

Influence of habitat and baiting strategy on oral rabies vaccine bait uptake by raccoons in the southeastern United States

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ABSTRACT

Oral rabies vaccination (ORV) is the primary tool for landscape level control and elimination of rabies virus in terrestrial wildlife species. However, there is currently a limited understanding of how different baiting strategies influence raccoon (*Procyon lotor*) uptake of ORV baits in non-agricultural habitats in the Southeastern United States, which may hinder the refinement of ORV management for raccoons in this region. Using a combination of new and existing data, we investigated the role of baited area (0.16 vs 3 km²), bait density (75 vs 150 baits/km²) and season (spring vs fall) on placebo ORV bait uptake by raccoons in four rural, non-agricultural habitats in the southeastern United States (bottomland hardwood forest, upland pine forest, riparian forest, and isolated wetlands). Increasing the baited area to 3 km² increased the proportion of raccoons that consumed baits by 140 % in riparian forests and decreased the proportion consuming baits by 70 % in bottomland hardwood forests. A greater proportion of raccoons consumed baits in riparian habitat in spring compared to fall, but bait density did not influence uptake in either season in this habitat. Increasing the bait density during spring in bottomland hardwoods increased the proportion of raccoons that consumed baits by 270 %, but there was no effect of increasing bait density in bottomland hardwoods during fall. We suggest that variation in habitat contiguity and seasonal resource availability influence how raccoons utilize these habitats which in turn impacts habitat-specific ORV bait uptake. The estimated proportion of raccoons that consumed baits did not exceed 60 % for any treatment, and for most treatments was less than 40 %. These low uptake rates indicate a need for research into additional strategies to maximize raccoon uptake of ORV baits across rural, non-agricultural southeastern landscapes.

1. Introduction

Raccoon (*Procyon lotor*) rabies virus (RABV) is enzootic across eastern North America (Elmore et al., 2017; Slate et al., 2020), and raccoons account for about one third of the RABV cases reported to the Centers for Disease Control and Prevention annually (Ma et al., 2021, 2022, 2023). Raccoon RABV can incur substantial economic and public health costs (Chipman et al., 2013; Anderson et al., 2014; Elser et al., 2016), and thus extensive efforts are undertaken to control and

eliminate raccoon RABV in free ranging populations.

The primary tool for landscape-level RABV control is oral rabies vaccination (ORV), which consists of the coordinated deployment of baits containing a RABV vaccine (Slate et al., 2005; Elmore et al., 2017; Fehlner-Gardiner, 2018). The ORV program to control and eliminate raccoon RABV historically contained the spread of this variant using a vaccine border along the Appalachian Mountains (Slate et al., 2008). The objective of ORV is to locally eliminate RABV and move ORV zones eastward with the eventual goal of eliminating raccoon RABV from the

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eastern United States (Davis et al., 2023). Wildlife rabies control through ORV in the US is coordinated by the National Rabies Management Program (NRMP) of the United States Department of Agriculture. On average, the NRMP distributes 8–10 million ORV baits annually in the United States, the majority of which are deployed aerially across rural landscapes using fixed-wing aircraft (Slate et al., 2005; Elmore et al., 2017).

The success of wildlife ORV depends on attaining levels of bait consumption that will produce the necessary vaccination coverage to achieve herd immunity (World Health Organization, 2018). While some studies have found ORV targeting rural areas can result in raccoon RABV control (Gilbert et al., 2018; Pedersen et al., 2019a; Johnson et al., 2021), others have found vaccination rates of raccoons in other areas may not reach thresholds needed for control (Boulanger et al., 2008; Sattler et al., 2009; Berentsen et al., 2018). Given the expansive footprint of the ORV program targeting raccoons in the US, there is an ongoing need for practical strategy refinement to maximize raccoon vaccine uptake in rural habitats to increase program effectiveness.

A primary decision in ORV programs is the density of baits to distribute that maximize uptake by target species while minimizing costs (Pedersen et al., 2019b). The NRMP employs a standard bait density of 75 baits/km² targeting raccoons in rural habitats, although in developed areas increasing the bait density to 150 or 300 baits/km² may be necessary to produce desired vaccination coverage (Gilbert and Chipman, 2020). An important component of efficiently allocating ORV resources, particularly across rural management areas, is understanding whether increasing bait densities may yield population vaccination rates which justify the costs (Slate et al., 2020).

The timing of bait deployment may also be influential as vaccination campaigns that temporally align with juvenile dispersal may vaccinate more individuals because juveniles are foraging independently (Boyer et al., 2011; McClure et al., 2020). For raccoons in North America, this period includes fall which may have the added advantage of promoting bait uptake due to the potential scarcity of alternative food resources (Boyer et al., 2011). The placement of baits on the landscape relative to raccoon home ranges and habitat use also influences the number of individuals to which the baits are available (Boyer et al., 2011; Berentsen et al., 2013; Beasley et al., 2015, 2024). Additionally, many nontarget species consume baits intended for raccoons, but the degree of non-target competition varies across habitats of the rural southeastern US (Dixon et al., 2023).

The influence of multiple factors on raccoon uptake of ORV baits fluctuates widely across different regions, which can require site-specific evaluation (Davis et al., 2024). Within the Southeastern US, habitats that may be targeted for such efforts include rural non-agricultural habitats (i.e., bottomland hardwood forest, upland pine forest, riparian forest, and isolated wetlands), but until recently there has been limited research into factors affecting raccoon uptake of ORV baits in these habitats (Helton et al., 2023). The study by Helton et al. (2023) deployed baits only across a 0.16 km² study grid during spring and estimated low uptake rates by raccoons across habitats. To maximize raccoon uptake of baits in these habitats, exploration of different baiting strategies, such as bait density and timing of bait deployment, is warranted. Verification that the low uptake rates observed by Helton et al. (2023) are not influenced by edge effects of small patches was evaluated by increasing the spatial footprint of baiting coverage of sampling grids. Analysis of bait consumption by Virginia opossums (*Didelphis virginiana*), the primary vertebrate bait competitor in the southeastern United States (Slate et al., 2020), across various baiting strategies could also inform the extent to which raccoon bait consumption is impacted by competition. In the current study, we deployed ORV baits across four rural non-agricultural habitats in South Carolina, USA to examine the influence of the following ORV baiting factors on bait consumption by raccoons and opossums: (1) increasing the size of the baited area grids, relative to sampling grids, from 0.16 km² to 3 km²; (2) increasing the bait density from 75 to 150 baits/km²; and (3) deploying baits in the fall

in addition to spring.

2. Methods

2.1. Study site

We conducted this study from 2021 to 2022 on the Savannah River Site (SRS), a 780 km² site owned by the United States Department of Energy in the upper Coastal Plain region of South Carolina, USA (33°19'N, 81°42'W; Fig. 1). The SRS was established in the 1950s as a nuclear production facility and operations today consist of facilities for nuclear materials processing, tritium extraction and waste disposal (White and Gaines, 2000). Since 1951, much of the SRS has been managed for timber harvest (originally slash pine [*Pinus elliottii*] and subsequently loblolly [*Pinus taeda*] and longleaf pine [*Pinus palustris*]), and pine plantations are harvested on a rotating basis and subject to management practices such as thinning and prescribed burning (White and Gaines, 2000). The SRS is primarily covered by evergreen forest (54 %) and woody wetlands (24 %), with other land cover types (e.g., developed, open water, mixed forest) collectively comprising 22 % of the land area (Yang et al., 2018).

We studied bait competition across the same four habitats on the SRS examined in Helton et al. (2023): upland pine forest, isolated wetlands, bottomland hardwood forest and riparian forest. Upland pine forest is characterized by mature stands of loblolly and longleaf pine (*Pinus palustris*) with land cover classified as evergreen by the National Land Cover Database (NLCD). Isolated wetlands are natural shallow ovoid or elliptical-shaped depressions that form ephemerally and are usually surrounded by evergreen or mixed forest NLCD classes (Workman and McLeod, 1990; White and Gaines, 2000). There are 195 such wetlands across the site ranging in size 0.1–50 ha (White and Gaines, 2000). Bottomland hardwood forests are classified as woody wetlands by the NLCD and are confined to the lower southwest portion of the site along the Savannah River and consist of seasonally flooded cypress-tupelo forests (*Taxodium distichum*-*Nyssa aquatica*), with oak (*Quercus* spp.) and hickory (*Carya* spp.) scattered throughout (White and Gaines, 2000). The average flow rate of the Savannah River during spring 2017–2022 (excluding spring 2020) was 4978, 4849, 16336, 12696, and 10861 ft³/sec, respectively (United States Geological Survey, 2023). Riparian forest is also classified as woody wetlands, but bottomland hardwood is largely one contiguous habitat on the SRS. In contrast, riparian forests are more fragmented and are embedded in a matrix of

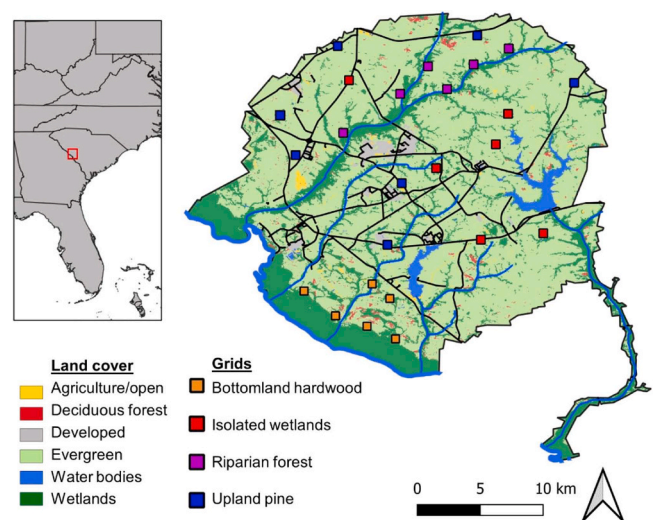


Fig. 1. Map showing locations of 24 grids where raccoons and opossums were trapped on the Savannah River Site, Aiken, South Carolina, USA (2017–2022) to assess uptake of placebo oral rabies vaccine baits.

upland habitat such as pine and hardwoods, existing in relatively narrow corridors along smaller rivers and creeks that feed into the Savannah River. This habitat is commonly produced by land conversion where native vegetation along waterways is left intact, resulting in the formation of a riparian zone (Stutter et al., 2021). Our riparian habitats were located along the upper portions of Tinker Creek and the Upper Three Runs Creek, both of which are relatively undisturbed and never received thermal effluent from nuclear reactors (Layman, 1993; Thomas et al., 2020).

2.2. Experimental design

All animal trapping and handling activities were conducted in accordance with the University of Georgia Animal Care and Use Guidelines under Animal Care and Use Protocol A208 06–024-A12. Field activities were approved by the Site Use Program of the Savannah River Site under Site Use Permit SU-20–42-R. We followed the methodology of Helton et al. (2023) for comparison of our results with the previous work. Briefly, we established six trapping grids separated by at least 5 km to maintain spatial independence (Hill et al., 2023c) in each of the four representative habitats. At each grid, we placed 25 Tomahawk® model 108SS live-capture box traps (Hazelhurst, WI) at intervals of 100 m in a 5 × 5 square configuration. We placed whole kernel corn on the ground adjacent to the trap and placed plaster tabs soaked in fish oil inside the traps as a lure (Webster and Beasley, 2019; Helton et al., 2023).

Our study was carried out concurrently with a mark-recapture study focused on estimation of habitat-specific density of raccoons and opossums within each of the four habitats (Bernasconi et al., 2022; Hill et al., 2023b). Each year, the 24 sampling grids were divided into three groups of eight and randomized with respect to habitat type and trapped consecutively. Eight grids were trapped concurrently during three consecutive 10-day sessions during fall (October–December) and trapped again in the same order during spring (February–March). In total, four seasons of trapping were conducted for this study: Fall 2020, Spring 2021, Fall 2021, and Spring 2022.

During Fall 2020 and Spring 2022, we examined bait uptake using placebo ONRAB® Ultralite baits (Artemis Technologies Inc., Guelph, Ontario, Canada) filled with a non-toxic biomarker, Rhodamine B (RB) (Sigma-Aldrich, Inc., St. Louis, Missouri, USA), as described in Helton et al. (2023). Ultralite baits consist of an oval blister pack measuring 30 × 14 × 10 mm with a rectangular lip extending to 40 × 20 mm encased in a waxy coating filled with water during manufacture. We used a 20 G 1'' needle and 3 mL syringe to extract 1 mL of water from the blister pack. We then injected 1 mL of distilled water containing RB at 150 mg/mL. Wax fragments from the external coating of the bait were retained and later melted and used to seal the puncture site.

Beginning in Spring 2021, we incorporated blood biomarkers propyl-iophenoxic acid (Pr-IPA) and ethyl-iophenoxic acid (Et-IPA) into baits (Berentsen et al., 2019). Because there was less than six months between fall and spring sessions, we implemented a staggered deployment of biomarkers, using Pr-IPA in Spring 2021 and Et-IPA in Fall 2021 (Figure S1). Baits containing IPA were identical to those containing RB and were prepared by Artemis, with 1 mL of distilled water in the blister pack and incorporating the appropriate isomer of IPA into the bait matrix at a concentration of 1 mg/bait. We resumed RB use in Spring 2022, given that a year and a half had passed since the previous use of RB in Fall 2020 and any RB markings from exposure in Fall 2020 would no longer be present due to whisker growth. The use of multiple unique biomarkers allowed retrospective examination among all sampled animals to document bait consumption to corresponding seasons even if an animal was not trapped within that season (Figure S1).

All baits were deployed 14–20 days before the start of the trapping session. For the current study, we established a 3 km² rectangle (baiting grid) centered on the trapping grid in which to deploy baits (Figure S2). During each season, three of the baiting grids within each habitat were

baited at 75 baits/km² and three were baited at 150 baits/km². Baiting grids consisted of four 1500 m transects spaced 500 m apart to simulate the aerial deployment methods used by the NRMP. For the 75 baits/km² treatment, we deployed a total of 225 baits in the 3 km² baiting area, which translated to one bait being deployed per 26.67 m along each transect. For the 150 baits/km² treatment, we doubled the number of baits deployed per linear meter along each transect. Although these baiting grids are much smaller than the scale at which landscapes are typically baited for ORV (often > 1000 km²), baiting grid sizes in our study were limited by our need to trap animals to assess bait uptake. Based on mean home range sizes of raccoons in these habitats (Hill et al., 2023c), extending the baiting grid any further would result in smaller chances of animals that consumed the baits also encountering the trapping grid. Trapping grid size was constrained by logistical limitations. A summary of the bait densities, baited area sizes, and biomarkers used in each year and season is provided in Table 1.

2.3. Animal handling

All raccoons and opossums were immobilized upon capture using intramuscular injection of Telazol (Fort Dodge Animal Health, Fort Dodge, IA) at a dosage of 5 mg/kg of estimated body weight (Gehrt et al., 2001; Beasley and Rhodes 2008; Smyser et al., 2010). At initial capture of an animal, it was marked with a pair of matching uniquely coded ear tags (bilaterally in each ear; Monel #3, National Band and Tag Company, Newport, KY), then weighed, sexed, and aged based on tooth eruption and wear (Grau et al., 1970). We also pulled two whiskers from each side of the face to evaluate evidence of RB uptake. Whiskers were pulled so that the entire whisker was retained for analysis, as fluorescent bands from recent RB consumption are at the proximal whisker end. All whiskers were placed in sealed plastic bags in dry dark storage until microscopic analysis for presence of RB.

To test for the presence of blood biomarkers, we used a 23 G 1'' needle to collect 3 mL of blood from the jugular vein of raccoons or a 25 G 5/8'' needle to collect blood from the caudal vein of opossums. Following all handling procedures, we placed animals in an inconspicuous location out of direct sunlight near the capture site and observed them until full recovery.

2.4. Laboratory methods

For microscopic analysis, whiskers were soaked in distilled water for ten minutes and then allowed to dry at ambient conditions for 15 min. We thoroughly cleaned dried whiskers with Kimwipes (Kimberly-Clark Corp., Irving, TX) and isopropyl alcohol to remove dirt and debris, always handling them with nitrile gloves and forceps. All four whiskers from an individual animal were then placed onto a single microscope slide with Fluoromount Aqueous Mounting Medium (Sigma-Aldrich) and covered with a cover slip. Tape was applied at the base of each slide to mask written data, and each slide was given a random number to

Table 1
Placebo oral rabies vaccine bait density, baited area size, and biomarker used in each year and season to examine uptake of baits by raccoons and opossums at the Savannah River Site, Aiken, South Carolina, USA (2017–2022).

Year	Season	Baited Area (km ²)	Bait density (baits/km ²)	Biomarker
2017	Spring	0.16	75	Rhodamine B
2018	Spring	0.16	75	Rhodamine B
2019	Spring	0.16	75	Rhodamine B
2020	Fall	3.0	75 or 150	Rhodamine B
2021	Spring	3.0	75 or 150	Propyl-iophenoxic acid
2021	Fall	3.0	75 or 150	Ethyl-iophenoxic acid
2022	Spring	3.0	75 or 150	Rhodamine B

avoid potential observer bias. We analyzed slides using an Olympus BX 61 fluorescent microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) with a tetramethylrhodamine isothiocyanate (TRITC) filter set (e.g. narrow-band excitation and red-shifted emission filters) under 4x and 10x magnification. If at least one of the four whiskers from an animal displayed fluorescent marker bands consistent with RB presence, the sample was scored as positive for bait uptake. All whiskers were scored independently by the same two observers and when contradictory scores were recorded, they were scored an additional time by a single third independent observer for a final determination (Smyser et al., 2010).

Immediately following collection of blood samples, we spun blood vials in a centrifuge for 15 min at 3000 rpm to separate the serum and stored samples at -20°C until analysis. We analyzed raccoon and opossum sera for the presence of Pr-IPA and Et-IPA using the liquid chromatography-mass spectrometry method described in Berentsen et al. (2019). We added 50 μL serum to a 1.5-mL microcentrifuge and then added 50 μL methyl-*i*-phenoxyc acid surrogate and 0.950 mL 0.5 % trifluoroacetic acid in acetonitrile. After centrifuging at 2000 rpm for 1 min, we added ~ 150 mg sodium chloride and centrifuged for 2 min at 14,000 rpm. We then pipetted 800 μL of the upper acetonitrile layer to a separate 1.5-mL tube. We removed the solvent from the tube with a flow of N_2 gas in a 45°C water bath. We added 250 μL acetonitrile, vortexed for 8–10 sec, and centrifuged briefly (~ 2 sec) to collect liquid in the bottom of tube. We added 750 μL ultrapure water, vortexed for 8–10 sec, and then centrifuged for 2 min at 14,000 rpm to clarify the sample. We assayed the samples using an ultra-high performance liquid chromatography instrument (1290 Series UHPLC, Agilent Technologies, Inc., Santa Clara CA).

The signal-to-noise ratio was used to determine the detection limit in serum. This was performed by comparing IPA responses observed in control serum fortified with approximately 475 ng/mL of each IPA with the baseline noise observed at the retention time of each IPA in control serum. We estimated the detection limit as three times the signal-to-noise ratio. The detection limit was 18 ng/mL for Et-IPA and 27 ng/mL for Pr-IPA. The quantitation limit was 59.0 ng/mL for Et-IPA and 88.9 ng/mL for Pr-IPA. Animals with IPA concentrations above the detection limits were considered to have consumed the respective bait, whereas those with concentrations below these levels were considered to have not consumed the bait.

2.5. Statistical analysis

For animals with multiple samples (e.g., blood and whiskers) collected during a single trapping session, we matched each sample with the corresponding bait season (for example, a Pr-IPA marker collected in Fall 2021 was matched with the baiting treatment on that grid in Spring 2021). Although animals could have consumed the bait at a grid other than where they were trapped, this was unlikely given their mean home range sizes and the spatial configuration of traps (Hill et al., 2023a, 2023c).

We modeled the probability of bait consumption using generalized linear models with a binomial error distribution and a logit link using the package ‘lme4’ (Bates et al., 2015) implemented in Program R version 4.0.4 (R Core Team, 2022). We incorporated year and grid as random effects in cases where the data supported this model structure. To address the baited area objective, we examined how increasing the baited area from 0.16 km^2 to 3 km^2 with a bait density of 75 baits/ km^2 influenced our estimates of bait uptake. We used all data from Helton et al. (2023), which included only 0.16 km^2 grids only baited at 75 baits/ km^2 , and our data from the 3 km^2 grids baited at 75 baits/ km^2 during spring to evaluate this objective. Only spring data were used for the longitudinal analysis, due to an uneven replication of seasonal ORV with size of area baited. To address our bait density-season objective, we examined how increasing the bait density from 75 to 150 baits/ km^2 and season influenced bait uptake using all data from the current study.

Analyses conducted to explore each objective consisted of three models examining different aspects of bait uptake. The first model assessed the probability of either species combined (raccoon or opossum) consuming the bait with the response variable as the animal testing positive or negative for the biomarker (interspecific model). The second model assessed factors influencing whether raccoons consumed the bait with the response variable as the raccoon testing positive or negative for the biomarker (raccoon model). Our third model examined factors influencing the proportion of raccoons consuming the bait with the proportion of raccoons on the grid positive for the biomarker as the response variable (grid-specific model).

The combinations of fixed effects varied by model (Table S1). All models for the baited area objective included baited area (0.16 or 3 km^2) and all models for the bait density-season objective included bait density (75 or 150 baits/ km^2) and season (fall or spring). All models for both objectives included habitat (bottomland hardwood, pine, riparian, or wetland) and the estimated density of raccoons and opossums on the grid during that season (Supplemental Information). The mean raccoon density across all habitats and grids was 3.7 animals/ km^2 (range 0–26.0) during fall and 2.8 animals/ km^2 (range 0–14.7) during spring. The mean density of opossums across all habitats and grids was 3.1 animals/ km^2 (range 0–18.7) during fall and 3.7 animals/ km^2 (range 0–31.4) during spring. Interactions between fixed effects were chosen based on their relevance and whether they were supported by the data (Table S1). For three-way interactions we also tested all constituent two-way interactions. Raccoon-only models also included the sex of the animal as well as a residency index because uptake may be related to the amount of time an animal spends on the grid (Smyser et al., 2010; Helton et al., 2023). In previous studies, residency index has generally been defined as the number of times the animal was caught during the trapping session (Smyser et al., 2010). Due to our retroactive sampling strategy, however, there were cases where we obtained a sample from a season in which the animal was never trapped. Consequently, we defined the residency index as the number of times the animal was trapped on the grid during the study.

We ranked the null and all possible model combinations based on sample-size corrected AIC (AICc), considering the model with lowest AICc to be the top model (Burnham and Anderson, 2002). We assessed the relative support for the top model by comparing models within 2 AICc units of the top model. If habitat was included as a parameter in the top model, we used the odds ratio to test for pairwise comparisons with a significance level of 0.05. We assessed the fit of the top model by calculating its marginal and conditional R^2 (Nakagawa and Schielzeth, 2013).

3. Results

We collected 701 biomarker (whiskers and sera combined) samples from 302 individual animals (190 raccoons and 112 opossums). For models evaluating the baited area objective, we combined our spring data from grids baited at 75 baits/ km^2 with data from Helton et al. (2023) for a total sample size of 472 (332 from raccoons and 140 from opossums). For the 0.16 km^2 baited area, 11.5 % of opossums and 29.0 % of raccoons were positive for the biomarker (Table 2). For the 3 km^2 baited area in spring, 4.8 % of opossum and 21.8 % of raccoon samples were positive. The top baited area interspecific model included habitat and species with the estimated probability of consumption approximately three times higher for raccoons compared to opossums (marginal $R^2 = 0.14$, conditional $R^2 = 0.20$, Table S2). The estimated probability of a raccoon testing positive for the biomarker ranged from 21 % to 35 % across habitats compared to 6–11 % for opossums (Table S3). Although habitat was included in the top model, no pairwise comparisons were significant.

The top raccoon model for the baited area objective included sex, raccoon density and the interaction between baited area and habitat (marginal $R^2 = 0.18$, conditional $R^2 = 0.45$, Table S4). Males were on

Table 2

Number of raccoons and opossums positive and negative for placebo oral rabies vaccine bait biomarker across four habitats and two baiting areas at the Savannah River Site, Aiken, South Carolina, USA (2017–2022).

Baited area	Habitat	Opossum			Raccoon		
		Negative	Positive	Proportion positive	Negative	Positive	Proportion positive
0.16 km ²	Bottomland	20	2	0.09	68	23	0.25
	Pine	13	2	0.13	38	21	0.36
	Riparian	24	2	0.08	32	8	0.20
	Wetland	12	3	0.20	36	19	0.35
	Total	69	9	0.12	174	71	0.29
3 km ²	Bottomland	30	2	0.06	35	2	0.05
	Pine	8	1	0.11	8	4	0.33
	Riparian	9	0	0.00	15	8	0.35
	Wetland	12	0	0.00	10	5	0.33
	Total	59	3	0.05	68	19	0.22
Grand Total		128	12	0.09	242	90	0.27

average 44 % more likely than females to test positive for the biomarker (Table S5). The estimated probability of a raccoon consuming a bait increased by about 12 % with every one animal/km² increase in raccoon density. The estimated probability of a raccoon testing positive for the biomarker decreased by 75 % in bottomland habitat when baited at 3 vs 0.16 km² ($p = 0.046$), but there was no difference in the other habitats between the baited areas (Fig. 2).

The grid-specific mean proportion of raccoons testing positive for the biomarker at 0.16 km² and 3 km² was 0.26 and 0.19 in bottomland, 0.42 and 0.38 in pine, 0.19 and 0.36 in riparian, and 0.38 and 0.54 in wetland, respectively. The top grid-specific model included the interaction between baited area and habitat (marginal $R^2 = 0.21$, conditional $R^2 = 0.48$, Table S6). The proportion of raccoons testing positive for the biomarker was 140 % greater at the 3 vs 0.16 km² baited area scale in riparian ($p = 0.014$), 70 % less in bottomland ($p = 0.006$), and there was no difference in the other habitat types (Fig. 3, Table S7).

For our bait density-season objective, the probability of opossums testing positive for the biomarker was 0.01 at 75 baits/km² during fall, 0.03 at 150 baits/km² during fall, 0.05 at 75 baits/km² during spring, and 0.02 at 150 baits/km² during spring (Table 3). For raccoons, the probability of raccoons testing positive for the biomarker was 0.39 at 75 baits/km² during fall, 0.48 at 150 baits/km² during fall, 0.22 at 75 baits/km² during spring, and 0.42 at 150 baits/km² during spring. The top interspecific model included bait density, season, species, and opossum density ($R^2 = 0.45$, Table S8). The model estimated that raccoons were about 11 times as likely to test positive for the biomarker compared to opossums and this difference was not affected by bait

density, season, or habitat (Table S9). Animals were about 24 % more likely to test positive for the biomarker in fall compared to spring. The probability of an animal testing positive for the biomarker decreased by about 4 % with each one animal/km² increase in opossum density.

The top raccoon model indicated no difference in the likelihood of raccoons testing positive for the biomarker between the two bait densities in fall, but raccoons were about twice as likely to test positive at the higher bait density in spring ($R^2 = 0.10$, Fig. 4, Table S10, Table S11). However, there was no difference in the probability of raccoons consuming a bait between fall and spring at the higher bait density ($p = 0.381$). The probability of a raccoon consuming a bait increased by about 7 % with each additional day trapped on the grid, and the probability decreased by about 4 % with each one animal/km² increase in opossum density.

The mean grid-specific proportion of raccoons positive for the biomarker across habitats, seasons, and baiting densities ranged from 0.19 (75 baits/km² in bottomland spring) to 0.53 (75 baits/km² in riparian fall; Table S12). The top grid-specific model (marginal $R^2 = 0.33$, conditional $R^2 = 0.53$, Table S13) indicated that the effect of bait density was dependent on season and habitat. Increasing the bait density did not influence the proportion of raccoons positive for the biomarker in any habitat in fall, but during spring the proportion positive was 3.7 times higher in bottomland ($p = 0.001$) and 2.5 times higher in wetlands ($p = 0.045$) at the higher bait density (Table S14, Fig. 5). In riparian habitat, the proportion of raccoons positive was about 50 % greater in spring compared to fall at both bait densities. In bottomland and wetland habitat, proportion positive was about five times and two times higher,

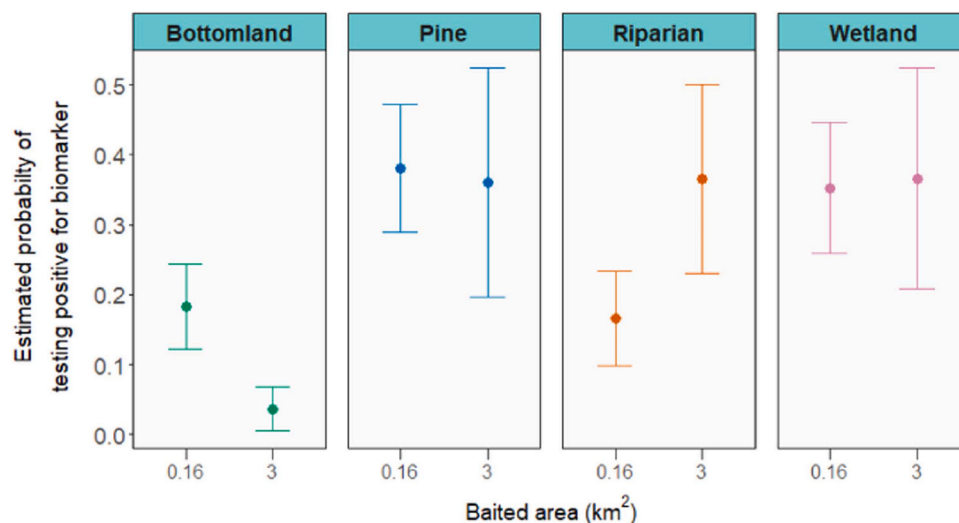


Fig. 2. Estimated probability of raccoons (based on the top model in Table S4) testing positive for oral rabies vaccine bait biomarker at two different baited area sizes across four habitats on the Savannah River Site, Aiken, SC, USA (2017–2022).

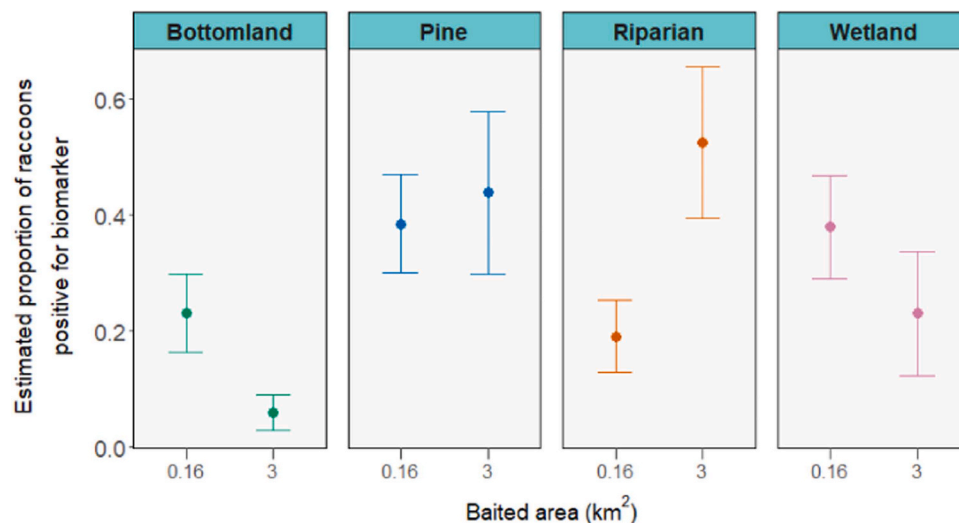


Fig. 3. Estimated proportion of raccoons (based on the top model in Table S6) on trapping grids testing positive for oral rabies vaccine bait biomarker at two different baited area sizes across four habitats on the Savannah River Site, Aiken, SC, USA (2017–2022).

Table 3

Number of raccoons and opossums positive and negative for placebo oral rabies vaccine bait biomarker across four habitats at two baiting densities during spring and fall at the Savannah River Site, Aiken, South Carolina, USA (2020–2022).

Season	Density (baits/km ²)	Habitat	Opossum			Raccoon		
			Positive	Negative	Proportion positive	Positive	Negative	Proportion positive
Fall	75	Bottomland	37	1	0.03	40	22	0.35
		Pine	9	0	0.00	9	7	0.44
		Riparian	8	0	0.00	19	12	0.39
		Wetland	13	0	0.00	13	10	0.43
		75 Total	67	1	0.01	81	51	0.39
	150	Bottomland	42	0	0.00	22	11	0.33
		Pine	9	1	0.10	18	19	0.51
		Riparian	8	0	0.00	14	18	0.56
		Wetland	10	1	0.09	7	9	0.56
		150 Total	69	2	0.03	61	57	0.48
		Fall Total	136	3	0.02	142	108	0.43
Spring	75	Bottomland	30	2	0.06	35	2	0.05
		Pine	8	1	0.11	8	4	0.33
		Riparian	9	0	0.00	15	8	0.35
		Wetland	12	0	0.00	10	5	0.33
		75 Total	59	3	0.05	68	19	0.22
	150	Bottomland	28	1	0.03	19	8	0.30
		Pine	9	0	0.00	20	17	0.46
		Riparian	3	0	0.00	18	15	0.45
		Wetland	9	0	0.00	8	8	0.50
		150 Total	49	1	0.02	65	48	0.42
		Spring Total	108	4	0.04	133	67	0.34
		Grand Total	244	7	0.03	275	175	0.39

respectively, in fall compared to spring at 75 baits/km² but there was no seasonal difference in either habitat at 150 km². The estimated proportion of raccoons positive for the biomarker decreased by 5.1 % with each one animal/km² increase in opossum density.

4. Discussion

Combining our data with previous work, we found that the effects of baited area, bait density, and bait season on raccoon uptake of placebo ORV baits in the southeastern US varied widely among rural non-agricultural habitats. None of the baiting factors examined at this spatial scale consistently increased bait uptake across all habitats and their effects across habitats were often divergent. We suggest that variation in habitat contiguity and resource availability influences how raccoons utilize these habitats which in turn impacts habitat-specific ORV bait uptake.

Raccoon uptake in bottomland hardwoods was consistently lower than in other habitats, which likely reflects increased availability of alternative food sources. Raccoons often concentrate foraging efforts along water bodies and the availability of water in bottomland hardwoods tends to produce greater prey abundance compared to other habitats (Stuewer, 1943; Chamberlain et al., 2003; Byrne and Chamberlain, 2011). As a result, raccoons in bottomland hardwoods may have been less food-motivated, consuming fewer baits compared to animals in habitats with lower food abundance. Additionally, there is greater bait consumption by competitors such as opossums and wild pigs (*Sus scrofa*) in bottomland hardwoods compared to upland pine (Dixon et al., 2023), and greater bait competition may have led to reduced consumption by raccoons in this habitat.

Unlike the other habitats we studied, bottomland hardwoods are also heavily influenced by seasonal flooding of the Savannah River. Raccoons inhabiting such wetlands often reduce activity and seek refuge in dens

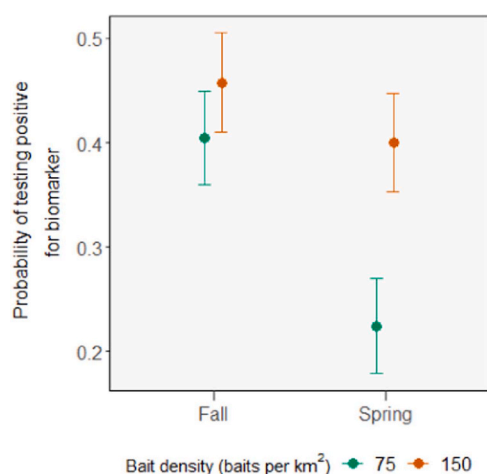


Fig. 4. Estimated probability of raccoons (based on the top model in Table S10) testing positive for oral rabies vaccine bait biomarker at two bait densities at the Savannah River Site, Aiken, SC, USA (2021–2022).

when water levels are high (Cagle, 1949). For our baited area objective, the only years with the 3 km² baited area included 2021 and 2022, which were years of greater inundation of swamplands as measured by the average flow rate of the Savannah River. Raccoons may not have ranged as widely during this time compared to other years which could account for the reduced bait uptake at 3 km² compared to 0.16 km². Since raccoons in other habitats of the SRS are not influenced in this way by the Savannah River, this would also explain why increases in baited area size did not have the same effect on raccoon bait uptake in other habitats sampled at SRS. This conclusion is also supported by differences in the proportion of raccoons positive for the biomarker across grids within the bottomland habitat. In two grids that abutted the Savannah River, the proportion positive decreased from about 36 % in the smaller baited area to 0 % at the larger baited area. Conversely, in one of the grids that was positioned further from the Savannah River, the proportion of raccoons positive actually increased from 19 % to 58 % when the baited area increased (no raccoons were trapped in the remaining three grids baited at 75 baits/km² during spring at the 3 km² baited area). Thus, there may have been unintended biases resulting from the location of the grids relative to the river and their assigned baited areas.

These divergent findings across habitats result in ambiguity regarding the effectiveness of using the 0.16 km² baited area

implemented in Helton et al. (2023) to test raccoon uptake of ORV baits in rural habitats of the southeastern US. The appropriate size area over which to experimentally allocate baits relative to the trapping grid (where animals are sampled for uptake) is determined by raccoon space use. When the baiting grid extends far beyond the trapping grid, there may be few animals with home ranges overlapping both the baiting and trapping grids in habitats where animals have small home ranges. However, a larger baited area may be necessary to fully encompass the movements of animals trapped in habitats where animals tend to have larger home ranges. During the breeding season when the baited area objective was studied, males in riparian have larger home ranges than in bottomland (3.20 ± 1.45 km² vs. 2.29 ± 1.32 km², respectively) (Hill et al., 2023c) which may explain why using the larger baiting grid increased uptake for animals in riparian whereas it did not for bottomland. The appropriate baited area size for examining uptake of ORV baits likely varies depending on habitat-specific raccoon movement patterns and may also depend on the grid size used in ORV operations, which may be substantially larger than the spatial scales at which we examined uptake (Davis et al., 2024).

Male raccoons were more likely to test positive for the biomarker than females only for the baited area objective (2017–2022), which was carried out in spring. This is likely related to movement patterns with male raccoons often having larger home ranges than females, leading to higher likelihood of encountering baits (Hill et al., 2023c). However, these sex-specific differences were not apparent in the bait density season objective, which included fall in addition to spring. Space use by raccoons at SRS tends to be more similar between the sexes during fall compared to spring with smaller or no differences in home range sizes (Hill et al., 2023c). Greater similarity in space use during fall likely accounts for the reduced effect of sex on bait uptake for the bait density season objective.

Riparian was the only habitat where bait uptake was greater in spring compared to fall. Riparian habitat is presumably resource abundant for raccoons due to the availability of food and water (Chamberlain et al., 2003; Owen et al., 2015). However, the relatively narrow patches of this habitat likely leads animals to also incorporate adjacent upland pine habitat into their home ranges (Hill et al., 2023b, 2023c). Soft mast is an important food item for raccoons in upland pine, but is not readily available until summer (Byrne and Chamberlain, 2011). As a result, raccoons during spring tend to shift foraging to areas with greater water availability where they can forage on vertebrates and invertebrates (Byrne and Chamberlain, 2011). Therefore, the higher bait consumption by raccoons in riparian habitat during spring may reflect greater raccoon

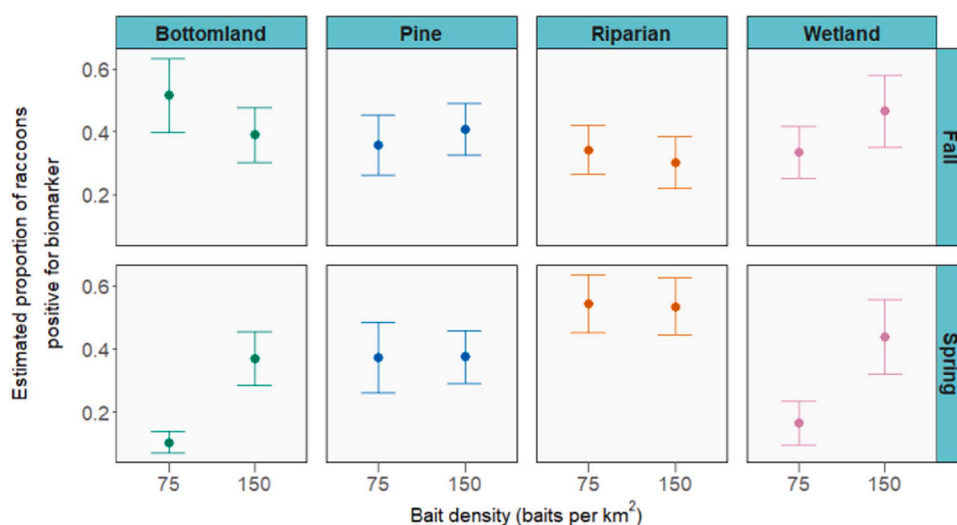


Fig. 5. Estimated proportion of raccoons (based on the top model in Table S12) on trapping grids positive for oral rabies vaccine bait biomarker during spring and fall across four habitats at the Savannah River Site, Aiken, SC, USA (2021–2022).

use due to increased foraging opportunities compared to surrounding habitat. The higher bait density did not increase uptake rates in riparian habitat in either season, suggesting that uptake was primarily driven by season rather than bait density.

Increases in bait density had the most pronounced effect on bait uptake in bottomland hardwoods, but only during spring. During winter and into spring, hard mast forms an important component of raccoon diets (Johnson, 1969), and hard mast is often highly abundant in bottomland hardwoods during this time (Chamberlain et al., 2003). It is possible that the high abundance of hard mast in this habitat during this time made raccoons more reluctant to consume baits, but that doubling the bait density increased their encounter rate which encouraged greater bait uptake. However, uptake at the higher density during spring in bottomland hardwoods did not differ from uptake at either density during fall.

There may be greater intraspecific bait competition at elevated raccoon densities, requiring higher bait densities to achieve desired vaccination rates (Slate et al., 2020). However, at lower raccoon densities, intraspecific bait competition plays a lesser role in raccoon bait uptake, which can lessen the impact of increasing the bait density. The latter appears to be the case at our site as raccoon densities in these habitats are comparatively low (Hill et al., 2023b) and increasing bait densities did not have a substantial influence on raccoon bait uptake. In fact, at very low raccoon densities, it may be possible to use bait densities lower than 75 baits/km² without sacrificing vaccination rates (Slate et al., 2020). Our results suggest that such a strategy may be possible across the rural non-agricultural habitats we examined, although additional studies in these habitats using lower bait densities are needed.

Bait consumption by raccoons was consistently higher than opossums across all habitats and bait treatments. A few of the models indicated reduced consumption by raccoons with increasing opossum density, but the effect was generally small. The reduced bait consumption of opossums compared to raccoons is likely the result of raccoons' larger body size which results in more expansive movement behavior leading to greater bait encounter probability (Ginger et al., 2003; Helton et al., 2023). Additionally, opossums tend to occur at low densities across these habitats on the SRS (Bernasconi et al., 2022). These results agree with a study at SRS using cameras which found opossums to be a minimal bait competitor, consuming only 5 % of over 1300 placebo baits deployed (Dixon et al., 2023). Competition with opossums, therefore, does not appear to influence bait uptake by raccoons in these habitats.

Despite the lack of competition with opossums, raccoon uptake rates remained low across all treatments, consistent with other studies at the SRS (Dixon et al., 2023; Helton et al., 2023). Fall baiting increased uptake relative to spring, which supports the current NRMP strategy of vaccinating during fall. However, even during fall, the percentage of raccoons positive for the biomarker did not exceed 60 % in any habitat and was often below 40 %. The target raccoon vaccination thresholds to interrupt RABV transmission are generally thought to be greater than 60 %, though vaccination thresholds for control and elimination at scale have not been validated by field data and the specific percentage likely varies by region and habitat (Rees et al., 2013; Berentsen et al., 2018). The proportion of raccoons consuming baits reported in this study is likely below the herd immunity thresholds that may be needed in these habitats, prompting interest in additional strategy refinements to increase raccoon uptake of ORV baits.

In our study, the IPA biomarkers were incorporated into the bait matrix whereas RB was in the sachet, which may have affected vaccine uptake. Animals sometimes consume the bait matrix without perforating the sachet, which may have resulted in overestimates of IPA compared to RB bait uptake. Additionally, only RB was used in the 0.16 km² baiting grids, which may have affected our results. Furthermore, most of our R^2 values were relatively low (< 0.50), suggesting that factors other than those which we investigated may influence raccoon ORV bait

uptake in these habitats.

We did not follow the fates of individual baits in this study, so it is not clear whether other competitors consumed the baits or whether raccoons chose to not consume the baits when they encountered them. Our methodology only allowed us to examine bait competition with opossums, but previous research at SRS suggests that vertebrates are not likely major competitors for baits (Dixon et al., 2023). However, invertebrates consume considerable ORV baits at our study site (Dixon et al., 2023), and deterring invertebrate consumption may increase the availability of ORV baits for raccoons. Altering the bait matrix or using alternative sachet types could increase uptake by making baits more attractive to raccoons. Differentially allocating baits based on predicted raccoon habitat-use patterns has been suggested as a possible strategy to increase bait uptake (Boyer et al., 2011; Mainguy et al., 2012; Tardy et al., 2014; Beasley et al., 2015; McClure et al., 2022). This may be effective in the habitats we studied considering the variation in ORV bait uptake as a function of habitat and season, although caution may be warranted in scaling recommendations directly from this study due to a limited spatial scale and habitat similarity relative to the NRMP ORV zone. Future research that examines how strategies such as these increase raccoon uptake of ORV baits may be necessary for effective ORV campaigns in rural non-agricultural habitats of the Southeastern United States.

CRedit authorship contribution statement

Jacob Hill: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Madison L. Miller:** Writing – review & editing, Formal analysis. **Richard B. Chipman:** Writing – review & editing, Conceptualization. **Amy T. Gilbert:** Writing – review & editing, Conceptualization. **James C. Beasley:** Writing – review & editing, Conceptualization. **Guha Dharmarajan:** Writing – review & editing, Conceptualization. **Olin E. Rhodes:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106320.

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