ABSTRACT

FULKERSON, KAYLA MARIE. From Habitat to Hand: Understanding the Impact of Educational Programming on Ambassador Snake Welfare. (Under the direction of Dr. Jennifer Campbell and Dr. Emily Lynch).

Zoos play a vital role in public education regarding animals and their conservation, and ambassador animals serve as powerful tools for engagement. While snakes are popular ambassadors within educational programs, little research has explored how these programs impact welfare. To redress this gap, we examined the effects of programming on six ambassador snakes housed at the North Carolina Zoo. Behavioral profiles were assessed both in-habitat and during education programs. In-habitat observations were gathered via cameras and behavior during programming sessions was gathered via live observations. To assess program-related behavior changes, we established a "no-program" phase (non-program days or pre-program time) and a "post-program" phase (after a program) to compare behavior and exposure. We report that snakes were significantly more likely to be exposed post-program (52.9%; $p < 0.001$) compared to non-program periods (21.4%; $p < 0.001$). Following a program, snakes were significantly more likely to both engage in interaction with a transparent boundary (undesired behavior; 8.7%; $p < 0.001$) and be active compared to non-program time periods (64%; $p < 0.05$), suggesting heightened arousal or disruption in typical behavioral patterns. Notably, snakes spent 95 - 99% of program time in positive behavioral states, with negative behaviors observed for only 12.6 minutes out of 38 hours across 110 programs. These findings enhance our understanding of the welfare of an understudied taxonomic group and provide valuable insights for evidence-based husbandry practices in ambassador animal programs.

From Habitat to Hand: Understanding the Impact of Educational Programming
on Ambassador Snake Welfare

by
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APPROVED BY:

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DEDICATION

To Pogo, Scutes, Slinky, Freckles, Gryphon, Bristol, and all our scaly companions who are too often misunderstood or overlooked - this is for you.

To the mentors who fueled my curiosity, believed in my dreams, and cheered me on every step of the way - thank you for everything.

BIOGRAPHY

Kayla Marie Fulkerson is originally from Keystone, Colorado, where she grew up skiing, hiking, and developing a deep love for animals and the outdoors. She spent much of her childhood exploring forests and observing wildlife, a curiosity that only grew stronger with time. This upbringing laid the foundation for two parallel passions: animal behavior/welfare research and environmental/outdoor education.

Kayla earned her B.A. in Animal Studies from Eckerd College in St. Petersburg, Florida. There, she studied African elephant behavior which deepened her interest in animal behavior and welfare science. During this time, she also began spending summers back in Colorado, working as an outdoor educator leading environmental programs for young students.

In 2023, Kayla moved to Raleigh to pursue her M.S. in Biology at NC State University, where she collaborated with the North Carolina Zoo to study the behavior and welfare of ambassador snakes.

Outside of academia, Kayla has found immense joy in leading student travel programs, practicing yoga, attempting to learn to cook, and strolling through her neighborhood here in Raleigh. As she completes her degree, Kayla hopes to build a career at the intersection of research and education - connecting people, animals, and environments through community-based conservation, global learning, and shared experience in our natural world.

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To my family - Ellen, Fred, and Ethan Fulkerson - thank you for the countless FaceTimes and for your endless love and encouragement. You've supported me both academically and emotionally every step of the way.

To my research assistant and lab mates - Maddie Greenway, Cassidy Hawkins, Jonah Peckham, and Faith Polando - thank you for your ideas, support, and the much-needed laughter during long days in the office.

Finally, to the Ambassador Animal team and snakes at the North Carolina Zoo - Cat, Brett, April, Mindy, Isaac, and Beth - thank you for making this project possible. I am so grateful for your help, trust, and patience.

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1. INTRODUCTION

Modern zoos and aquariums (hereafter referred to as zoos) have evolved significantly from their origins as menageries focused on entertainment into multifaceted institutions prioritizing conservation and education (Conway, 1969; Minteer & Collins, 2013; Miranda et al., 2023). No longer limited to displaying animals for collection and public amusement, today's zoos play active roles in shaping conservation policy, supporting global research, advancing public health and well-being, and fostering environmental awareness (Spooner et al., 2023). Among these expanding responsibilities, they conduct in situ and ex situ conservation (Binding et al., 2020; Moloney et al., 2023; Staerk et al., 2024; Ziegler, 2015) and research to help preserve biodiversity on both local and global scales (Conde et al., 2011; Greenwell et al., 2023). Simultaneously, education has emerged as a foundational pillar to not only share the conservation work being done, but to foster connections between people and the natural world. Through interpretive signage, live demonstrations, school outreach, and other public engagement strategies, zoos aim to build empathy for wildlife and promote conservation-minded behavior (Fukano et al., 2021; Skibins & Powell, 2013).

A central component of these educational efforts is the use of animal ambassadors - individual animals that help translate conservation messages into meaningful, personal experiences for the public (Beer et al., 2023). An ambassador animal is typically defined as one that participates in public engagement activities by either (a) leaving its primary enclosure to be displayed to visitors during programs or events, or (b) being part of an experience that involves direct physical proximity or tactile contact with visitors, regardless of whether it leaves its enclosure (AZA Board of Directors, 2022). These ambassadors serve as living representatives of their wild counterparts and are used to cultivate empathy (Akerman, 2019), encourage pro-conservation behaviors (Neves et al., 2025), and enhance the public's connection to nature (Baird, 2018; Spooner et al., 2021).

Although ambassador animal programs are widely used in zoos their effectiveness at reaching learning outcomes is not well understood (Godinez & Fernandez, 2019), as existing studies offer mixed results. Some evidence suggests that ambassador animals can enhance audience understanding of ecological concepts (McNally et al., 2025; Whitehouse-Tedd et al., 2022), strengthen emotional connections to animals (Rank et al., 2021), and promote more positive attitudes toward wildlife (Fischer et al., 2024; Jerger et al., 2022; Learmonth et al., 2021; Miller et al., 2013). However, other research indicates that these effects may be short-lived (Neves et al., 2025) or less substantial than previously assumed (Clifford-Clarke et al., 2021; Kirsch et al., 2024; Spooner et al., 2021). At the same time, concerns have been raised about whether potential educational benefits justify exposing animals to potentially stressful conditions inherent in ambassador roles (D'Cruze et al., 2019; Learmonth, 2020).

Research on ambassador animal welfare has lagged behind studies on exhibit animals, despite growing recognition of the need for evidence-based husbandry to promote wellbeing (Binding et al., 2020; Melfi, 2009; Ward et al., 2018; Whitham & Wielebnowski, 2013). Of the few studies conducted, most have focused on mammals, with mixed findings: some report elevated stress hormones and negative behaviors (Anderson et al., 2002; Baird et al., 2016), while others observe no significant stress-related changes (Farrand et al., 2014; Kozlowski et al., 2021; Majchrzak et al., 2015; Miller et al., 2011; Powell et al., 2020). Research on birds, invertebrates, and reptiles is even more limited, despite reptiles being the most frequently used ambassador animals in zoos and aquariums (Ambassador Animal Scientific Advisory Group, 2020; D'Cruze et al., 2019; Lewbart & Zachariah, 2023).

According to a 2020 survey by the Association of Zoos and Aquariums Ambassador Animal Scientific Advisory Group (AASAG), reptiles accounted for approximately 1,661 ambassador animals, with snakes comprising 727 individuals across 109 species. Ball pythons (*Python regius*) were the most represented species (~ 95 individuals). Although they are popular, reptiles - and snakes in particular - remain underrepresented in welfare research. Their subtle behaviors, limited behavioral repertoires, and physiological differences from mammals present challenges for welfare assessment (Benn et al., 2019; Warwick et al., 2013; Whittaker et al., 2021). Only one study to date has examined welfare outcomes in ambassador snakes, finding minimal changes in fecal glucocorticoid levels but a reduction in time spent resting in open or visible areas by ball pythons (Doyon-Degroote, 2023), leaving it unclear whether these shifts reflect stress or habituation. This lack of research is concerning given the widespread use of snakes in ambassador roles and the potential welfare impacts of frequent handling, transport, and exposure to novel stimuli. Research is needed to inform evidence-based practices and ensure high standards of welfare for this understudied, but highly essential group of species.

Our study aims to contribute to the sparse but growing body of research on the welfare impacts of ambassador programming on snakes. We first examine how participation in programs influences types of behaviors they display during programs. We then assess how program participation influences their behavioral profiles upon returning to their habitats, including how long it takes for them to return to baseline levels of exposure (likelihood of the snake spending time visible or out in the open). We expect minimal negative behaviors during programs, potentially due to habituation or familiarity with handling. We hypothesize that snakes will be more active and demonstrate a higher frequency of undesirable behaviors following program participation. Additionally, we predict a gradual decline in exposure over time after returning from the program. This research will contribute to our understanding of best practices for monitoring the well-being of an understudied taxonomic group, which will empower animal care staff to make informed, evidence-based decisions regarding the husbandry of snakes. Ensuring the welfare of frequently used ambassador snakes supports ethical conservation education and reinforces institutional commitments to high animal care standards.

2. METHODS

Methodology and animal use were reviewed and approved by the North Carolina Zoo's Research Review Committee.

2.1 Study Subjects and Program Details

This study was conducted from April to July 2024. Study subjects included six snakes housed in the Ambassador Animal Department at the North Carolina Zoo (Table 1). Snakes were housed individually in habitats of varying sizes and substrate compositions, located within a temperature-controlled room, not accessible to the public. The snakes participated in both on-site (n=110) education programs at the North Carolina Zoo and off-site (n=24) programming during the research period. In order to reduce variability, only on-site programs were included in this study. Snakes were offered prey anywhere from every two days to once every other week (per species-specific norms). They were not used for programming within 36-48 hours after feeding to allow for proper digestion, or during shedding (from the onset of pre-shed through completion) to minimize discomfort. Events such as veterinary procedures, shedding, and enclosure changes were also noted as they occurred. In this study, snakes could be used for programming up to three times per day, five days per week as long as substantial breaks occurred between sessions and their total program time did not exceed one hour per day. However, this occurred rarely - snakes were used more than once in a day only five times during the study period.

All snakes were transported a short distance by hand in a carrier to one of two pre-determined locations at the North Carolina Zoo, and programs took place in accordance with established zoo and department protocols. Location A (n = 61) was a high-traffic area near the zoo entrance, along the path to an underwater exhibit viewing area. Crowd sizes fluctuated greatly (~20 - 275+), sometimes varying even within a single program. Location B ($n = 49$) was a gated but publicly accessible space designed for young children and families, where crowd sizes remained consistently small (~12 - 50). When on program, snakes were handled by a trained specialist, and the presentation method was consistent across all trials. Zoo staff handling the snake determined if the public could have a touch interaction with the snake.

Table 1. Summary of the individual history and characteristics of the six ambassador snakes observed during the study.

Species	Age (Years)	Sex	Years at NC Zoo
Ball Python *(Python regius)*	34	1.0	Since 2001 Ambassador since 2010
Eastern Indigo Snake (*Drymarchon couperi*)	5	0.1	Born at NC Zoo in 2019 Ambassador since 2020
Eastern Rat Snake (*Pantherophis alleghaniensis*)	10	1.1	Since 2015 Ambassador since 2015
Macklots Python (*Liasis mackloti)*	23	1.0	Since 2012 Ambassador since 2012
Northern Pine Snake (*Pituophis melanoleucus)*	12	1.0	Since 2015 Ambassador since 2015

2.2 Behavioral Data Collection

2.2.1 On-program Behavioral Observations

We exclusively observed on-site programs at the North Carolina Zoo between April 18 and July 10, 2024, during which one morning and one afternoon program were observed each Thursday through Sunday. Programs were video recorded (Apple Inc., 2024) and data were subsequently analyzed using ZooMonitor (Lincoln Park Zoo, 2022) and an ethogram to conduct continuous sampling of all behaviors exhibited by the snakes during the programs (Augustine et al., 2022; Table 2). Variables, including weather, crowd size, and presenter comfort, influenced program length. Estimated crowd count and ambient temperature (AccuWeather, Inc., 2024) were recorded at the conclusion of the program.

Table 2. Ethogram to be used for continuous on-program behavioral observation (adapted from (Hamilton et al., 2022; Spain et al., 2020; Warwick et al., 2013; Whittaker et al., 2021; and notes from animal care team).

Behavior	Definition	Behavior Category
Resting	The snake is stationary in a relaxed (not clenched) body posture while being held in hand or on the chat tree	Positive-inactive
Stationary	Neither head nor body moving but snake is not resting (i.e. appeared to be attentive to surroundings)	Positive-active
Locomotion	Partial or whole-body movement that causes a change in location via undulating. Can be motion in hands, or on the chat tree	Positive-active
Investigate	Exploratory movement of the head and neck toward nearby objects or surroundings, making contact or coming close to contact	Positive-active
Clutching	The snake grips the handler or presentation object intensely	Negative
Hyperactivity	The snake displays abnormally high levels of physical activity for the species	Negative
Hissing	Vocalization resulting from air passing through the glottis and vibrating a small structure inside ** Pine snakes are known to vocalize - not as a defensive mechanism.	Negative
Pre-Strike	Body is tensely posed in an "S" shape	Negative
Striking	The snake performs high-velocity, aggressive lunges toward an individual or object	Negative
Tail Wagging	The snake vibrates its tail rapidly, moving it back and forth on the same plane	Negative
Hiding head under body	The snake deliberately conceals its head under objects, the handler, or its own body	Negative
Head flattening	The head is flattened and widened, appearing more broad and triangular in shape	Negative
Cloacal evacuations when handled	The snake excretes a malodorous or fecal substance from the cloaca	Negative
Other	Any behavior not previously described	N/A

2.2.2 In-habitat Video Observations

Cameras (Wyze Cam v3 1080P Wired Indoor/Outdoor Home Security Camera, 2025) were attached in locations that provided an unobstructed view of the habitat. These cameras recorded 24-hour continuous footage from April 18, 2024 - July 10, 2024 using both day and night vision (n = 12,096 hours).

We employed interval scan sampling at the 00:00 and 00:30 marks of each hour, from 06:00 to 17:59, and recorded the behavior and exposure of snakes within their enclosures (Table 3; Altmann, 1974; Hamilton et al., 2022). For analysis, behaviors were grouped into 3 categories: active, inactive, and interaction with a transparent boundary (ITB; Table 3). ITB was categorized separately because, although it involves movement, is an established behavioral indicator of negative welfare and was a behavior of interest in this study (Warwick et al., 2013; Whittaker et al., 2021). The total number of observation days varied by individual snake due to technical difficulties and natural shed cycles (Table 4). Only observations where the snakes were clearly visible to the observers or their location could be determined through additional video review were included, in order to avoid making assumptions about their location or behavior. This filtering retained 94% of samples for the in-habitat exposure analysis and 32% for the in-habitat behavior analysis, as specific behaviors could not be reliably coded when snakes were partially obscured or only their location was discernible. Two observers confirmed inter-observer reliability (IOR) using a series of 10 selected observation days, resulting in an IOR rate of 93.3% (Caro et al., 1979).

Table 3. Ethogram used for in-habitat behavioral and exposure identification (Hamilton et al., 2022; Spain et al., 2020; Warwick et al., 2013; Whittaker et al., 2021)**.**

Activity	Category	Description
Behavior		
Locomotion	Active	Partial or whole-body movement that causes a change in location via undulating
Resting	Inactive	The snake is stationary in a relaxed (not clenched) body posture
Stationary	Active	Neither head nor body moving but snake is not resting (i.e. appears to be attentive to surroundings, head is in an upright posture)
Digging	Active	The snake is using its snout to push aside the substrate
Investigation	Active	Exploratory movement of the head and neck toward nearby objects or surroundings, making contact or coming close to contact
Interacting with transparent boundaries (ITB)	ITB	The snake is demonstrating repetitive movement directed along glass boundaries of the enclosure; must complete the same circuit at least three times to count as ITB
Not visible	N/A	The snakes behavior being displayed is not visible or is unclear
Out of enclosure	N/A	The snake is determined to be out of the enclosure during the observation period
Other	N/A	Any behavior not previously described
Exposure		
Hiding	N/A	At least 50% of the snake’s body is hidden from view beneath substrate or enclosure furnishings
Exposed	N/A	At least 50% of the snake’s body is visible and not covered by substrate or furnishings
Not visible	N/A	The snake is positioned in a manner where observers cannot determine exposure
Out of enclosure	N/A	The snake is determined to be out of the enclosure during the observation period

Table 4. Total number of in-habitat observation days per individual snake.

Snake	Number of Days
Ball Python (*Python regius*)	67
Eastern Rat Snake (*Pantherophis alleghaniensis*)	66
Eastern Rat Snake (*Pantherophis alleghaniensis*)	64
Eastern Indigo Snake (*Drymarchon couperi*)	41
Northern Pine Snake (*Pituophis melanoleucus*)	69
Macklots Python (*Liasis mackloti*)	62

2.3 Statistical Analysis

All analyses were conducted using the statistical software R (v 4.4.1; R Core Team 2024) and all plots were made using *ggplot2* (Wickham & Chang, 2016).

2.3.1 On-program Behavior Statistical Analysis

Behaviors identified individually through continuous sampling were grouped into three categories - positive active, positive inactive, and negative - for analysis (Table 2). Descriptive statistics and probabilities were used to identify potential trends in on-program behaviors. For each snake, the proportion of time spent in each behavioral category during programs was calculated by summing the total duration of that behavior across all observed programs and dividing it by the total time the snake spent on programs (Hamilton et al., 2022). Additionally, to explore potential influences on behavior, we examined descriptive statistics for each behavioral state across contextual variables, including program location, temperature, handler identity, and presentation method. These summaries included means, standard deviations, and ranges within each category, providing a basis for interpreting patterns in behavior.

2.3.2. In-habitat Statistical Analysis - Exposure

Generalized linear mixed models (GLMMs) (lme4 package, v1.1-26; Bates et al., 2015) were constructed to test the impact of program participation on a snake's exposure in the off-exhibit habitat. We grouped all observations gathered before a program or on a non-program day into a “No program” phase, and all observations following a program (through the end of the day) into a “Post program” phase. If a snake participated in multiple programs in a single day, the relative post-program timeline was re-set after each program. Preliminary analyses indicated that time of day did not significantly influence behavior, supporting this phase-based grouping approach. The program phase was included as a fixed effect (no program, n=6,633; post program, n=985) with exposure (hiding, n=5549; exposed, n=2069) as a binary outcome variable. Snake identity (n=6) was included as a random effect due to repeated observations from each snake.

To broaden our understanding of exposure likelihood and consider time as a confounding variable in interpreting exposure, we also explored the effect of time of day and time post program on snake exposure. Across all "No program" observations, which served as a control (n=6633), a GLMM was constructed with exposure as the outcome variable (hiding, n=5110; exposed, n=1523) and time of day as a fixed effect, measured in 30-minute intervals between 06:00 and 17:59 (n = 24/day). Snake ID was included as a random effect.

The same model structure was used to assess exposure for "Post program" observations (hiding, n=409; exposed, n=523).

To estimate the probability of exposure on "No program" days, we used the model coefficients to calculate predicted probabilities for each observation. We then averaged these values to establish a baseline exposure probability for non-program days. This baseline served as a reference point to compare changes in exposure probability on "Post program" days. We reported 95% confidence intervals for all estimates.

2.3.3 In-habitat Statistical Analysis - Behavior

We then tested the effects of programming on snake behavior. To do this, the mlogit package (v1.1-1; Croissant, 2020) was used to construct a multinomial logistic regression, where the outcome variable, behavior, had three levels: active (n=1215), inactive (n=1215), and ITB n=221). Program phase was included as a fixed term (no program, n = 2042; post program, n = 609) and snake identity was included as a random effect. This dataset only included observations where snakes were inside their enclosures and visible (n =2,651). The model was initially run twice, once using *active* and once using *inactive* as the reference level. Because results were consistent across both models, we report only the model where *inactive* was the reference category for clarity.

3. RESULTS

3.1 On-program Behavioral Analysis

A total of 110 programs were observed during this study. Program duration ranged from ~ 8 to ~ 37 minutes (min = 8.45, max = 36.4, mean = 20.4, sd = 5.25; Table 5). In the programs we observed, indicators of stress were minimal. Overwhelmingly, snakes exhibited behavior that indicated positive welfare while on programs (Figure 1). The snakes spent an average of 97.92% of time on programs in positive behavioral states (min = 95.94%, max = 99.85%, SD = 1.68; Table 6). Negative behaviors were observed for only 12.6 minutes out of 38 hours across 110 programs. When they were present, negative welfare indicating behaviors made up, on average, 2.07% of the time on a program (min = 0.15%, max = 4.06%, SD = 1.69; Table 6). The most frequently observed negative behaviors included hissing, clutching, and hyperactivity. The Ball Python and the female Eastern Rat Snake had the fewest instances of negative behavior (accounting for ~ 0.14 - 0.15 % of time on a program; Table 6). The Eastern Indigo Snake, Northern Pine Snake, and male Eastern Rat Snake had the most instances of negative welfare, which accounted for, on average, ~ 3.2 - 4 % of time spent on a program (Table 6). The infrequency of negative behaviors precluded meaningful analysis of other recorded predictors, such as location, temperature, handler identity, and presentation method (Table 7).

Table 5. Summary of program participation and duration for each ambassador snake. Number of programs, minimum, maximum, average, and standard deviation of program duration (minutes) are shown for each individual.

Snake	**Number of Programs**	**Minimum Program Duration**	**Maximum Program Duration**	**Average Program Duration**	**Standard Deviation Program Duration**
Eastern Indigo Snake (*Drymarchon couperi*)	14	13.12	27.57	18.22	4.58
Macklots Python (*Liasis mackloti)*	16	13.30	24.02	18.34	3.58
Northern Pine Snake (*Pituophis melanoleucus)*	14	13.05	26.70	18.90	3.62
Ball Python *(Python regius)*	28	14.67	36.58	23.61	5.89
Eastern Rat Snake (*Pantherophis alleghaniensis*)	21	15.90	26.30	20.32	3.05
Eastern Rat Snake (*Pantherophis alleghaniensis*)	17	8.65	32.07	23.22	5.80

Figure 1. Average percentage of program time snakes spent in positive and negative behavioral states. Snakes spent an average of 97.9% of program time in positive states (range: 95.9–99.9%), with negative behaviors occurring rarely (average: 2.1%, range: 0.15–4.1%) across 110 programs (38 hours total).

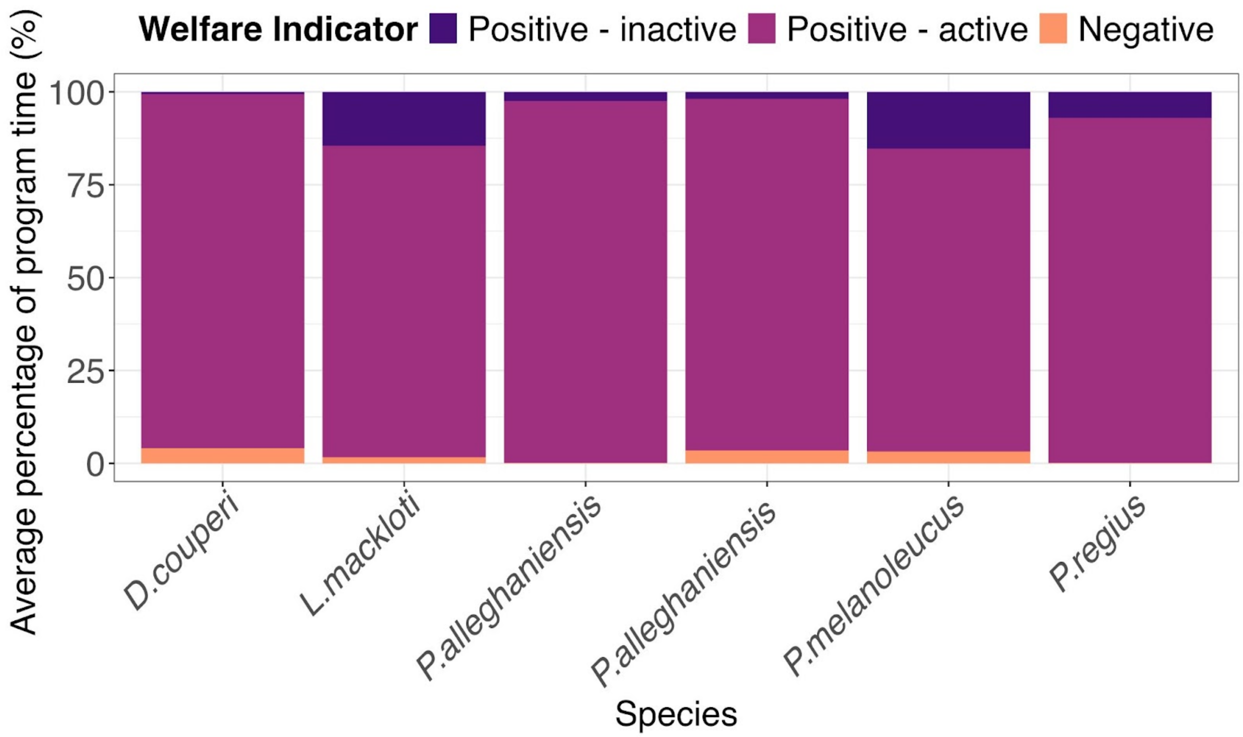


Table 6. Average percentage of program time snakes spent in each behavioral category. Percentages represent the mean proportion of time spent in negative indicators, positive-active behaviors, positive-inactive behaviors, and other behaviors during programs for each individual snake.

Snake	**Average Negative Indicator (%)**	**Average Positive-Active Behavior (%)**	**Average Positive - Inactive Behavior (%)**	**Average Other Behavior (%)**	**Total %**
Eastern Indigo Snake (*Drymarchon couperi*)	4.06	95.35	0.59	0	100
Macklots Python (*Liasis mackloti)*	1.62	83.89	14.49	0	100
Northern Pine Snake (*Pituophis melanoleucus)*	3.20	81.50	15.30	0	100
Ball Python *(Python regius)*	0.15	92.88	6.95	0.02	100
Eastern Rat Snake (*Pantherophis alleghaniensis*)	0.15	97.28	2.56	0	100
Eastern Rat Snake (*Pantherophis alleghaniensis*)	3.27	94.95	1.78	0	100

Table 7. Presentation variables recorded during public presentations.

Presentation Variable	Eastern Indigo Snake (*Drymarchon couperi*)	Macklots Python (*Liasis mackloti*)	Northern Pine Snake (*Pituophis melanoleucus*)	Ball Python *(Python regius)*	Eastern Rat Snake (*Pantherophis alleghaniensis*)	Eastern Rat Snake (*Pantherophis alleghaniensis*)
Presentation Location						
Location A	11	7	9	14	12	8
Location B	3	9	5	14	10	8
Care Staff						
Primary Animal Care Staff	13	8	8	8	8	8
Not Primary Animal Care Staff	1	8	6	20	12	8
Public Tactile Interaction Allowed						
Yes - Touch	4	8	5	16	10	8
No - No Touch	10	8	9	12	12	8
Presentation Style						
On Hand	14	5	14	25	14	9
Chat Tree	0	11	0	3	7	7
Temperature						
Mean	77.25	80.60	81.50	78.41	78.94	79.64
Min	70	69	72	65	64	72
Max	84	89	89	86	89	88

3.2 In-habitat Behavioral Analysis

A total of 8,064 individual observations were made between 06:00 and 17:59, between the months of April to July (excluding observations where the snake was not visible/out of enclosure).

3.2.1 In-habitat Analysis - Exposure

During the "No program" phase, snakes had a 21.4% predicted probability of being exposed (Figure 2; 95% CI: 0.16–0.28), with an observed exposure rate of 27% (Figure 3; OR = 0.28, 95% CI: 0.20–0.40, $p < 0.001$). In contrast, during the "Post program" phase, the predicted probability of exposure increased to 52.9% (Figure 2; 95% CI: 0.44–0.62), and snakes were over four times more likely to be exposed compared to the "No program" phase (Figure 3; OR = 4.08, 95% CI: 3.53–4.70, $p < 0.001$).

We report that exposure significantly decreases over time following a program (Figure 4). Within 30 minutes of returning from a program, snakes were predicted to be exposed in their habitats 69% of the time (estimate = 0.80, $p = 0.067$). The predicted probability of exposure steadily decreased with time following a program. Several time points post-program were associated with significantly reduced odds of exposure compared to the immediate post-program period, including hour 3.5 (estimate = -0.93 $p < 0.01$), hour 4 (estimate = -1, $p < 0.01$), hour 4.5 (estimate = -1.26, $p < 0.001$), and hour 6 (estimate = -1.13, $p < 0.05$), indicating a decline in exposure over time. The calculated average baseline probability of exposure days with no programs was 22.33%.

Figure 2. Predicted probability of snake exposure by program phase.

Predicted probabilities (±95% CI) were estimated from a GLMM with program phase as a fixed effect and snake ID as a random intercept. The predicted probability of exposure was 21.4% (CI: 0.16–0.28) in the "No program" phase and 52.9% (CI: 0.44–0.62) in the "Post program" phase.

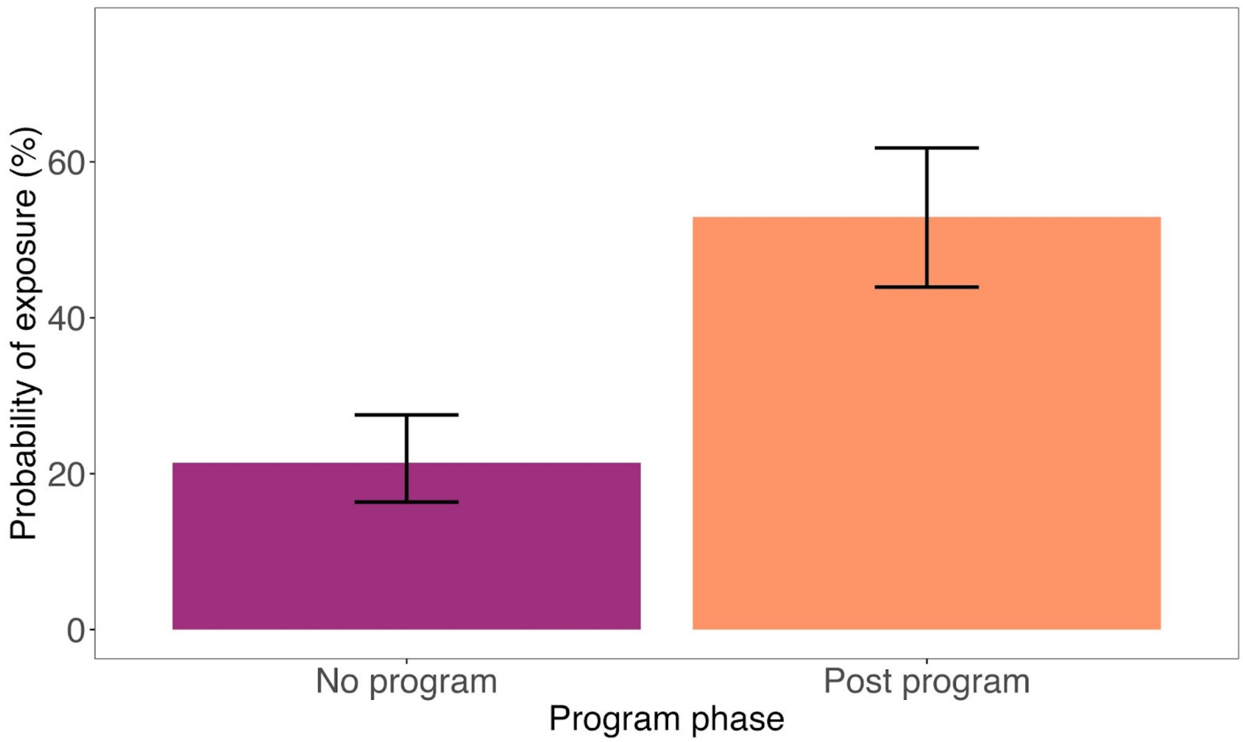


Figure 3. Odds ratios of snake exposure by program phase.

Odds ratios (±95% CI) estimated from a GLMM with program phase as a fixed effect and snake ID as a random intercept. Snakes were significantly more likely to be exposed during the "Post program" phase (OR = 4.08, 95% CI: 3.53–4.70, $p < 0.001$) compared to the "No program" phase (OR = 0.28, 95% CI: 0.20–0.40, $p < 0.001$). The horizontal dotted line at OR = 1 represents no difference in odds.

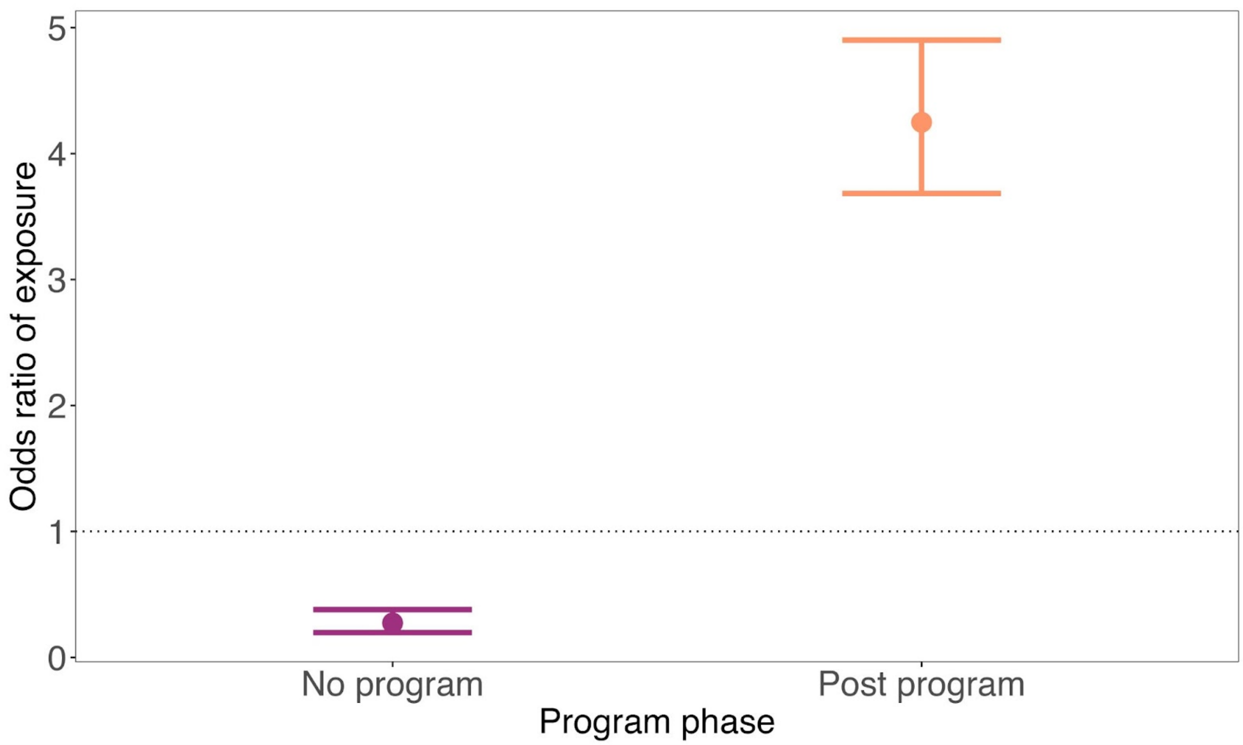
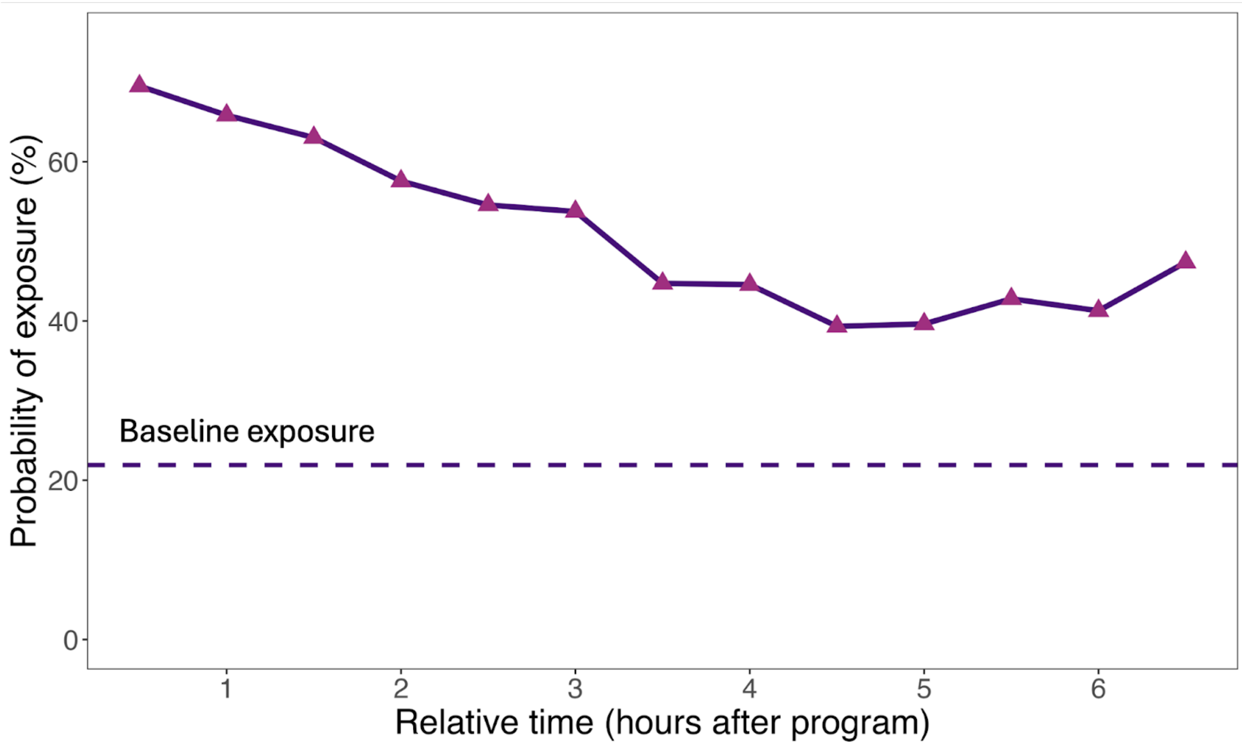


Figure 4. Predicted probability of exposure over time following program participation. Predicted probabilities (±95% CI) were estimated from a GLMM with relative time as a fixed effect and snake ID as a random intercept. Exposure was highest within 30 minutes post-program (69%; estimate = 0.80, $p = 0.067$) and decreased significantly at multiple later time points (e.g., hour 3: $p < 0.05$; hour 4.5: $p < 0.001$) but did not reach baseline before our observations ended. Dashed line shows baseline exposure probability on non-program days (22.33%).

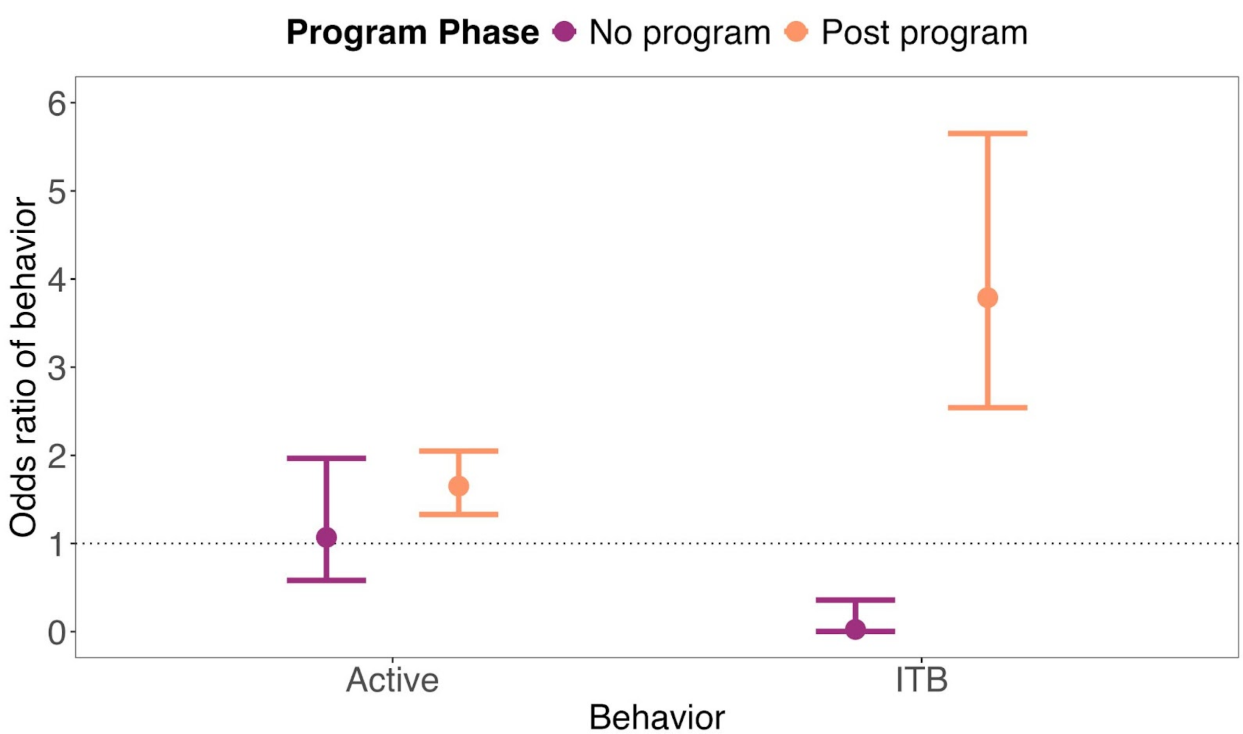


3.2.2 In Habitat Analysis – Behavior

We found that during the non-program phase, snakes were significantly less likely to exhibit ITB behavior compared to inactive behavior (OR = 0.03, CI: 0.002-0.36, estimate = - 3.69, $p < 0.01$; Figure 5). In contrast, after returning from a program, snakes were 3.8 times more likely to exhibit ITB compared to being inactive (OR = 3.81, CI: 2.56-5.68, estimate = 1.34, $p < 0.001$; Figure 5). Active behavior was also significantly more likely (1.6 times) to occur than inactivity in the post-program phase (OR = 1.64, CI: 1.33–2.04, estimate = 0.5, $p < 0.001$; Figure 5).

Figure 5. Odds ratios of active and ITB behaviors by program phase.

Odds ratios (±95% CI) from a multinomial GLMM comparing each behavior to inactivity. Snakes were less likely to exhibit ITB during the no program phase (OR = 0.03, $p < 0.01$) but more likely to exhibit ITB (OR = 3.81, $p < 0.001$) and active behavior (OR = 1.64, $p < 0.001$) post-program. The horizontal dotted line at OR = 1 represents no difference in odds.



4. DISCUSSION

Our findings suggest that ambassador program participation influences snake behavior in measurable ways. Outside of, or before programming, the snakes were more likely to be hiding or inactive. During a program, snakes exhibited primarily positive welfare-indicating behaviors. However, following programs, we report increased exposure, activity levels, and undesired behaviors. While snakes may not exhibit negative welfare indicators during programs, the programs caused significant changes in behavior immediately upon return to their habitats. These shifts could reflect residual arousal, behavioral inertia, or stress as they raise considerations for welfare monitoring and management.

During ambassador programs, snakes spent 95–99% of the time exhibiting positive welfare states. Negative behaviors, including hissing, clutching, hyperactivity, and cloacal evacuation, were rare and totaled only 12.8 minutes across all observations. This low frequency of negative behaviors is unsurprising, as these snakes were accustomed to handling, either as former pets or through early habituation in human care. Early-life handling has been shown to reduce behavioral and physiological stress responses in adulthood across species, including mice (Anisman et al., 1998), rabbits (Pongrácz & Altbäcker, 1999), and trinket snakes (*Coleognathus helena*; Mehta, 2009) - highlighting how early experiences shape responses to novel or stressful situations (Hogan et al., 2011; Richardson et al., 2011). Accordingly, the steady positive welfare indicators observed during programs may reflect the snakes' early-life exposure to handling, contributing to their tolerance, and potentially their success, in ambassador roles.

In the absence of programming, we found that snakes typically remain hidden and inactive, consistent with reported patterns of inactivity and hiding in captive snakes (Carter et al., 2021; Hamilton et al., 2022; Huo et al., 2025). Hamilton et al., (2022) observed over 97%

inactivity in Catalina Island rattlesnakes, and Carter et al., (2021) found similar patterns of inactivity and hiding in other species during the COVID-19 pandemic. Augustine et al., (2022) noted that regularly handled snakes were exposed over 90% of the time but remained less active. These tendencies are likely influenced by the absence of ecological drivers like foraging or thermoregulation in captivity (Kelly et al., 2025). The increase in post-program visibility may reflect snakes being placed in open areas instead of hides, which could prolong their exposure. While we might expect a stressed snake to seek shelter (Bonnet et al., 2012; Claunch et al., 2023) this was infrequently observed. Our findings contradict the results of the only other study on ambassador snake welfare where Doyon-Degroote (2023) found that Ball Pythons exhibited reduced visibility and increased use of hides post-program. This suggests that context, species differences, and husbandry practices influence the interpretation of increased visibility as a welfare indicator.

In contrast to their behavior in the "No program" phase, or during programs, snakes exhibited clear behavioral shifts after program participation. They were significantly more likely to be visible, active, and to display ITB. For example, exposure probability increased from 21.4% to 52.9%, and snakes were 1.6 times more likely to be active and 3.81 times more likely to perform ITB compared to baseline. This increase in activity likely reflects residual arousal or behavioral inertia, as seen in reptiles exposed to novel stimuli or frequent handling (Augustine et al., 2022; Burghardt, 2013; Lynch et al., 2025; Warwick et al., 2013). Huo et al., (2025) reported heightened activity following brief handling sessions, while Doyon-Degroote (2023) noted increased exploratory behaviors and ITB in ambassador ball pythons post-program. However, while increased activity may be a positive welfare indicator (Brereton & Fernandez, 2022; Miller et al., 2020; Spain et al., 2020), it is less established in reptiles, and the rise in ITB raises concerns about whether this reflects positive engagement or frustration.

ITB has been associated with escape attempts and stereotypic-like behaviors in reptiles (Benn et al., 2019; Rose et al., 2017; Warwick et al., 2013), with perceptual challenges around transparent barriers contributing to these behaviors (Warwick et al. 2013). Additionally, many of the genera in our study - *Pantherophis, Drymarchon, and Pituophis* - are known for covering large distances in free ranging environments (*Pantherophis obsoletus,* (Howze et al., 2019); *Drymarchon couperi*, (Breininger et al., 2011); and *Pituophis melanoleucus,* (Gerald et al., 2006). Program handling may activate these species' locomotive drives, which cannot be satisfied within enclosure limits, leading to redirected behaviors such as ITB when placed back in a confined space. While larger enclosures allow more natural movement (Hoehfurtner et al., 2021) the inability to engage in large-scale locomotive patterns post-program may cause frustration. Despite the increased ITB, which typically resolved within hours, this suggests a transient, not chronic, welfare concern.

We found that the behavioral effects of ambassador programming were short-lived, with snakes gradually returning to baseline over time. After programming, snakes initially became more visible, but exposure declined over the next six hours, suggesting a slow return to their typical hiding behavior rather than a rapid transition and immediate retreat into shelter. This pattern of gradual decrease in visibility aligns with previous studies on reptiles, such as Greenberg, (1976) who found that Blue Spiny Lizards (*Sceloporus cyanogenys*) showed delayed shelter-seeking responses. While the decline in exposure was observed as an average across all snakes, individual patterns varied - some snakes returned to baseline more quickly than others. This variability highlights the importance of considering individual differences in behavioral responses. Overall, while ambassador programming temporarily increases exposure, snakes ultimately begin to resume their natural sheltering tendencies, indicating a capacity to adjust and return to baseline following temporary disruption.

Cumulatively, our results suggest that ambassador programs have relatively minor short-term effects on snake welfare, with most individuals tolerating or adapting well to programming. Certain variables and contexts show nuanced effects, but the variability highlights the importance of considering individual differences when choosing and using program animals, as Martin et al. (2024) recommends. Anecdotally, we observed that snakes sometimes exhibited stress-related behaviors when being retrieved for or returned from programs - likely due to abrupt transitions. These sensitive moments warrant further study. Incorporating choice-based training and continued staff education in interpreting snake behavior may help reduce transitional stress and support positive welfare outcomes.

We acknowledge several limitations in this study. First, the small sample size and inclusion of multiple species may have affected the results. Individual snakes were included as random effects to account for variability; however, species- and individual-level differences were not sufficiently consistent or robust to warrant separate analyses. Despite this limitation, the taxonomic and life history diversity represented reflects typical ambassador collections, supporting the generalizability of the findings. Second, behavioral data were recorded only when snakes were clearly visible to ensure observational accuracy. While this was not a major limitation for exposure analyses (94% of samples retained), it reduced the sample size for behavioral observations, as only 32% of samples met visibility criteria. However, many of the excluded observations likely reflected resting behavior, as snakes tend to remain hidden when inactive (Carter et al., 2021; Hamilton et al., 2022; Huo et al., 2025). This approach minimized assumptions about behavior when snakes were obscured and their exclusion likely had minimal impact on the interpretation of activity-related patterns. Third, observations were confined to 06:00–17:59, potentially omitting crepuscular and nocturnal activity. Although this restricted timeframe limits behavioral inference outside daylight hours, it corresponds to operational periods when ambassador animals are most frequently handled and observed. Finally, the study did not incorporate physiological measures. Although physiological indicators can complement behavioral assessments, such measures are not always feasible in routine zoological settings. This study prioritized behavioral monitoring as a practical approach to welfare assessment, consistent with previous validations in reptile species (Doyon-Degroote, 2023; Hamilton et al., 2022; Hollandt et al., 2021; Krishnan et al., 2022; Watters et al., 2009). Future research incorporating both behavioral diversity and physiological metrics may provide a more comprehensive evaluation of welfare (Miller et al., 2020). For understudied taxa such as snakes, systematic behavioral documentation remains a critical foundation for advancing welfare science, particularly for individuals in ambassador roles.

Based on our findings, we recommend several management strategies to support improvement of ambassador snake welfare. Providing enrichment after programs may help channel increased arousal levels and facilitate a smoother return to baseline behavior. Prior studies have demonstrated the benefits of environmental and physical enrichment for captive snakes (Almli & Burghardt, 2006; Hoehfurtner et al., 2021; Nagabaskaran et al., 2022, 2025), including reductions in ITB (Krishnan et al., 2022). We encourage care teams to implement novel enrichment post-program to redirect arousal into species-typical behaviors. Offering food after programs may also support welfare, provided this aligns with species-specific metabolic needs. Scheduling feeding directly after participation could reinforce program involvement and simulate natural energy expenditure associated with foraging.

We further recommend 24-hour behavioral monitoring, particularly for nocturnal species, to capture a more comprehensive picture of post-program recovery (Gandia, 2023). Particularly for nocturnal species like ball pythons (Vosjoli, 2004), this could provide a clearer picture of when snakes fully return to baseline and better inform management practices. Integrating physiological measures (e.g., fecal glucocorticoid metabolites) and longitudinal behavioral data may help identify welfare concerns that are not apparent through behavior alone, such as chronic stress or learned helplessness (Baird et al., 2016; Doyon-Degroote, 2023).

Finally, we advocate for incorporating choice- and control-based management practices to enhance welfare, an approach increasingly adopted across zoological settings (Brando & Norman, 2023; Martin et al., 2024; Rose & Lewton, 2024; Rust et al., 2024). Although reptiles were once considered untrainable (Szabo et al., 2021), recent studies have demonstrated successful training in snakes and other reptiles (Fleming & Surski, 2013; Plass, 2024; Sinclair, 2023; Williams et al., 2022). Expanding training opportunities for snakes may enhance agency, reduce stress, and better support their roles as ambassador animals

Future research should build on these findings by addressing several priority areas for ambassador snake welfare. First, seasonal variation in educational programming should be considered alongside snakes' natural physiological cycles. While many zoos offer educational programs year-round, peak demand typically occurs during the spring and summer months (Aylen et al., 2014; Perkins, 2021; Su & Lin, 2018). In contrast, many snake species undergo brumation during the cooler fall and winter months (Cloudsley-Thompson, 1999; Gangloff & Greenberg, 2023; Holden et al., 2021). Comparing activity budgets influenced by ambassador programs to the typical seasonal fluctuations observed in brumating snakes may help determine whether program participation disrupts or aligns with natural patterns, informing optimal scheduling to support welfare. Second, research should evaluate the feasibility of personality assessments to identify individuals best suited for ambassador roles. Studies have shown that snakes exhibit individual differences in boldness (Mayer et al., 2016) and personality traits (Gibert et al., 2022; Waters et al., 2017). Early-life assessments may facilitate intentional selection and improve program outcomes. Future studies should also investigate how presentation variables - including handling duration, environmental context, audience size, and transport distance - affect welfare indicators such as behavior and glucocorticoid levels.

As zoos continue to prioritize conservation and education, ambassador animals will remain integral to these efforts. Ensuring ethical use requires advancing our understanding of best practices in programming, management, and care. This need is particularly urgent for snakes, a taxon widely used in ambassador programs yet underrepresented in welfare research. Addressing their behavioral, physiological, and psychological needs will improve program outcomes and elevate welfare standards. These findings underscore the importance of flexible, evidence-based management that balances educational goals with individual animal welfare.

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