Exposure of a population of invasive wild pigs to simulated toxic bait containing biomarker: implications for population reduction

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Abstract

BACKGROUND: An international effort to develop an acute and humane toxic bait for invasive wild pigs (Sus scrofa) is underway to curtail their expansion. We evaluated the ability to expose a population of wild pigs to a simulated toxic bait (i.e., placebo bait containing a biomarker, rhodamine B, in lieu of the toxic ingredient) to gain insight on potential population reduction. We used 28 GPS-collars and sampled 428 wild pigs to examine their vibrissae for evidence of consuming the bait.

RESULTS: We estimated that 91% of wild pigs within 0.75 km of bait sites (total area = 16.8 km²) consumed the simulated toxic bait, exposing them to possible lethal effects. Bait sites spaced 0.75–1.5 km apart achieved optimal delivery of the bait, but wild pigs ranging ≥ 3 km away were susceptible. Use of wild pig-specific bait stations resulted in no non-target species directly accessing the bait.

CONCLUSION: Results demonstrate the potential for exposing a large proportion of wild pigs to a toxic bait in similar ecosystems. Toxic baits may be an effective tool for reducing wild pig populations especially if used as part of an integrated pest management strategy. Investigation of risks associated with a field-deployment of the toxic bait is needed.

Keywords: biomarker; feral swine; integrated pest management; pesticide; Sus scrofa; toxicant; wild boar; wildlife damage management

1 INTRODUCTION

Invasive wild pigs (Sus scrofa; hereafter– wild pigs), also termed feral hogs, feral pigs, or feral swine, are a widely distributed and destructive invasive species throughout parts of North America, Australia, South America, Africa, and many island nations. The invasion of wild pigs is associated with extensive agricultural, ecological, and control costs, which also occur in their native range. Annual economic losses have been estimated at $1.5 billion across the USA from crop damage, predation of livestock, spread of disease, and the cost of control. Another estimate suggests $190 million of losses in crop yields per year across just 10 high-producing states. In addition, wild pigs harm native ecosystems and natural resources by reducing plant species diversity, predating sensitive species, and destroying the habitat and nests of valued native species. Wild pigs are expanding in distribution and population density throughout the USA and Australia. These increases are attributed to intentional and accidental introductions by humans, high reproductive potential, lack of predators, human alterations to the landscape that improve its suitability for wild pigs, and the adaptability of wild pigs to occupy a variety of landscapes and opportunistically feed on many food items. Populations of wild pigs rebound quickly following drastic declines, therefore annual removal of 50–60% of the population may be required to keep a population from increasing, and this may be as high as 80% in some areas. Reduction of wild pig populations has been demonstrated using trapping, snaring, recreational hunting, professional sharpshooting, and aerial shooting. These methods can be labor intensive or costly, and are typically not applied in a consistently coordinated manner to sufficiently keep populations from increasing across large regions. Additional effective and cost-effective tools are needed to help control existing populations of invasive wild pigs and curtail their expansion and associated damage across large spatial scales. Toxic baits offer a potentially cost-effective option for controlling invasive wild pigs that could be broadly applied, provided
that appropriate criteria are met for safety, humaneness, and efficacy.\textsuperscript{32,33} Specifically, a toxic bait containing the active ingredient sodium nitrite (SN), has been under development through a collaborative research effort between the National Wildlife Research Center (NWRC) of the United States Department of Agriculture, Texas Parks and Wildlife Department (TPWD), Invasive Animal Cooperative Research Center (IACRC), and Animal Control Technologies Australia Pty Ltd.\textsuperscript{34}

Initial studies with SN toxic bait have demonstrated a high level of palatability and efficacy for wild pigs,\textsuperscript{34,35} and low secondary risks to non-target scavenger species.\textsuperscript{36} A wild pig-specific bait station has also been developed that successfully excludes non-target species, including the capable and ubiquitous raccoon (\textit{Procyon lotor}), preventing non-target species from directly accessing the toxic bait inside the bait stations while still allowing access by wild pigs.\textsuperscript{37} Usage of the bait station by wild pigs, however, required a 2–3-week pre-baiting period for pigs to become accustomed to the bait station.\textsuperscript{38} Notwithstanding these findings, no studies have directly evaluated the potential for SN toxic bait on a population of free-ranging wild pigs using a bait station.

Field studies of SN toxic bait in the USA have been limited because new pesticides are evaluated and carefully regulated by the US Environmental Protection Agency. At the time of this study, testing of the toxic bait in a field setting was not yet permitted, but tests involving placebo bait (non-toxic) were allowed. In particular, adding a biomarker to the placebo bait can provide valuable understanding of the potential for a simulated deployment of SN toxic bait to expose a population of wild pigs to the toxic bait and possible lethal effects. The biomarker, rhodamine B (RB), has been demonstrated to be an effective biomarker for marking the vibrissae of wild pigs\textsuperscript{39,40} 14 days after consumption,\textsuperscript{39} and without being aversive or toxic to wildlife.\textsuperscript{41}

Our objective was to evaluate population-level exposure of wild pigs in a natural ecological setting to placebo bait using a biomarker in lieu of the toxic ingredient, SN. Specifically, we evaluated the spatial extent and proportion of a wild pig population that consumed the biomarker bait across a large property with abundant wild pigs in south-central Texas, USA. We also evaluated how the movement behavior of wild pigs influenced the likelihood of wild pigs consuming the bait. We discuss the results in terms of the potential for uptake of the placebo bait to mimic effects that could be observed using toxic bait. Results from this study will be used to help refine toxic baiting strategies.

\section{Methods}

\subsection{Study area}

All research was conducted on a restricted access military property, Camp Bullis (112.9 km\textsuperscript{2}), operated by Joint Base San Antonio, TX, USA during July–September 2017. Permission to conduct research on this property was provided by the Natural Resources Office of Joint Base San Antonio, located on Camp Bullis. The property lies in the Edwards Plateau and Blackland Prairie ecoregions of south-central Texas.\textsuperscript{42,43} Vegetation communities are dominated by a mosaic of cedar–oak woodlands and grasslands on rocky soils and limestone outcrops.\textsuperscript{44,45} Average daily temperatures ranged from 23 to 33 °C, and daily precipitation averaged 2.7 mm (National Climatic Data Center). Wild pigs were considered absent from Camp Bullis until 2006, followed by suspected release(s) or immigration from surrounding areas resulting in an abundant population throughout the property (Cooksey ML, U.S. Army Environmental Command, private communication). Since then, population control has mostly consisted of limited recreational hunting. Camp Bullis was completely fenced, but wild pigs were able to move in or out the property through gaps in the fence.

\subsection{Capture and collaring wild pigs}

We captured wild pigs during 19–29 January 2017 using box traps, corral traps, air-powered net cannons (WCS Net Blaster\textsuperscript{TM}, Wildlife Control Supplies, East Granby, CT, USA), and drop nets within each of three target areas in Camp Bullis (Fig. 1). We targeted capturing and collaring up to four wild pigs per trapping location. We immobilized adult wild pigs (> 36 kg) in traps using a mixture of 3.3 mg kg\textsuperscript{-1} Telazol\textsuperscript{®} (200 mg mL\textsuperscript{-1}) and 1.5 mg kg\textsuperscript{-1} xylazine 100 mg mL\textsuperscript{-1} (Wildlife Pharmaceuticals, Inc., Windsor, CO, USA)\textsuperscript{46} or a 0.17 mg kg\textsuperscript{-1} dose from a 1:1:1 solution of medetomidine (10 mg mL\textsuperscript{-1}), butorophenol (50 mg mL\textsuperscript{-1}), and midazolam (50 mg mL\textsuperscript{-1}) (Wildlife Pharmaceuticals, Inc.) via intramuscular injection. We fitted each wild pig with a Global Positioning System (GPS) satellite-transmitting collar (VERTEX PLUS-2 Collar, VECTRONIC Aerospace GmbH, Berlin, Germany) equipped with an ultra-high frequency (UHF) proximity sensor and a uniquely identifiable ear tag (Y-Tex Cattle Tags, Y-Tex\textsuperscript{®} Corporation, Cody, WY, USA, and 7X Ear Tags, Premier1Supplies, Washington, IA, USA). After handling was complete, we delivered antagonist drugs and released the animals. All procedures were approved by the Institutional Animal Care and Use Committees from Texas A&M University-Kingsville's (2015-08-20) and the United States Department of Agriculture/Animal Plant and Health Inspection Service/Wildlife Services (USDA/APHIS/WS), National Wildlife Research Center (QA-2632 and QA-2724).

In total, we attached GPS collars to 37 wild pigs (20 females, 17 males) and ear-tagged an additional two females that were too small for GPS collars. Prior to initiation of baiting, three of those wild pigs were killed by adjacent landowners, four collars slipped off, and two collars malfunctioned. Overall, the sample of GPS collared animals consisted of 28 adult wild pigs (15 females, 13 males). In addition to the wild pigs marked with collars and ear tags during 2017, wild pigs with uniquely identifiable ear tags (Alfflex A Cattle Tags, Alfflex USA Inc., Dallas, TX, USA) remained in the study area from a study conducted in 2016.\textsuperscript{47} It was not known how many remained, but the original sample consisted of 42 wild pigs. We opportunistically used these animals as they were observed, to boost our sample of identifiable wild pigs.

\subsection{Baiting wild pigs}

We focused our baiting efforts in the same three areas where we collared wild pigs (Fig. 1). We laid 0.75 km\textsuperscript{2} grids over each of the three areas. We used this grid size so that any wild pig living within the grids should encounter one or more bait sites per 0.75 km\textsuperscript{2}, based on previous studies.\textsuperscript{38,48} Initially we overlaid 1.5m\textsuperscript{2} grids over each of three target areas in Camp Bullis, and pre-baited two or three sites within each grid cell using 11.3 kg andpre-baited two or three sites within each grid cell using 11.3 kg of whole-kernel corn at each site (n = 80 bait sites total; Table 1).

We monitored the bait sites with motion-activated, remote cameras (RECONYX PC900, RECONYX, Inc., Holmen, WI, USA) mounted on T-posts or trees 5 m away from bait at heights of ~ 1.5 m. We programmed cameras to record three images at 5-s intervals upon detection of motion, with 1-min quiet periods between triggers. We also placed UHF-emitting stationary ID tags (VECTRONIC Aerospace GmbH) at 5 m from each bait site, 15 cm above the ground to record proximity events (i.e., visitation to bait sites) by collared wild pigs that approached within ~ 25 m of each site. Proximity events were logged on GPS collars, recording the date and location.
Figure 1. Study area (Joint Base San Antonio, Camp Bullis, TX, USA) for a biomarker deployment of placebo bait targeting invasive wild pigs during July–September 2017. The study area contained three independent subsections for deploying 0.75 km² baiting grids (i.e., northwest, northeast and south).

time of each event. We re-visited bait sites daily, refreshed bait as needed, and inspected camera imagery for evidence of wild pig and non-target species visitation.

After 7 days of pre-baiting, we reduced the number of sites in each of the three baiting areas to the 10 best grid cells, and selected the single best bait site within each grid cell for a total of 10 bait sites per baiting area (Fig. 1). Specifically, we selected the grid cells and bait sites ranking in the highest category: (i) consistent visitation by wild pigs (i.e., \( \geq 2 \) days in a row), (ii) consistent visitation by a single family group of wild pigs (i.e., one or more female with multiple piglets), (iii) consistent visitation by more than one family group, and (iv) consistent visitation by family groups that were independent from any family groups observed at nearby bait sites. Ultimately, our goal was to have independent family groups consistently visiting each of the 30 final bait sites. We focused on maximizing the number of family groups visiting bait sites because removing females and juveniles would lead to the greatest reduction in the population of wild pigs, 49,50 and would be the most efficient method for using a toxic bait for wild pigs. 38

On day 8, we deployed wild pig-specific bait stations at all 30 of the baiting sites. Each bait station (137 cm length \( \times \) 36 cm width \( \times \) 17 cm height) consisted of two back-to-back troughs with overhanging lids that could be positioned from propped completely open to secured closed with 13 kg of magnetic resistance. 37,38,51 We secured bait stations to the ground by wiring the handles to two T-posts to prevent flipping or removal by wild pigs. We also began implementing a baiting strategy with deliberate stages for accustoming wild pigs to using the bait station and consuming placebo bait (Table 1). 38 Placebo bait consisted of a black, peanut paste with crushed grains. 35 Specifically, we used a pig-informed strategy which entailed advancing each bait site to the next stage of baiting after we observed wild pigs accessing
and consuming the bait for two consecutive nights at the previous stage. Thus, each bait station progressed independently through all of the baiting stages. However, we synchronized all sites for final stage (i.e., deployment of the biomarker) to eliminate confounding of multiple exposures with the biomarker through time.

We used a pilot study to confirm that the addition of RB to placebo bait did not influence the palatability of the bait for wild pigs (Kinsey J, Texas Parks and Wildlife Department, unpublished data). For bait containing the biomarker, we attempted to match the concentration of RB needed to reliably mark wild pigs, i.e., 15 – 30 mg kg\(^{-1}\) body weight of wild pigs\(^{32}\); with the same quantity of bait that would be needed to reach the LD\(_{99}\) (i.e., 400 mg kg\(^{-1}\) body weight) from SN toxic bait (HOGGONE\(^{®}\), Animal Control Technologies Australia PTY Ltd, Victoria, Australia) loaded with 10% SN.\(^{32}\) Specifically, we used the finding that free-ranging wild pigs consume ∼ 300 g of placebo bait during a feeding bout\(^{35}\) and in just a few mouthfuls. Therefore, we loaded the placebo bait with 0.5% RB by considering the conservative case of a large wild pig (e.g., 75 kg) consuming 300 g of bait. This animal would have received a dose of 20 mg kg\(^{-1}\) RB, equivalent to 400 mg kg\(^{-1}\) of SN if the toxic version of the bait was consumed. This provided a conservative estimate because a previous pen study found that smaller wild pigs consumed an average of 479 g of SN toxic bait,\(^{34}\) and thus would have received a larger dose. Using the 0.5% concentration of RB, we considered any wild pig that was identified as positive for consuming RB as potentially susceptible to consuming a lethal dose of SN toxic bait.

### 2.4 Population density camera grids

Immediately following biomarker deployment (Table 1), we established a camera grid containing 10 cameras within each of the three baiting areas in Camp Bullis to evaluate the population density of wild pigs (Fig. 1). We overlaid a second grid, but with 0.2 km\(^2\) cells, across each of the baiting areas using a Geographic Information System (ArcGIS v10.2, ESRI, Redlands, CA, USA), and selected 15 adjacent grid cells for potential camera locations. We visited each of these grid cells and selected the 10 best cells with recent activity of wild pigs (e.g., evidenced by fresh rooting, feces, tracks, wallows, or visual observations of wild pigs). We placed a remote camera (RECONYX PC900) ≤ 75 m from the centroid of these grid cells. All cameras were mounted on T-posts or trees facing north, ∼ 1.2 m off the ground, at an interior angle of 70°. Cameras were set to capture three time-lapse images at 20-s intervals, every 15 min. We secured a hog-pipe (1 m long × 10 cm diameter polyvinyl chloride pipe with ∼ 1 cm holes for trickling out bait), containing ∼ 16 kg of whole-kernel corn, to another T-post 5 m north of each camera to slowly disperse bait and continually attract wild pigs to the cameras for multiple days. We also placed an additional ∼ 16 kg of whole-kernel corn around the hog-pipe. We opted to use bait and attract pigs for estimating density under the assumption that our detection probability would more closely match our biomarker marking probability that also used bait. We left the sites undisturbed for 10 consecutive days before returning to retrieve the cameras and hog-pipes. We determined that the bait was diminished after 6 days, therefore we only used the first 5 days of camera data to estimate abundance.

For each image, we recorded the total number of wild pigs observed that were marked adults, unmarked adults, and non-independent wild pigs (i.e., juveniles or piglets) using the Colorado Parks and Wildlife Photo Warehouse Database.\(^{53}\) We also recorded the IDs of marked adults when possible, otherwise these animals were recorded as marked but not identifiable. Non-independent wild pigs were animals estimated to be < 27 kg and observed in the presence of an adult wild pig.

### 2.5 Sampling of wild pigs for biomarker consumption

Fifteen days after biomarker deployment (Table 1), we used box and corral traps to begin trapping and sampling wild pigs for biomarker consumption. All wild pigs captured were immediately

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**Table 1.** Study schedule for the deployment of placebo bait with 0.5% biomarker (rhodamine B) at 30 bait sites on Joint Base San Antonio, Camp Bullis, TX, USA beginning on 11 July 2017. Baiting was immediately followed with population density estimation using camera grids, and ultimately with sampling wild pigs for biomarker exposure. Study activity demonstrates our methodology for simultaneously accustoming wild pigs to the novel bait (i.e., by slowing the increasing bait ratio offered) while accustoming wild pigs to access the bait station (i.e., by slowly advancing until lids were closed using magnetic resistance).

<table>
<thead>
<tr>
<th>Stage of study</th>
<th>Days of study</th>
<th>No. of days</th>
<th>Study activity</th>
<th>Corn</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-baiting and biomarker deployment</td>
<td>1–5</td>
<td>5</td>
<td>Pre-baiting – locate wild pigs</td>
<td>11.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>6–7</td>
<td>2</td>
<td>Pre-baiting – introduce placebo</td>
<td>11.3</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>8–9</td>
<td>2</td>
<td>Pre-baiting – introduce bait stations, lids propped to 25 cm</td>
<td>11.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10–12</td>
<td>3</td>
<td>Pre-baiting – bait station lids propped to 5 cm</td>
<td>11.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>13–14</td>
<td>2</td>
<td>Pre-baiting – bait station lids closed with 0 kg of magnetic resistance</td>
<td>7.9</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>15–16</td>
<td>2</td>
<td>Pre-baiting – bait station lids closed with 13 kg of magnetic resistance</td>
<td>2.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>17–18</td>
<td>2</td>
<td>Pre-baiting – bait station lids closed with 13 kg of magnetic resistance</td>
<td>1.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>19–20</td>
<td>1</td>
<td>Simulated toxic bait deployment (biomarker deployment)</td>
<td>0.5</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>21–31</td>
<td>10</td>
<td>Remove bait sites</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Density estimation</td>
<td>32–34</td>
<td>3</td>
<td>Remove bait sites</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>35–54</td>
<td>19</td>
<td>Remove bait sites</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Biomarker sampling</td>
<td>55–56</td>
<td>2</td>
<td>Remove bait sites</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
and humanly killed via cranial gunshot, following the American Veterinary Medical Association guidelines.\textsuperscript{54} We recorded the GPS location at which each animal was captured. We also recorded sex and estimated ages according to tooth eruption and wear.\textsuperscript{55} We consolidated ages into three categories; (i) piglets (0–30 weeks), (ii) juveniles (30 weeks–18 months), and adults (>18 months). We used forceps to collect 8–12 vibrissae from the muzzle of each wild pig, ensuring collection of the entire lengths of vibrissae. We placed the vibrissae samples from each wild pig into a uniquely labeled Whirl-Pak® Write-On Bags (4 oz., Nasco, Fort Atkinson, WI, USA) and stored the bags at room temperature and out of direct sunlight.

To supplement our trapping efforts, the Texas Wildlife Services of USDA/APHIS conducted aerial shooting of wild pigs via helicopter during the last 2 days of the study (Table 1). During the aerial operation, ground crews immediately located and sampled all wild pigs that were shot and located. The sampling of wild pigs took place throughout the entire property of Camp Bullis at a variety of distances from the baiting sites (Figs 1 and 2).

\section*{2.6 Biomarker evaluation}
We examined an average of 7.9 (SE = 0.02) vibrissae per wild pig for evidence of biomarker consumption. Specifically, we removed dirt and debris from vibrissae using a disposable wipe (Kimwipes®, Kimtech Science®, Roswell, GA, USA) wetted with deionized water. We then mounted the vibrissae onto glass microscope slides (Fisherfinest™; Fisher Scientific, Pittsburgh, PA, USA) using clear adhesive tape. We examined the length of each vibrissae under 10x magnification using a fluorescent microscope (Olympus BX43; Tokyo, Japan) with a triple multiband excitation filter (69000-ET-DAPI/FITC/TRITC, Chroma® Technology Corp, Bellows Falls, VT, USA) to examine for banding due to RB consumption. We considered any wild pig as positive for RB consumption if we detected a band on more than one of the vibrissae.\textsuperscript{56} We recorded the number of vibrissae with RB banding from each animal. We used a blind, double-observer approach for the first 50 wild pigs and realized 100% corroboration between observers on the RB status of the animals. Thus, we used single observer for the remaining samples unless any questionable vibrissae were encountered.

\section*{2.7 Data analyses}
\subsection*{2.7.1 Movement analysis}
We estimated the home ranges for the GPS-collared wild pigs during a 3-week pre-trial before pre-baiting began (19 June–10 July 2017), to provide a baseline for comparison of subsequent movement behaviors during the trial. Specifically, we used the adehabitatHR package\textsuperscript{57} in Program R (v3.3.3; R Foundation for Statistical Computing, Vienna, Austria) to employ a movement-based kernel density estimator MKDE.\textsuperscript{58,59} We calculated the distances from the centroids of home ranges to the nearest bait sites. We used UHF proximity records to detect whether wild pigs visited the bait sites, and at what frequency. Specifically, we examined how many of the collared wild pigs visited the bait sites during the final night of baiting when the RB bait was deployed.

\subsection*{2.7.2 Biomarker analysis}
For analyzing biomarker data, our response data for each wild pig was binomial (1 = yes, 0 = no) for whether the animal tested positive for consuming RB. We evaluated whether the distance between the sampling location and nearest bait site influenced the probability of consuming the RB bait. We also examined whether sex and age classes influenced that probability. Specifically, we used binomial generalized linear mixed-models with logit links and restricted maximum likelihood in package lme4\textsuperscript{60} in Program R. We accounted for non-independence among family groups of wild pigs by creating a grouping factor based on the proximities of sampling locations. For this analysis, we considered any animals that were sampled ≤400 m from each other (i.e., ~25% of average home range diameter) as belonging to the same group, and used group IDs as a random effect in the analyses.

\subsection*{2.7.3 Population impacted by biomarker bait}
We estimated the abundance of adult wild pigs in each of the three camera grids using a mark–resight analysis.\textsuperscript{61} Specifically, we used the combination of collared and ear-tagged animals on the landscape as uniquely identifiable animals. Location data were not available for all identifiable animals (i.e., ear tagged animals), therefore we used a non-spatial, zero-truncated Poisson-log normal model,\textsuperscript{62} implemented in Program MARK,\textsuperscript{63} to estimate the number of unmarked animals in each of the three camera grids.

\section*{Figure 2. Distribution of sampling distances and predicted probabilities for wild pigs to consume placebo bait based on distance between the locations of sampling and the nearest bait sites in south–central Texas, USA during July–September 2017.}
We evaluated all combinations of varying or non-varying by camera grid for the following response variables: intercept, individual heterogeneity (i.e., variability in resight probability for individual animals), number of unmarked individuals in the population, and number of marked animals for estimating the number of unmarked animals in each camera grid (Appendix 1).

Models were compared using the second-order Akaike’s information criterion (AICc), whereas models \( \leq 2 \Delta \text{AICc} \) of the top model were considered competitive.\(^6\) We used the top model to generate estimates of the number of unmarked animals in each camera grid, we then added the number of marked animals to estimated abundance, and finally converted to density by dividing by the area that the camera grid sampled. We designated this area by creating a buffer around the camera grid based on the mean maximum distance moved (MMDM) for the collared wild pigs during 40 random selections of five consecutive-day increments prior to this study. We also incorporated information from the non-independent wild pigs (i.e., juveniles and piglets) into the estimates of abundance by extrapolating the ratio of non-independent wild pigs to adult wild pigs from each of the camera grids. We calculated the standard error of the abundance estimates using the delta method.\(^6\)

To estimate the proportion of the population that consumed the biomarker bait, we assumed that the density calculated from each camera grid was representative of the associated baiting area. We modified the density estimation formula from distance sampling Eqn 1\(^6\); to estimate the number of wild pigs that tested positive for consuming the biomarker bait as a function of distance from the bait sites. Our modification assumed a homogeneous population where the probability of consuming the biomarker bait declined with distance to the bait site, using the distance function \( g(r) \) with respect to distance \( r \) generated from the biomarker analysis for all wild pigs above. We used a 0.75 km radius of inference from our camera grid, which we deployed bait sites. Finally, we used the estimates of density \( \hat{D} \) calculated above, and solved the below equation for \( n \), the number of animals that consumed the biomarker bait, and thus would have succumbed to a toxic bait. To calculate the proportion of the population impacted by the biomarker \( \beta \) we divided the estimated number of animals impacted by the bait \( \hat{n} \) by the total number of animals in the area (i.e., density \( \times \) the total area within a radius of \( w \); Eqn 2).

\[
\hat{D} = \frac{n}{2\pi \int_0^\infty r g(r)dr} \quad (1)
\]

\[
p = \frac{\hat{n}}{\hat{D} \pi w^2} \quad (2)
\]

3 RESULTS

3.1 Movements near bait sites

The average home range size during the 3 weeks prior to baiting was 1.68 km\(^2\) (SE = 0.26) for females and 1.81 km\(^2\) (SE = 0.32) for males. On average, the distance between the centroids of those home ranges to the nearest bait stations was 0.72 km (SE = 0.07) for females and 1.22 km (SE = 0.28) for males. Twenty-seven of the 28 GPS collared wild pigs visited one or more bait sites. The remaining wild pig was a female that lived 2.8 km from the nearest bait site, the third-farthest distance from a bait site for any collared animal in this study. On average, females visited a total of 2.6 different bait sites (SE = 0.36) and males visited 4.1 (SE = 0.58) for the duration of baiting. Similarly, all of the 27 collared wild pigs visited one or more bait sites during the final night of baiting when the biomarker was deployed. During this night, females visited an average of 1.75 bait sites (SE = 0.28) and males visited 2.25 (SE = 0.33).

Using camera imagery from the night of biomarker deployment, we observed visitation by raccoons (five sites), white-tailed deer (Odocoileus virginianus; six sites), wild turkeys (Meleagris gallopavo; six sites), and squirrels (Sciurus spp.; two sites). Yet, none of these non-target species were observed gaining access to bait stations while locked with magnetic resistance with biomarker bait inside.

3.2 Biomarker results

We sampled whiskers from 428 wild pigs (254 females and 172 males), including the marked wild pigs sampled above, throughout the study area (Fig. 1). We collected 363 of the wild pigs via trapping, and 65 via helicopter shooting. Of the wild pigs deemed positive for RB, we identified RB banding in an average of 6.9 (SE = 0.08) vibrissae. Overall, we identified 317 wild pigs (74.1%) as testing positive for RB from consuming the RB bait, sampled from an average distance of 0.61 km (SE = 0.04) from the nearest bait sites. We identified 33 marked wild pigs (collars and/or ear tags) visiting the bait stations during the night that biomarker was deployed. We were able to sample 18 of those, of which 16 (88.9%) consumed the bait with RB.

Distance to the nearest bait site was the most influential factor determining whether a wild pig consumed the RB bait (\( \beta = -3.17, 95\% \text{ CI} -4.66 \) to \(-2.22; \text{Fig. 2} \)). Males were slightly more likely to have consumed the RB bait than females (\( \beta = 1.5, 95\% \text{ CI} 0.16 \) to \(2.18 \)). Additionally, juveniles were more likely to have consumed the RB bait than adults (\( \beta = 0.75, 95\% \text{ CI} 0.04 \) to \(1.49 \)), and adults had similar probabilities as piglets (\( \beta = 0.76, 95\% \text{ CI} -0.13 \) to \(1.65 \)). We found similar probabilities for wild pigs that were sampled via trapping or helicopter (\( \beta = -0.02, 95\% \text{ CI} -1.75 \) to \(1.50 \)), when accounting for distance to the nearest bait site.

3.3 Population impacted by biomarker bait

The top model for explaining variation in the estimates of abundance for the three camera grids contained 99% of the model set weight, and suggested the resighting rate and number of unmarked individuals varied among the grids (Appendix 1). Abundance estimates among the grids ranged from 28 to 89 adult wild pigs, or 36–96 including piglets and juveniles (Table 2). The MMDM buffer was 1829 m, and translated to density estimates of 1.4–4.7 adult wild pigs per km\(^2,\) or 1.8–5.1 wild pigs per km\(^2\) including piglets and juveniles. We estimated that 91% (95% CI 60% to 99%) of wild pigs sampled within 0.75 km of the bait sites (i.e., 5.6 km\(^2\) for each baiting area, 16.8 km\(^2\) total for all three areas) consumed the biomarker bait.

4 DISCUSSION

The potential for delivery of SN toxic bait to large proportions of wild pigs appeared high in proximity to the bait sites. Our findings suggest that, during one night, 91% of the population of wild pigs within a total area of 16.8 km\(^2\) accessed and consumed enough bait to potentially deliver lethal doses of SN following 18 days of pre-baiting. Furthermore, we found evidence that wild pigs \( \geq 0.75 \) km from the bait sites consumed the biomarker bait within reasonably high frequency (i.e., \( \sim 0.50 \) probability of consumption at 1.5 km), suggesting that more wild pigs were also susceptible to consuming the bait. If similar results can be
demonstrated with SN toxic bait, the potential lethality would exceed the recommended 50–60% needed annually to keep populations from growing,20 and therefore has promising potential for reducing populations of wild pigs. However, similar to other toxic baiting programs for wild pigs, toxic baiting alone may not be sufficient for eradication,46–63 but will be useful as part of an integrated pest management approach.

Our findings build on previous research in New Zealand which found that 11 of 12 wild pigs (92%) succumbed to a similar SN toxic bait in a field setting.64 Similarly, a pen study in the USA found 95% efficacy for SN toxic bait on groups of wild pigs.34 For comparison with other toxic baiting strategies, a study in Australia found 63% consumption of biomarker baits by wild pigs using aerial baiting at 18 baits km$^{-2}$ over an area of 70 km$^2$.65 Additionally, wild pigs ground-baited with sodium monofluoracetate (compound 1080) in Australia at a density of 33 baits km$^{-2}$ over 400 km$^2$ resulted in a 58% reduction in wild pigs.66 We expect that our extensive pre-baiting procedures may have led to a more efficacious delivery of bait in our study, although the method did require substantially more time and effort. Increasing the time and effort for pre-baiting has been shown to increase the number of wild pigs becoming accustomed to use the bait station.38

To our knowledge, no studies using other methods of control have been published in south-central Texas for direct comparison with our results. For comparison with other methods in other ecosystems, aerial shooting of wild pigs in Australia resulted in nearly 80% reductions in populations of wild pigs (i.e., more than 6–10 wild pigs per km$^2$) in 5–9 days.69,70 A study in Texas, USA found that 3 days of aerial shooting resulted in 66% reduction in populations.71 However, as densities of wild pigs declined to fewer than two to six wild pigs per km$^2$ (i.e., similar to densities observed in this study), the efficacy of aerial shooting decreased drastically.72 Reductions in populations from trapping had varied success, with reports of 62–83% reductions in populations that were exposed to traps, and 28% for the overall population using up to 330 trap nights.24,73,74 Ground shooting has been less effective but may be necessary to remove the last remaining wild pigs.20 Hunting wild pigs (e.g., public hunts) is the least effective method,20,75 and was hypothesized to actually increase populations of wild pigs because of increased food resources (i.e., bait) that hunters supply to a population.76

An important finding from this study was that most of the collared wild pigs visited more than one bait site during the final night with biomarker deployment, leading to two relevant conclusions. First, the 18-day baiting strategy for locating and accustoming wild pigs to the placebo bait and bait stations was adequate. Overcoming neophobic tendencies of wild pigs is a principle challenge for any baiting program.77,78 Wild pigs visited the bait sites throughout the accustoming stages and were not deterred by the novel bait and bait stations. Similar results were also found in a recent study in which it took $\geq$ 15 days for most wild pigs to access bait stations.38 Patient baiting strategies are similarly important for other methods of removal such as whole-sounder trapping, which typically takes 7–14 days and may require multiple corral traps at the same location.79 The second conclusion was that the spacing of the bait stations (i.e., one bait site per 0.75 km$^2$) was adequate for exposing all wild pigs in between bait sites to the bait, and may have been expanded to expose more wild pigs. Our predicted results showed that the probability of consuming the biomarker bait was $\geq$ 50% up to 1.5 km away from a bait site (Fig. 2). Therefore, bait sites placed at a density of one site per $\leq$ 1.5 km$^2$ should theoretically expose all encompassed wild pigs to the bait. Alternatively, spacing the bait sites at farther distances will allow for a larger geographic focus but a lower overall proportion of wild pigs will be removed.80

Our results showed evidence that consumption of the biomarker bait varied by sex of wild pigs. Despite males and females having similarly sized home ranges, males visited $\sim$ 37% more bait sites and thus had more encounters with the bait. Males are also thought to be less risk averse than females,67,81 and thus may become accustomed to the bait and bait station more quickly. Additionally, we observed some males aggressively defending bait stations from other wild pigs similar to observations in another baiting study,71 which may have led to females having a lower probability of consuming bait at those sites. To overcome these defensive behaviors, deploying multiple bait stations in close proximity may allow easier access for subordinate females and young. Alternatively, the toxic bait could be used initially to remove the aggressive males, and then allow access by subordinate females and young. Aggressive males that defend the bait station may hinder learning to access the bait station in subdominant wild pigs, thus applicators may have to re-initiate the pre-baiting routine after those dominant animals are removed. Importantly, accustoming and ultimately removing females will most effectively reduce populations of wild pigs through time.20

Contrary to other toxic baiting studies,66,67 we found evidence that consumption of bait varied by age class. Adults and piglets had the same probabilities of consuming the bait, likely because piglets are highly dependent on adults. Whereas, juveniles had higher probabilities of consuming the bait possibly because these wild pigs dispersed away from their family groups at $\sim$ 16 months of age$^{22,81}$ and were more naive and susceptible to consuming the novel bait. Regardless, removing juveniles is highly important for the long-term reduction of populations of wild pigs, because these animals reach sexual maturity and can begin breeding within their

### Table 2. Model averaged estimates of density using the top model of the resight analysis of wild pigs on Joint Base San Antonio, Camp Bullis, TX, USA during August 2017. Density estimates were calculated using buffers of 1829 m around study areas, based on a MMDM analysis. Estimates of density from adults were extrapolated to include piglets and juveniles using the non-independent-to-adult ratio. Densities from post-simulated toxic baiting represent the density of wild pigs estimated to be remaining within 0.75 km of bait sites following one night of toxic bait deployment.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Density of adult wild pigs km$^{-2}$ (95% CI)</th>
<th>Density of all wild pigs km$^{-2}$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-trial</td>
<td>Post-simulated toxic baiting</td>
</tr>
<tr>
<td>Northeast</td>
<td>2.2 (1.5–3.0)</td>
<td>0.2 (0.02–1.2)</td>
</tr>
<tr>
<td>Northwest</td>
<td>1.4 (1.2–1.7)</td>
<td>0.1 (0.01–0.6)</td>
</tr>
<tr>
<td>South</td>
<td>4.7 (3.3–6.8)</td>
<td>0.4 (0.03–2.7)</td>
</tr>
<tr>
<td>Average</td>
<td>2.8 (2.0–3.8)</td>
<td>0.2 (0.02–1.5)</td>
</tr>
</tbody>
</table>

www.soci.org NPSnow
first year.20 This susceptibility of juveniles to the bait further suggests that toxic bait will be a useful tool for population control. Multiple applications of SN toxic bait, or another method of control, may be used to further suppress populations of wild pigs.

Important caveats limiting these findings should be discussed. First, implications from this study are restricted to a population of wild pigs during a hot and dry season in south–central Texas. We expect that food resources during this time were scarce, and the wild pigs were concentrated near sources of water, hence aiding our ability to target these animals with bait. Regardless, we expect toxic baiting applications should similarly take advantage of these times of year, therefore our findings should be applicable to most applications when wild pigs readily utilize bait sites.

Secondly, not all wild pigs succumbed to SN toxic baits. Approximately 95% succumbed to SN toxic bait in a pen trial in the USA,34 and 90% and 92% succumbed in pen and field trials, respectively, with a similar bait in New Zealand.68 In addition, baits containing RB in lieu of SN may be more palatable to wild pigs. A microencapsulated coating is used to hide the aversive salty taste of SN, but degradation or defects in the coating may reduce the palatability and subsequent consumption by wild pigs. Palatability of SN toxic baits for free-ranging wild pigs is an important line of future research. Also, this study did not account for group behaviors of wild pigs that were exposed to SN, where some animals may experience rapid onset of toxicosis and lethality. Symptomatic animals may alert other wild pigs, and reduce feeding behaviors by those animals. We expect that extensive pre-baiting may overcome this because it is designed to encourage a feeding frenzy where all wild pigs feed at once.38,51 Also, symptomatic animals do not experience extended periods of distress,36 thus may not alert other group members to the risks.

Another important consideration for using SN toxic baits in the field is the risk to non-target wildlife. Risks of secondary poisoning for scavengers of wild pig carcasses were reportedly low,36 risks of non-targets directly accessing bait in the bait stations were similarly low,37,51 but the risk of non-targets accessing dropped bait by wild pigs outside bait stations is currently unknown. Notably, upon morning inspections, we observed almost undetectable amounts of biomarker bait left outside the bait stations by wild pigs. Observations from camera imagery showed wild pigs readily consumed dropped bait outside bait stations, but it is possible that some non-target species consumed dropped bait also. Furthermore, if an actual toxic bait were used there may be fewer wild pigs to clean up dropped bait once the animals start experiencing intoxication symptoms or death. A field evaluation of SN toxic bait is needed to truly evaluate the risks dropped bait may pose in a natural setting. Finally, American black bears (Ursus americanus) overlap with portions of the current distributed range of wild pigs in the USA.35 Although the bait station employed in this study kept out the non-target species present, development of a black bear-resistant bait station will be needed to deploy toxic bait for wild pigs in those areas.

5 CONCLUSION

We demonstrated that a simulated toxic bait can be deployed across a large property and expose a substantial proportion of a wild pig population to consuming the bait, provided guidelines are followed. Bait sites should be spaced 0.75 – 1.5 km apart, and closer will provide better efficacy. Approximately 18 days of coordinated pre-baiting should be sufficient for accustoming wild pigs to using the wild pig-specific bait stations and readily consuming a novel bait. Multiple bait stations at a baiting site may be needed if resource protecting by dominant wild pigs is observed. The risk to non-target species (other than black bears) from directly accessing bait inside the bait station appears minimal, although more research is needed on bait that may be dropped by wild pigs. Incorporating SN may reduce consumption of the bait by wild pigs, thus a field study evaluating the efficacy of SN toxic bait is needed because addition of the toxicant could affect the behavioral ecology of bait consumption.

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Appendix

Model results for mark–resight analysis (zero-inflated Poisson log-normal) of wild pigs in south–central, Texas, USA during August 2017. Parameters included in the models were the intercept (alpha), individual heterogeneity (sigma), number of unmarked individuals in the population (U), and number of marked animals (r). We also allowed each of the parameters to be constant (.) or vary by camera grid (g). The top model AICc value was 228.27

<table>
<thead>
<tr>
<th>Model</th>
<th>Δ AICc</th>
<th>w</th>
<th>K</th>
<th>Deviance</th>
</tr>
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<tr>
<td>alpha(g) sigma(.) U(g) r(.)</td>
<td>0.00</td>
<td>8</td>
<td>206.01</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>10</td>
<td>207.55</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>10</td>
<td>208.21</td>
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<tr>
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<td>12</td>
<td>204.52</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>3</td>
<td>238.94</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>10</td>
<td>217.17</td>
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<tr>
<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>6</td>
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<td>8</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>231.02</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
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<td>3</td>
<td>311.92</td>
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<td>alpha(g) sigma(.) U(g) r(.)</td>
<td>98.12</td>
<td>0</td>
<td>319.53</td>
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</table>

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