require sophisticated laboratory instrumentation and specialized analytical chemistry expertise,

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which can come at a substantial cost. Together, these factors can limit the accessibility to Pb exposure data in wildlife biology.

Recently, low-cost Pb testing instruments designed for human exposure screening have become readily available and used in wildlife studies (Craighead and Bedrosian 2008, McLelland et al. 2011, Rogers et al. 2012, Langner et al. 2015) and by conservation or animal care organizations for measuring Pb concentrations in avian blood (Samour and Naldo 2002, Parish et al. 2007, McLelland et al. 2010, Finkelstein et al. 2012). These instruments (e.g., LeadCare[®] 2; Magellan Diagnostics, North Billerica, MA, USA) utilize anodic stripping voltammetry (ASV) to determine Pb concentrations in whole blood without the need for intricate digestion or spectrometry instrumentation. The ASV instruments are portable enough to use in the field, comparatively nontechnical to operate, and produce results in <5 min, making them a logistically and economically attractive option that can provide near-real-time results. Despite the benefits listed above, these instruments were designed specifically for determination of Pb concentrations in human blood in nonclinical medical settings, raising question about their accuracy with blood from nonhuman animals, such as birds.

Some avian studies have found correlations between Pb concentrations determined with these instruments and those determined by either GFAAS or ICP-MS (Parish et al. 2007, Craighead and Bedrosian 2008, Langner et al. 2015). However, those studies also found evidence of a negative bias associated with the portable ASV analyzers. Thus, these portable instruments may report lower Pb concentrations than actually occur in avian blood (Craighead and Bedrosian 2008, Langner et al. 2015). Any consistent, substantial bias associated with these portable instruments has implications for cross-study comparisons if these data are not adjusted to account for this bias. Further, the manufacturer of the ASV instruments recommends using only fresh whole blood for analysis, potentially precluding previously frozen blood from Pb analysis with ASV units and limiting the utility of these instruments. Finally, the simple interface of these instruments does not have built-in quality assurance–quality control (QA–QC) triggers that flag concentrations that do not meet predetermined QA–QC standards (e.g., reference standards, duplicates, blanks, etc.), increasing the likelihood that they may be used without appropriate QA–QC documentation. Despite these potential data-validity issues, a comprehensive treatment of the utility of these instruments and the extent or consistency of bias is still lacking, as is the existence of general guidelines for best practices in using these instruments for wildlife research purposes. Failure to critically evaluate the utility of portable Pb analyzers for quantitative wildlife science and conservation efforts risks diminishing the value of published information; or worse, informing animal well-being, conservation, and resource management decisions based upon systematically flawed information.

To facilitate a clearer understanding of the applicability of these instruments for wildlife research purposes, we comprehensively evaluated the use of portable Pb

analyzers in the published literature, and coupled that assessment with our own empirical analysis of original data. We sought to determine the comparability of data from these instruments relative to spectrometry-based analyses, and develop equations to facilitate conversion (if necessary) of ASV-based Pb concentrations to make them compatible with data from more conventional, broadly accepted methodologies. We then quantified the extent of bias in the portable ASV analyzer results across a range of Pb concentrations, while also determining whether there are species-specific differences in the relationship between Pb concentrations from portable analyzers in comparison with those from GFAAS or ICP-MS. We also tested whether freezing samples, a common preservation method for avian blood samples, influenced Pb concentrations determined using the portable ASV instrument. We summarize these results, present model functions for converting data derived from one method to another method, and provide guidance on the appropriate application of the portable ASV instruments for wildlife ecology and conservation efforts.

METHODS

Review of Portable ASV Instrument Use in the Literature

To examine published studies that have utilized the ASV for Pb determination or provided guidance on the use of ASV for wildlife research, we searched the Thompson Reuters ISI Web of Science[®] on 29 September 2017 for the years 2000–2017, using all possible combinations of these topic words: anodic stripping voltammetry, blood lead, LeadCare[®], lead poisoning, lead toxicosis, portable lead analyzer, and secondary poisoning. We included additional studies that were not identified by our search but cited by those we reviewed. Reviewed works included peer-reviewed research journal articles, conference proceedings, and book chapters. From each study, we documented whether 1) the bias in Pb concentrations determined via ASV was reported or acknowledged; 2) the ASV results were validated with split samples using concentrations determined via some generally accepted form of spectroscopy; 3) biased ASV Pb concentrations were adjusted using linear regression models developed from paired ASV and spectrophotometry data; 4) any quality assurance measures, such as duplicates and internal standards, were included and reported; and 5) ASV and GFAAS–ICP-MS were reported without correction as equivalent concentrations. Not all studies presented empirical data, some published papers included only guidelines on the use of ASV for Pb monitoring of wildlife. For those studies, we only considered whether the authors 1) acknowledged the bias in Pb concentrations determined via ASV; 2) discussed how biased ASV Pb concentrations should be adjusted using linear regression models developed from paired ASV and spectrophotometry data; and 3) reported ASV and GFAAS–ICP-MS without correction as equivalent concentrations. We identified and included in our review 25 total studies.

Empirical Assessment of Portable ASV Instrument

We also used an empirical approach with original data to evaluate the relationship between paired Pb concentrations derived from ASV and GFAAS or ASV and ICP-MS using published (Parish et al. 2007, Craighead and Bedrosian 2008, Langner et al. 2015) and unpublished data. We collected blood from 7 bird species (California condor [*Gymnogyps californianus*], bald eagle [*Haliaeetus leucocephalus*], common raven [*Corvus corax*], golden eagle [*Aquila chrysaetos*], red-tailed hawk [*Buteo jamaicensis*], Swainson's hawk [*B. swainsoni*], and turkey vulture [*Cathartes aura*]) sampled throughout Arizona, California, Oregon, Montana, and Wyoming, USA. We captured birds using nonlethal (Bal-chatri noose mats, bow nets, nest visits, net launchers, or walk-in traps) or lethal collection methods (shotgun and nontoxic shot—common ravens and turkey vultures only). We collected blood from the metatarsal or brachial vein, or via cardiac puncture (lethal collections only) using 19–27-gauge hypodermic needles. All captures and collections were covered under state (Montana Fish, Wildlife, and Parks; 209, 573, Oregon Department of Fish and Wildlife; 062-13, 009-14, 064-15) and Federal Scientific Collection Permits (20786, 22637, 23353, MB28361A-0, MB04889B-1), approved Institutional Animal Care and Use Permits (University of Montana-Missoula; 013-07EG-DBS-060807, Oregon State University; 4428, National Park Service; PWR-PINN-Condor-2016.A3), and endangered species permit (TE157291-1). We stored blood samples cool (but not frozen) in the field in ethylenediaminetetraacetic acid (EDTA) or heparinized blood tubes and assayed them as fresh blood using an ASV instrument (see below). Immediately after Pb determination using ASV, we froze and stored blood samples at -20°C .

Determination of Blood Lead Concentrations

Specifics of previously published data, including blood collection details, analytical details, and QA–QC can be found in reviewed papers (e.g., Parish et al. 2007, Craighead and Bedrosian 2008, Langner et al. 2015). We determined Pb concentrations in paired blood samples using ASV and ICP-MS (all species) or ICP-MS and GFAAS (California condor). For the ASV method, we analyzed fresh blood samples for Pb following the manufacturer's guidelines for the LeadCare[®] or LeadCare[®] 2. Briefly, we pipetted 50 μL of fresh blood into a vial containing a manufacturer-provided dilute hydrochloric acid solution, capped the vial, and gently inverted it 10 times. We then added a drop of the blood–hydrochloric acid mixture on the sensor of the ASV instrument, which reported the Pb concentration in $\mu\text{g}/\text{dL}$. We used the same ASV procedure to measure the concentration of Pb in previously frozen samples after they were thawed at room temperature and vortexed to homogenize the blood. We included internal quality assurance standards provided by the instrument manufacturer and we analyzed every 10–15 blood samples in duplicate. Recoveries averaged $99.4\% \pm 5.0\%$ for manufacturer supplied quality assurance standards and the absolute relative percent difference for duplicates averaged $12.7\% \pm 5.0\%$.

We digested blood samples in concentrated trace-metal grade nitric acid following Andersen (1996) prior to GFAAS or ICP-MS analysis. Digests were fortified with an internal indium standard. We determined Pb concentrations of digested blood samples using either a PerkinElmer Elan DRCII (PerkinElmer, Waltham, MA, USA) or Thermo Scientific X-Series II CCT ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA). We prepared calibration curves in aqueous solutions using certified commercial Pb standards. We used certified reference materials (blood [National Institute of Standards and Technology SRM 955c]), method blanks, and duplicates for method validation. Recoveries averaged $97.4\% \pm 4.2\%$ for certified reference materials, with the absolute relative percent difference for duplicates averaging $9.0\% \pm 2.6\%$.

Statistical Methods

We analyzed samples for ASV analysis data using LeadCare[®] or LeadCare[®] 2 instruments. Accordingly, as a first step we determined whether data generated from both ASV instrument models were equivalent. We used an analysis of covariance (ANCOVA) model with LeadCare[®] as the independent variable, species as a categorical factor, LeadCare[®] 2 concentrations as a covariate, and a species \times LeadCare[®] 2 interaction to test for slope differences between LeadCare[®] Pb and LeadCare[®] 2 Pb among species. The species \times LeadCare[®] 2 interaction ($F_{1,24} = 1.77$, $P = 0.20$) was not significant, indicating that the slope of the relationship between LeadCare[®] or LeadCare[®] 2 did not differ by species. After removing the interaction and the species effect, LeadCare[®] 2 concentrations significantly predicted LeadCare[®] concentrations ($F_{1,26} = 158.48$, $P < 0.001$, $r^2 = 0.86$); and the slope of the relationship (0.89 ± 0.14) was not statistically different from 1 ($t_{27} = 1.57$, $P = 0.13$); so we treated Pb concentrations from LeadCare[®] and LeadCare[®] 2 models as equivalent (hereafter, $\text{Pb}_{\text{ASVfresh}}$). In all subsequent models, we removed the species effect when interactions were not significant to simplify model development.

To determine whether Pb spectrometry data ($\text{Pb}_{\text{spectrometry}}$) generated by GFAAS and ICP-MS (California condor only) were equivalent, we used an ANCOVA model with $\text{Pb}_{\text{ASVfresh}}$ as the independent variable, analysis-type (GFAAS or ICP-MS) as a categorical factor, $\text{Pb}_{\text{spectrometry}}$ as a covariate, and an analysis-type \times $\text{Pb}_{\text{spectrometry}}$ interaction to test for slope differences between the forms of spectrometry used. The analysis-type \times $\text{Pb}_{\text{spectrometry}}$ interaction ($F_{1,130} = 0.01$, $P = 0.93$) was not significant, indicating that the slope of the relationship between ICP-MS and ASV and GFAAS and ASV did not differ. Consequently, we considered GFAAS and ICP-MS data obtained from California condors to be equivalent for subsequent models. We then used a linear regression model to assess the relationship between $\text{Pb}_{\text{spectrometry}}$ concentrations and $\text{Pb}_{\text{ASVfresh}}$ concentrations, with $\text{Pb}_{\text{spectrometry}}$ as the independent variable and $\text{Pb}_{\text{ASVfresh}}$ as the dependent variable. The intent of this initial model was to quantify the relationship

between $Pb_{\text{spectrometry}}$ and Pb_{ASVfresh} concentrations and develop simplified model functions that would allow ASV users to predict $Pb_{\text{spectrometry}}$ concentrations regardless of species. Next, we assessed the relationship between $Pb_{\text{spectrometry}}$ concentrations and Pb_{ASVfresh} concentrations across all species using an ANCOVA model that included $Pb_{\text{spectrometry}}$ as the independent variable, species as a categorical factor, Pb_{ASVfresh} concentrations as a covariate, and a species \times Pb_{ASVfresh} interaction to test for slope differences between $Pb_{\text{spectrometry}}$ concentrations and Pb_{ASVfresh} concentrations among species. We observed a species \times Pb_{ASVfresh} interaction ($F_{6,439} = 4.70$, $P < 0.001$), indicating that slopes differed among species, and then conducted pairwise tests to assess those differences. Bald eagle and California condor slopes did not differ from each other ($t_{204} = 1.53$, $P = 0.13$), but differed from 50% of the remaining species (common raven, golden eagle, red-tailed hawk; all $P < 0.05$). All of the remaining species (hereafter, other species) had similar slopes to one another (all $P > 0.14$). To account for these apparent differences in slope in subsequent models, we included bald eagle and California condor data together, with data from the other species as a separate model.

To examine the relationship between previously frozen blood Pb concentrations determined by ASV (hereafter, $Pb_{\text{ASVfrozen}}$) and Pb_{ASVfresh} concentrations, we tested an ANCOVA model that included Pb_{ASVfresh} as the independent variable, species as the categorical factor, $Pb_{\text{ASVfrozen}}$ as a covariate, and a species \times $Pb_{\text{ASVfrozen}}$ interaction term to test for slope differences between $Pb_{\text{ASVfrozen}}$ concentrations and Pb_{ASVfresh} concentrations among species. The interaction was not significant ($F_{1,35} = 0.35$, $P = 0.56$), so we removed both the interaction and species effect from the model. Lastly, to examine the relationship between $Pb_{\text{ASVfrozen}}$ and $Pb_{\text{spectrometry}}$ concentrations, we tested an ANCOVA model that included $Pb_{\text{spectrometry}}$ as the independent variable, species as a categorical factor, $Pb_{\text{ASVfrozen}}$ as a covariate, and a species \times $Pb_{\text{ASVfrozen}}$ interaction term to test for slope differences between $Pb_{\text{ASVfrozen}}$ concentrations and $Pb_{\text{spectrometry}}$ concentrations among species. The interaction was not significant ($F_{1,8} = 0.00$, $P = 0.98$), so we removed both the interaction and species effect from the model. The final form of the linear regression models tested were as follows:

$$\text{Bald eagle-California condor } \ln Pb_{\text{spectrometry}} = \beta_0 + \beta_1(\ln Pb_{\text{ASVfresh}}) + \varepsilon \text{ (Model 1)}$$

$$\text{Other species } \ln Pb_{\text{spectrometry}} = \beta_0 + \beta_1(\ln Pb_{\text{ASVfresh}}) + \varepsilon \text{ (Model 2)}$$

$$\text{Other species } \ln Pb_{\text{ASVfresh}} = \beta_0 + \beta_1(\ln Pb_{\text{ASVfrozen}}) + \varepsilon \text{ (Model 3)}$$

$$\text{Other species } \ln Pb_{\text{spectrometry}} = \beta_0 + \beta_1(\ln Pb_{\text{ASVfrozen}}) + \varepsilon \text{ (Model 4)}$$

where other species in Model 2 consisted of common ravens, golden eagles, red-tailed hawks, Swainson's hawks, and turkey vultures; other species in Model 3 consisted of common ravens

and turkey vultures; and other species in Model 4 consisted of common ravens, golden eagles, and turkey vultures.

To examine the potential bias in the Pb_{ASVfresh} -generated data (% difference between the ASV and $Pb_{\text{spectrometry}}$ concentrations), we calculated Pb_{ASVfresh} -derived concentrations as a percentage of $Pb_{\text{spectrometry}}$ -derived concentrations and plotted it relative to the $Pb_{\text{spectrometry}}$ -derived concentration. Finally, to determine whether the type of blood storage tube (EDTA or heparin) influenced Pb_{ASVfresh} concentrations, we tested an ANCOVA model that included Pb_{ASVfresh} EDTA as the independent variable, species as a categorical factor, Pb_{ASVfresh} heparin as a covariate, a species \times Pb_{ASVfresh} heparin interaction term to test for slope differences between Pb_{ASVfresh} heparin concentrations and Pb_{ASVfresh} EDTA concentrations among species. The interaction was not significant ($F_{1,14} = 1.48$, $P = 0.26$), so we removed it and the species effect from the model. Prior to all statistical analyses, we natural-log-transformed Pb concentrations to improve normality of residuals and homogenize the variance structure. We set significance for all analysis at $\alpha = 0.05$.

RESULTS

Pb Determination Using Anodic Stripping Voltammetry in the Peer-Reviewed Literature

We identified 25 published studies that either measured blood Pb concentrations from avian and mammalian wildlife (18 avian and 4 mammalian species) or discussed ASV use in monitoring Pb exposure in wildlife (Table 1). Blood Pb concentrations determined via ASV were only validated with GFAAS or ICP-MS in 28% of those studies. Overall, 32% of the studies acknowledged the existence of low bias in Pb concentrations determined via ASV relative to more standardized techniques such as ICP-MS, but ASV concentrations were only adjusted for this bias in 20% of all studies. Importantly, documentation of analytical quality assurance or quality control (QA-QC) results were rarely presented across the 25 studies. Only a single study (4%) reported results from analysis of duplicates and 8% of studies presented recoveries of standard reference materials. Finally, 20% of studies reported uncorrected ASV values simultaneously with ICP-MS concentrations without acknowledging any bias between the 2 methods nor making any adjustments to correct for the bias. We also observed that 8% of the studies diluted blood samples reported to be "High" on the ASV using saline solution, but presented no validation details for this method.

Paired ASV_{fresh} and Pb_{spectrometry} Concentrations

Across all species, ASV_{fresh} Pb concentrations significantly predicted $Pb_{\text{spectrometry}}$ -derived concentrations ($F_{1,451} = 1,800.40$, $P < 0.001$, $r^2 = 0.80$; Fig. 1; Table 2). At the species group level, bald eagle-California condor ASV_{fresh} Pb concentrations significantly predicted $Pb_{\text{spectrometry}}$ -derived concentrations ($F_{1,206} = 633.44$, $P < 0.001$, $r^2 = 0.75$; Fig. 2A; Table 2), as was also the case for other

Table 1. Summary of published wildlife studies that have used anodic stripping voltammetry to measure lead exposure between 2000 and 2017.

Species	Scientific name	Citation
Bald eagle	<i>Haliaeetus leucocephalus</i>	Cruz-Martinez et al. 2012
Bald eagle		Neumann 2009
Bald eagle–common loon	<i>Gavia immer</i>	Kornetsky et al. 2013
Bald eagle–golden eagle	<i>Aquila chrysaetos</i>	Cowan and Blakley 2016
Black bear–cougar–grizzly bear–wolf	<i>Ursus americanus–Puma concolor–U. arctos–Canis lupus</i>	Rogers et al. 2012
Black scoter–Stellar’s eider	<i>Melanitta nigra–Polysticta stelleri</i>	Brown et al. 2006
Black vulture	<i>Coragyps atratus</i>	Van Wettere et al. 2012
California condor	<i>Gymnogyps californianus</i>	Aguilar et al. 2012
California condor		Parish et al. 2007
California condor		Green et al. 2008
California condor		Hall et al. 2007
California condor		Rideout et al. 2012
Cinereous vulture	<i>Aegypius monachus</i>	Kenny et al. 2013
Cinereous vulture		Kenny et al. 2015a
Cinereous vulture–Egyptian vulture–griffon vulture	<i>Neophron percnopterus–Gyps fulvus</i>	Rodriguez-Ramos et al. 2009
Common raven	<i>Corvus corax</i>	Craighead and Bedrosian 2008
Golden eagle		Langner et al. 2015
Griffon vulture		González et al. 2017
Kea	<i>Nestor notabilis</i>	McLelland et al. 2010
Kea		Reid et al. 2012
Lanner falcon–peregrine falcon–saker falcon	<i>Falco biarmicus–F. peregrinus–F. cherrug</i>	Samour and Naldo 2002
Rock pigeon	<i>Columba livia</i>	Cai and Calisi 2016
Swamp harrier	<i>Circus approximans</i>	McLelland et al. 2011
Unspecified		Fallon et al. 2017
White-backed vulture	<i>Gyps africanus</i>	Kenny et al. 2015b

species ($F_{1,243} = 1,199.05$, $P < 0.001$, $r^2 = 0.83$; Fig. 2B; Table 2). ASV_{fresh} Pb concentrations were 37.6% and 29.6% lower than $Pb_{spectrometry}$ -derived concentrations for bald eagles–California condors and other species, respectively. The bias (% difference between the ASV_{fresh} and $Pb_{spectrometry}$ concentrations) for both condors and bald eagles (Fig. 3A) and other species (Fig. 3B) exceeded 0% at Pb_{ICPMS} concentrations below 5 $\mu\text{g/dL}$, but were negative across the data range above 20 $\mu\text{g/dL}$.

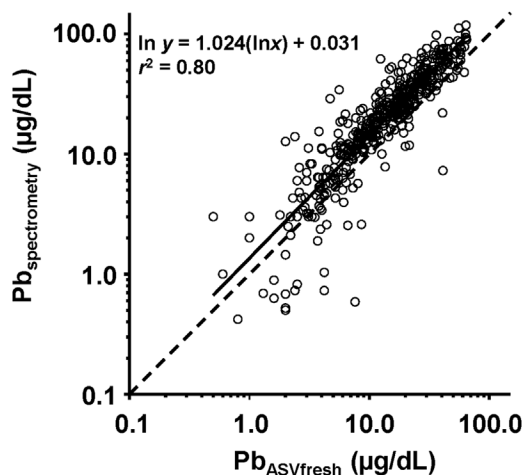


Figure 1. Linear relationship of lead (Pb) concentrations between fresh anodic stripping voltammetry ($Pb_{ASVfresh}$) and spectrometry Pb concentrations ($Pb_{spectrometry}$; graphite-furnace atomic absorption spectrometry–inductively coupled plasma mass spectrometry) across 7 avian species: bald eagle, California condor, common raven, golden eagle, red-tailed hawk, Swainson’s hawk, and turkey vulture. Diagonal dashed line indicates the zero intercept with a slope of 1 (i.e., perfect correspondence). We collected all blood samples between 2000 and 2016 throughout Arizona, California, Oregon, Montana, and Wyoming, USA.

Paired ASV_{fresh} and ASV_{frozen} Pb Concentrations

Despite using the same instrument for analysis, Pb concentrations of frozen blood samples differed from the same blood sample analyzed fresh using ASV ($t_{38} = 2.96$, $P = 0.005$). There was a linear relationship between Pb concentrations of previously frozen blood and fresh blood determined via ASV ($F_{1,37} = 418.26$, $P < 0.001$, $r^2 = 0.92$; Fig. 4A; Table 2), but the Pb concentrations of previously frozen blood were 16.2% greater than the paired ASV-derived concentrations that were analyzed fresh. However, the bias was not consistent along the concentration gradient; below 10 $\mu\text{g/dL}$ there was relatively large bias, but between 20 and 50 $\mu\text{g/dL}$ the bias decreased (Fig. 3C).

$Pb_{ASVfrozen}$ Versus $Pb_{spectrometry}$ Data

Despite a relatively small sample size, $Pb_{ASVfrozen}$ concentrations significantly predicted $Pb_{spectrometry}$ concentrations ($F_{1,11} = 198.97$, $P < 0.001$, $r^2 = 0.95$; Fig. 4B; Table 2). $Pb_{ASVfrozen}$ concentrations were 20.1% lower than the paired $Pb_{spectrometry}$ -derived concentration. The bias between those methods for $Pb_{ASVfrozen}$ samples was substantially lower than the bias associated with the paired ASV_{fresh} and $Pb_{spectrometry}$ concentrations and the paired ASV_{fresh} and ASV_{frozen} -derived Pb concentrations (Fig. 3A–D).

Anticlotting Agent Effects on ASV-Derived Pb Concentrations

$Pb_{ASVfresh}$ concentrations stored in heparinized blood tubes significantly predicted EDTA blood-tube concentrations ($F_{1,22} = 82.85$, $P < 0.001$, $r^2 = 0.79$); and the slope of the relationship (0.83 ± 0.18) was not statistically different from 1 ($t_{23} = 1.88$, $P = 0.07$). $Pb_{ASVfresh}$ concentrations from heparinized blood tubes were $10.6\% \pm 7.1\%$ greater, on average, than $Pb_{ASVfresh}$ concentrations from EDTA blood tubes ($t_{23} = 2.88$, $P = 0.008$).

Table 2. Linear regression functions for lead (Pb) concentrations in 7 avian species describing relationships between natural-log fresh whole-blood anodic stripping voltammetry ($Pb_{ASVfresh}$) –derived lead (Pb) concentrations, natural-log spectrometry Pb concentrations ($[Pb_{spectrometry}]$: graphite-furnace atomic absorption spectrometry–inductively coupled plasma mass spectrometry), and natural-log previously frozen whole-blood ASV–derived Pb concentrations ($Pb_{ASVfrozen}$). All models were run with each variable as both the response and independent variable to allow back-calculation in either direction. We collected all blood samples between 2000 and 2016 throughout Arizona, California, Oregon, Montana, and Wyoming, USA.

Blood analysis type	$Pb_{spectrometry}$			$Pb_{ASVfresh}$			$Pb_{ASVfrozen}$		
	β_0	β_1	r^2	β_0	β_1	r^2	β_0	β_1	r^2
All species ^a $Pb_{ASVfresh}$	0.303	1.024	0.80						
Bald eagle and California condor $Pb_{ASVfresh}$	0.892	0.857	0.75						
Other species ^b $Pb_{ASVfresh}$	0.031	1.091	0.83				0.003 ^c	1.044	0.92
All species $Pb_{spectrometry}$				0.277	0.781	0.80			
Bald eagle and California condor $Pb_{spectrometry}$				−0.110	0.880	0.75			
Other species $Pb_{spectrometry}$				0.382	0.762	0.83	−0.590 ^c	1.099	0.95
Other species $Pb_{ASVfrozen}$	0.666 ^c	0.862	0.95	0.194 ^d	0.879	0.92			

^a Bald eagle, California condor, common raven, golden eagle, red-tailed hawk, Swainson’s hawk, and turkey vulture.

^b Common raven, golden eagle, red-tailed hawk, Swainson’s hawk, and turkey vulture.

^c Common raven, golden eagle, and turkey vulture.

^d Common raven and turkey vulture.

DISCUSSION

Portable ASV-based Pb analyzers can be valuable tools for simple and rapid determination of Pb exposure in wildlife. However, these instruments were specifically calibrated for human blood and not designed for wildlife studies, so substantial (but correctable) bias exists in comparison with more traditional Pb analysis techniques. As a result, improper use of these instruments is relatively common in wildlife studies, with <33% of the studies acknowledging the potential bias of ASV-derived data. Additionally, only a subset of those adjusted ASV concentrations to either a GFAAS or ICP-MS equivalent concentration. We observed that 1 in 5 studies reported ASV and spectrometry-derived data interchangeably, despite the documented bias between the methods. Moreover, very few studies provided any evaluation of data quality via QA–QC reporting. In fact, only 4% of studies reported data on instrument precision (through analysis of duplicates) and only 8% evaluated accuracy through certified reference standards. It is unclear if this paucity of QA–QC data are a result of lack of application or simply lack of reporting. Either way, the failure to provide QA–QC measures prevents independent verification of data quality, reducing the confidence in results from such studies. Quality assurance and quality control measures serve as a cornerstone for ecotoxicology and environmental chemistry disciplines for verifying the accuracy and precision of analytical results. However, conservation implications of contaminant exposure in wildlife are resulting in more of these studies being published in ecology and wildlife journals that are less familiar with analytical chemistry standards. Even with the development of less complex instrumentation (such as ASV Pb analyzers), it is important that this practice be adopted by journals outside the ecotoxicological disciplines to ensure data of adequate analytical quality are being published. Accepted QA–QC measures for analytical chemistry should be consulted and applied, as would be done in laboratories determining Pb

concentrations using GFAAS or ICP-MS (EPA 1998). Details on blood-tube collection type, sample handling, and storage should also be included.

Although not a primary objective of our literature review, we routinely observed differences in how data were handled during analysis throughout these studies that can have implications for interpretation of results. Specifically, contaminant concentration data are typically log-normal distributed, and failing to log-transform these data can result in biased arithmetic mean estimates, heterogeneous residuals, and variance that increases with increasing concentrations, all of which violate the basic assumptions of linear models. This is particularly important if model functions are to be used to convert ASV concentrations to GFAAS–ICP-MS equivalent concentrations. We illustrate how much difference this can make using our log model functions for the other species group (i.e., not bald eagle and California condor) and an ASV Pb concentration of 10.0 $\mu\text{g}/\text{dL}$ (log 2.3), which yields a back-calculated equivalent spectrometry Pb concentration of 12.7 $\mu\text{g}/\text{dL}$. Using model functions from an untransformed data set would produce an estimated concentration (14.2 $\mu\text{g}/\text{dL}$) that is 12% greater. Thus, failing to account for how the distribution of a contaminants data set influences model outcomes could lead to further biased results and subsequent erroneous interpretations.

To facilitate conversions of Pb concentrations from one analytical method to another, we provide regression equations from our different paired analyses, including frozen versus fresh blood analyses. Further, we provide a prediction tool for ASV users to predict GFAAS–ICP-MS equivalent concentration from fresh ASV values (Table S1, available online in Supporting Information or by contacting the corresponding author). Similar to other wildlife studies, we found that avian blood Pb concentrations were underestimated when analyzed via ASV from 30–38% (Parish et al. 2007, Craighead and Bedrosian 2008, Langner et al. 2015). However, the consistent differences between the 2 methods make it possible to apply these correction factors and adjust

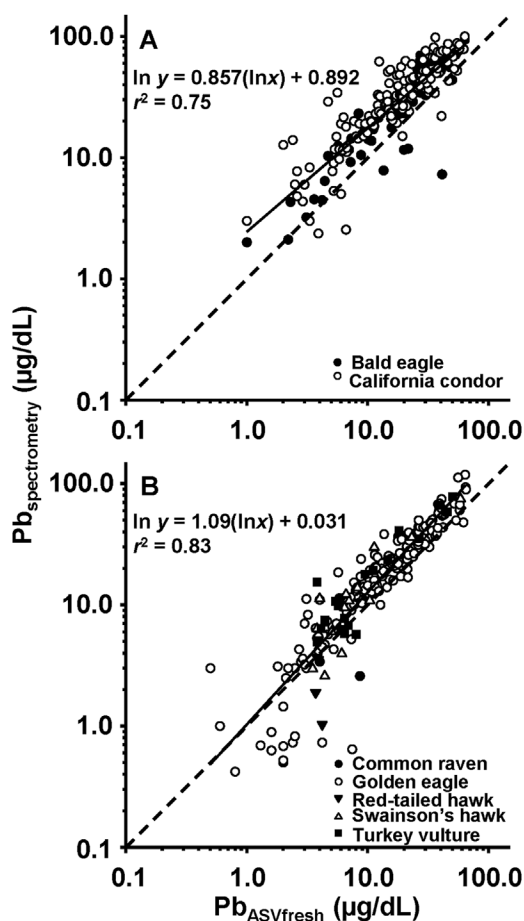


Figure 2. Linear relationship lead (Pb) concentrations between fresh anodic stripping voltammetry ($Pb_{ASV_{fresh}}$) and spectrometry Pb concentrations ($Pb_{spectrometry}$: graphite-furnace atomic absorption spectrometry–inductively coupled plasma mass spectrometry) for (A) bald eagle and California condor, and (B) common raven, golden eagle, red-tailed hawk, Swainson’s hawk, and turkey vulture. Diagonal dashed line indicates the zero intercept with a slope of 1 (i.e., perfect correspondence). We collected all blood samples between 2000 and 2016 throughout Arizona, California, Oregon, Montana, and Wyoming, USA.

the concentrations into comparable units. Additionally, differences that we found between species groups in the relationship between ASV and spectrometry-based Pb highlight the importance of using one of our species-group-specific equations. However, conversions of data from species not assessed in this study would require additional validation of ASV results with either GFAAS or ICP-MS.

Mechanisms responsible for the negative bias associated with ASV-derived blood Pb concentrations are not clear, but several factors associated with blood chemistry and sample handling–storage may be involved. Thiols (organosulfur compounds—e.g., glutathione, metallothionein) can interfere with the ASV electrode surface because the sulfhydryl groups bind and block active sites on the ASV test sensor electrode, resulting in lower Pb concentration estimates (Taylor et al. 2004). Some thiol-rich proteins, such as metallothioneins, increase in avian blood when birds are exposed to Pb, resulting in a negative feedback loop that can proportionally increase the bias as Pb exposure increases

(Scheuhammer 1996, Vanparys et al. 2008, Lucia et al. 2012, Pikula et al. 2013). Similarly, blood collection tubes containing K_2EDTA as an anticoagulant also interfere with ASV sensor binding if there is an excess of EDTA associated with insufficient blood volumes being collected relative to tube volume (Bowen and Remaley 2014, Magellan Diagnostics 2016a). Correspondingly, using paired heparin–EDTA blood collections tubes, we found that Pb concentrations of blood stored in EDTA tubes were 11% lower than paired blood samples stored in heparinized tubes. Finally, prolonged blood contact with the rubber stopper of some blood collection vacuum tubes (including EDTA tubes) may result in a sulfur-based curing agent being released into the blood, which can also suppress the Pb response during analysis with the ASV (Bowen and Remaley 2014, Magellan Diagnostics 2016b).

Interestingly, Pb concentrations determined via ASV on previously frozen samples were more representative of Pb concentration determined via ICP-MS than on fresh samples determined via ASV. In both cases, we observed strong relationships between previously frozen and fresh blood ASV Pb concentrations and previously frozen ASV and ICP-MS blood Pb concentrations. Although the previously frozen blood ASV values were lower than the equivalent ICP-MS values, that bias was more consistent and generally lower than what was observed during the fresh blood ASV to spectrometry-based Pb comparison. The mechanism for this is unclear, but could be the result of freezing on sulfhydryl groups that could potentially impair Pb binding on the ASV. Freezing of tissues is known to decrease sulfhydryls (e.g., glutathione; Morton 1969, Stuiver et al. 1988, Wang 1994, Gadea et al. 2011) and subsequent thawing results in a continued reduction of glutathione (Stuiver et al. 1988). Alternatively, freezing samples may result in more complete lysing of red blood cells and increased availability of free Pb for detection by the ASV unit. Regardless, the practice of freezing blood samples before analysis on the ASV could potentially yield more accurate results if these findings are further validated experimentally and with additional species.

Application of ASV Pb Testing Instruments in Research and Conservation

The emergence and availability of ASV instruments for measuring blood Pb concentrations in wildlife research has opened many new avenues for improved risk characterization and real-time conservation applications. Among the most common applications are wildlife rehabilitation and field Pb testing for endangered avian species and raptors (Redig and Arent 2008, Scott 2016, Fallon et al. 2017). However, as with any technique, there are weaknesses in the capabilities of these instruments that can influence their utility. For example, the bias that we demonstrated with these instruments can result in misapplication of spectrometry-derived treatment benchmarks if not properly accounted for. This is important because recent guidelines for evaluating and assigning treatment options to Pb-poisoned birds are based

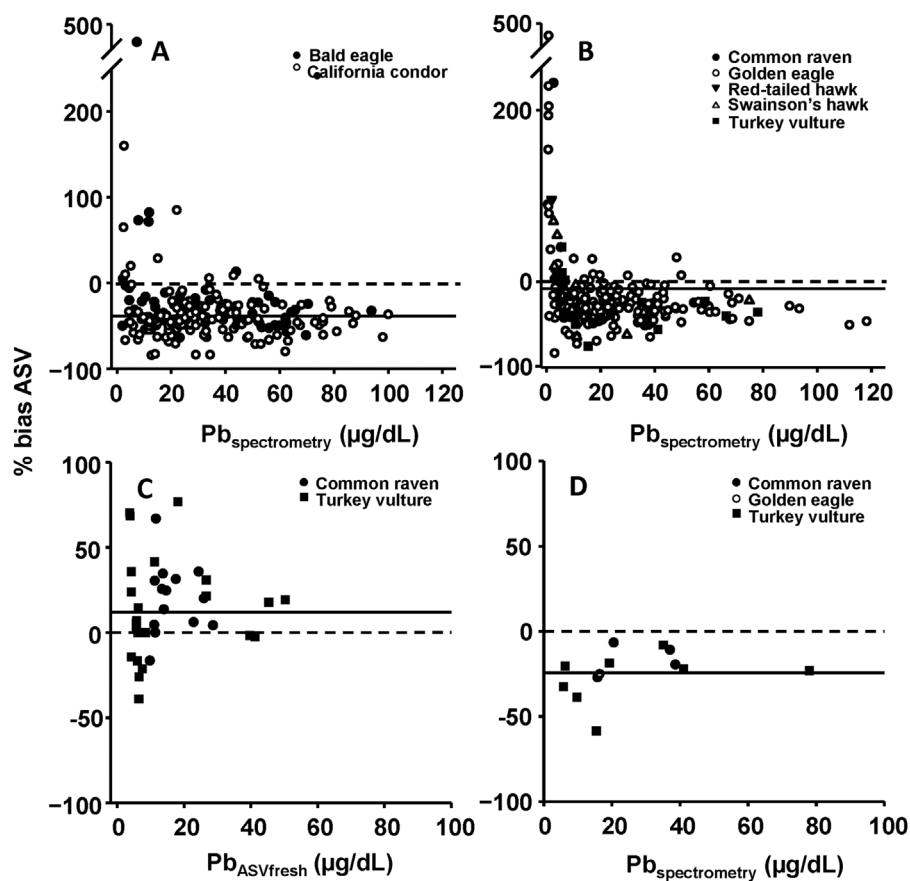


Figure 3. Relationship of lead (Pb) concentrations between the bias (difference between the fresh anodic stripping voltammetry [$Pb_{ASVfresh}$] and spectrometry Pb concentrations [$Pb_{spectrometry}$]: graphite-furnace atomic absorption spectrometry–inductively coupled plasma mass spectrometry) as a percentage of the spectrometry methods for (A) bald eagle and California condor $Pb_{spectrometry}$ and $Pb_{ASVfresh}$ concentrations, (B) remaining species $Pb_{spectrometry}$ and $Pb_{ASVfresh}$ concentrations, (C) $Pb_{ASVfresh}$ and previously frozen blood ASV Pb concentrations ($Pb_{ASVfrozen}$), and (D) $Pb_{ASVfrozen}$ and $Pb_{spectrometry}$ concentrations. Bias percentages are plotted against the independent blood Pb concentration. Dashed horizontal line indicates perfect agreement between $Pb_{ASVfresh}$ and $Pb_{spectrometry}$ or $Pb_{ASVfrozen}$, and the solid horizontal line indicates the average difference. We collected all blood samples between 2000 and 2016 throughout Arizona, California, Oregon, Montana, and Wyoming, USA.

upon clinical observations from eagles whose blood Pb concentrations were determined using primarily GFAAS (Kramer and Redig 1997). As an example of the influence the bias may have on treatment thresholds, we evaluated guidelines for treatment of Pb-exposed raptors (Fallon et al. 2017). Birds with blood Pb concentrations $\geq 60 \mu\text{g/dL}$ are recommended to be held for chelation therapy, those with Pb concentrations below $40 \mu\text{g/dL}$ are suggested for release, and birds with concentrations in between should be assessed for clinical signs of Pb poisoning. If uncorrected ASV values were applied to those benchmarks, the actual blood Pb concentrations would be 90 and $59 \mu\text{g/dL}$, respectively. In contrast, our regression equations indicate that ASV-derived concentrations of 41 and $27 \mu\text{g/dL}$ would result in actual blood Pb concentrations matching the suggested treatment or release benchmarks, respectively.

One further issue with using the ASV units for either research or conservation are the “Low” and “High” concentration limits. Anodic stripping voltammetry instruments report Pb concentrations that are below $3.3 \mu\text{g/dL}$ as “Low.” However, the inherent bias as well as the poor sensitivity of ASV at those concentrations indicate that Pb

concentrations reported as “Low” may be considerably greater than that “Low” cutoff. In fact, we found that “Low” ASV concentrations were as high as $6.2 \mu\text{g/dL}$ when measured with ICP-MS. Similarly, ASV instruments report Pb concentrations above $65 \mu\text{g/dL}$ as “High.” Samples reading as “High” should be reanalyzed via GFAAS or ICP-MS, and not diluted and analyzed via ASV as some studies have done (McLelland et al. 2010, Reid et al. 2012). Diluting human blood using saline solution consistently overestimated Pb concentrations by two-fold or greater when analyzed using an ASV because of changes to the blood matrix (Neri et al. 2014). However, one alternative is to use blood samples with very low Pb concentrations (e.g., $<2 \mu\text{g/dL}$ verified by GFAAS–ICP-MS) as the diluting agent because the blood matrix and the LCA reagent–mixture is not changed (Neri et al. 2014). If blood is used to dilute “High” samples, the blood should be from the same species. Verifying that blood samples used for dilution are in fact low requires either GFAAS or ICP-MS analysis; thus, these sample will not be fresh. Validating that frozen low-concentration blood can be used to dilute “High” samples is still required.

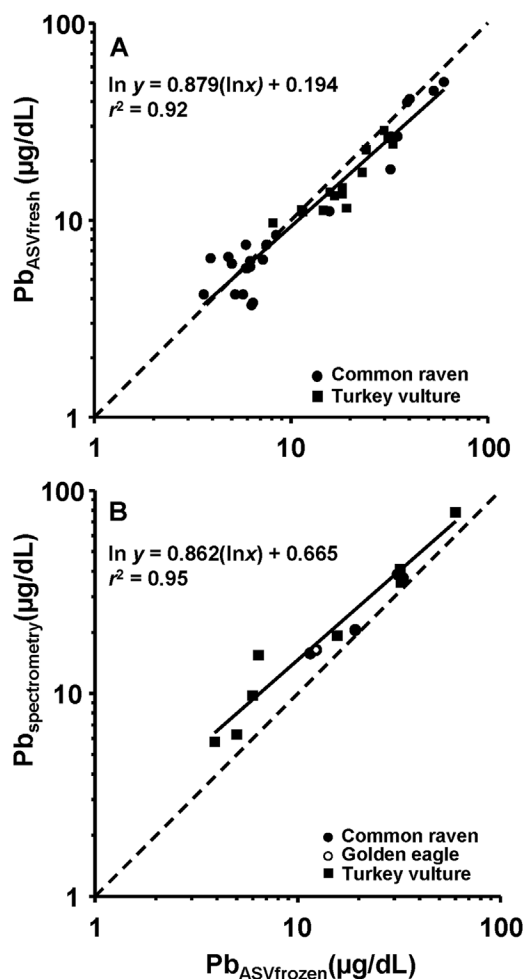


Figure 4. Linear relationship in lead (Pb) concentrations of 3 avian species between (A) previously frozen anodic stripping voltammetry ($Pb_{ASVfrozen}$) and fresh ASV Pb concentrations ($Pb_{ASVfresh}$), and (B) $Pb_{ASVfrozen}$ and spectrometry Pb concentrations (inductively coupled plasma mass spectrometry [$Pb_{spectrometry}$]). Diagonal dashed line indicates the zero intercept with a slope of 1 (i.e., perfect correspondence). We collected all blood samples between 2000 and 2016 throughout Arizona, California, Oregon, Montana, and Wyoming, USA.

Guidelines for Appropriate Application

Based on the findings of our analyses, we have developed 6 key guidelines to facilitate the use of ASV instruments for measuring Pb concentrations in wildlife, and applying those data to resource management issues.

1. Blood tubes—EDTA blood collection tubes should be filled to the manufacturer-recommended volume with blood to ensure appropriate mixing and concentration of EDTA in the sample and prevent analytical interference of EDTA with Pb determination. If this is not possible, then either sodium- or lithium-heparin tubes can be used with ASV.
2. Blood tube stoppers—to avoid potential sulfur-based bias in blood samples determined via ASV, blood should be transferred to polypropylene cryovials or similar storage vials after mixing in the blood collection tube.
3. Validation—data adjustment—Blood Pb concentrations generated with ASV instruments from species not reported

in this study should be validated with either GFAAS or ICP-MS Pb measurements. However, we also provide robust “all-species” model functions to predict provisional spectrometry-derived Pb concentrations (S1). Use of these functions may be adequate for many circumstances, but will likely be less precise than species-specific model functions. If researchers are validating the relationship between ASV and GFAAS–ICP-MS for a new species, then at minimum, 15–20 samples are needed (Schmidt 1971, Green 1991), and those samples must uniformly span the analytical range of the ASV instrument (3.3–65 $\mu\text{g}/\text{dL}$). Thus, sample sizes greater than 15–20 are likely needed in many cases to achieve this range.

4. QA–QC—reporting of QA–QC measures (e.g., duplicates, internal standards, certified reference materials, etc.) should be required for all wildlife Pb publications to allow readers to assess the validity of the analytical results.
5. “Low” and “High” blood samples—ASV concentrations reported as “Low” or “High” should be reanalyzed via GFAAS or ICP-MS to determine the exact Pb concentrations if the data are to be used for any quantitative purpose.
6. Data interpretation—direct interpretation of Pb concentrations derived from ASV should not be used in statistical analyses with physiological, survival, or behavior effects unless they are adjusted to a GFAAS–ICP-MS equivalent concentration. Nor should ASV-derived concentrations be used to calculate the proportion of individuals exceeding traditional clinical thresholds without appropriate concentration adjustment. Further, given the potential bias associated with glutathione and the ASV system, ASV-derived Pb concentrations should not be used in correlational analyses with glutathione or oxidative stress biomarkers.

MANAGEMENT IMPLICATIONS

Lead exposure is a significant conservation threat to many wildlife species, and accurate documentation of the scope and magnitude of risk to Pb exposure requires appropriate analytical techniques. Our results document the potential bias that can occur when using ASV-derived Pb concentrations from portable Pb analyzers, as well as quantify the variance in Pb concentration data introduced by those instruments. Our findings indicate that failure to properly adjust ASV-derived concentrations to GFAAS or ICP-MS equivalents can result in underestimating potential risk to wildlife associated with Pb exposure by up to 38%. However, our conversion equations, prediction tool (S1), and use guidelines provide tools that researchers and wildlife managers can use to ensure Pb concentration data in avian blood are representative of actual exposure, and of adequate quality.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site. The prediction tool allows for converting LeadCare[®] Pb concentrations to a GFAAS/ICP-MS equivalent concentration and then generates associated Pb concentration toxicity categories.