

RESEARCH ARTICLE

Environmental, individual and social traits of free-ranging raccoons influence performance in cognitive testing

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ABSTRACT

Cognitive abilities, such as learning and flexibility, are hypothesized to aid behavioral adaptation to urbanization. Although growing evidence suggests that cognition may indeed facilitate persistence in urban environments, we currently lack knowledge of the cognitive abilities of many urban taxa. Recent methodological advances, including radio frequency identification (RFID), have extended automated cognitive testing into the field but have yet to be applied to a diversity of taxa. Here, we used an RFID-enabled operant conditioning device to assess the habituation, learning and cognitive flexibility of a wild population of raccoons (*Procyon lotor*). We examined how several biological and behavioral traits influenced participation and performance in testing. We then compared the cognitive performance of wild raccoons tested in natural conditions with that of wild-caught raccoons tested in captivity from a previous study. In natural conditions, juvenile raccoons were more likely to habituate to the testing device, but performed worse in serial reversal learning, compared with adults. We also found that docile raccoons were more likely to learn how to operate the device in natural conditions, which suggests a relationship between emotional reactivity and cognitive ability in raccoons. Although raccoons in both captive and natural conditions demonstrated rapid associative learning and flexibility, raccoons in captive conditions generally performed better, likely owing to the heightened vigilance and social interference experienced by raccoons in natural conditions. Our results have important implications for future research on urban carnivores and cognition in field settings, as well as our understanding of behavioral adaptation to urbanization and coexistence with urban wildlife.

KEY WORDS: Carnivore, Cognition, Flexibility, Learning, RFID, Urban

INTRODUCTION

Cognition, the process by which organisms acquire, process, store and act on information from the environment, is central to an

organism's ability to overcome social and ecological challenges (Shettleworth, 2010). Cognition is hypothesized to be especially important for organisms living in novel, complex and moderately predictable environments, where they must quickly process and respond appropriately to various environmental stimuli and conditions (Mettke-Hofmann, 2014). For these reasons, cognition has been proposed as an important mechanism for the persistence of wildlife in urban environments (Sol et al., 2013). Cities are characterized by tremendous heterogeneity across spatial and temporal scales, presenting organisms with novel and diverse stimuli and conditions (Griffin et al., 2017; Lambert et al., 2021). Animals that can use cognition to capitalize on anthropogenic resources while evading threats are more likely to survive in urban environments. Yet the degree to which cognition plays a role in urban persistence remains largely undetermined and is likely related to several factors, such as a particular species' ecology, stage of urban invasion and perception/tolerance by humans (Barrett et al., 2019; Sayol et al., 2020; Wright et al., 2010). As such, we are very much still in the early stages of understanding the complex link between animal cognition and urban living (Lee and Thornton, 2021).

Several cognitive abilities have been proposed as particularly important for urban wildlife. Habituation, for example, is a non-associative form of learning that allows animals to decrease their responsiveness to harmless stimuli with increased exposure (Blumstein, 2016). Although habituation to humans may be an adaptive strategy for some urban species that have frequent, innocuous interactions with humans, it can also be problematic for species perceived as a nuisance by humans (Barrett et al., 2019; Schell et al., 2021). Another example is associative learning, which allows animals to form an association between cues, or between a particular stimulus and a response (Papini, 2002). This type of learning is highly conserved across different species, and can be adaptive when aiding animals in fitness-related tasks, such as finding food and mates or avoiding predation (Ginsburg and Jablonka, 2010). However, associative learning speed and ability have been shown to vary among individuals and species, and the causes and consequences of such variation are not fully understood (Morand-Ferron, 2017; Sih and Del Giudice, 2012). Finally, behavioral flexibility is a broad term used to describe an animal's ability to change its behavior in response to change and variation in its environment (Audet and Lefebvre, 2017; Lea et al., 2020). It is often cited as one of the most critical mechanisms for urban invasion and persistence, as it allows animals to respond appropriately to new and diverse obstacles and opportunities in a flexible manner (Sol et al., 2013). Despite our growing sense of the importance of cognitive abilities such as habituation, learning and behavioral flexibility for animals living in urban environments, direct measures of such abilities remain limited.

Our current understanding of the role of cognition in urban living is largely based on indirect metrics of cognition (e.g. relative brain

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size) and comparisons of cognition between urban and non-urban populations following predictions outlined by the cognitive buffer hypothesis (CBH). The CBH states that large brains facilitate domain-general cognitive abilities that allow animals to modulate new and flexible behaviors that enhance their survival and fitness (Allman et al., 1993; Sol, 2009). In line with this hypothesis, several studies have found correlational evidence linking large relative brain size to invasion success across several taxa (amphibians and reptiles: Amiel et al., 2011; birds: Sol et al., 2005; mammals: Sol et al., 2008), and, in some cases, the invasion of urban environments by birds (Carrete and Tella, 2011; Sayol et al., 2020). There is also some experimental evidence demonstrating that urban individuals have superior learning and problem-solving abilities compared with their non-urban counterparts (e.g. birds: Audet et al., 2016). However, results from these studies are not always in agreement and are generally limited to birds (Griffin et al., 2017; Lee and Thornton, 2021; but see Johnson-Ulrich et al., 2021; Chow et al. 2021). Given that the use of urban spaces, resources and stimuli differs greatly across species, this taxonomic bias towards birds currently limits our understanding of the relationship between cognition and urban living (Lee and Thornton, 2021).

In addition to current taxonomic limitations, the causes and consequences of individual variation in cognitive ability represents a significant gap in our knowledge (Boogert et al., 2018). Moreover, there are many individual-level traits that influence participation and performance in cognitive testing, which can complicate interpretation of results (Thornton and Lukas, 2012). For instance, studying animal cognition in the field is a challenging yet valuable approach, as animals living in natural conditions are exposed to environmental pressures and socio-ecological challenges that are difficult to replicate in captive and laboratory conditions for many species (Griebing et al., 2022). Participation of free-ranging animals is voluntary, however, and therein subject to various motivational factors, including energetic demands and hunger (Morand-Ferron et al., 2015a). In addition, certain behavioral traits, such as docility and boldness, may also increase the likelihood of participation, or even determine performance, in cognitive tasks (Boogert et al., 2018; Hare et al., 2002; Sih and Del Giudice, 2012). Because individual variation is not always considered or accounted for, especially when data collected from individuals are pooled to facilitate interpopulation comparisons such as urban versus non-urban, within-population variation in cognitive ability related to behavioral and biological traits such as age, sex or personality in urban wildlife is not well known (Lee and Thornton, 2021). Thus, directly assessing cognitive abilities of importance in a broader diversity of urban species while identifying sources of individual variation can greatly improve our understanding of how animals persist in urban environments.

Raccoons (*Procyon lotor*) are medium-sized, generalist carnivores that demonstrate incredible success in novel and urban environments yet have been understudied within the fields of animal behavior and cognition. The raccoon's native range in North America is currently expanding, and raccoons are now in many parts of Europe and Asia owing to human-mediated introductions (Hadidian et al., 2010; Timm et al., 2008), trends that are only expected to increase globally as increasing temperatures associated with climate change create more favorable conditions for highly adaptive species such as raccoons (Louppe et al., 2019). Historical records of raccoon behavior and distribution suggest that raccoon exploitation of urban areas has been longstanding, and raccoons are commonly found in high densities in North American cities today (Hadidian et al., 2010; Zeveloff, 2002). Although the cognition of

raccoons has been generally underexplored, raccoons are well known for their intelligence and innovative foraging strategies. This perception not only stems from contemporary observations of raccoon behavior, but is also reflected in notes by early naturalists (e.g. Audubon and Bachman, 1851) as well as in some North American Indigenous cosmologies (Justice, 2021; Pacini-Ketchabaw and Nxumalo, 2015). In addition, recent studies have provided new insights into raccoon brain morphology (Jacob et al., 2021; Jardim-Messeder et al., 2017) and demonstrated the rapid learning, flexibility and innovative problem-solving abilities of raccoons (Daniels et al., 2019; Stanton et al., 2017, 2021), all of which point to enhanced cognitive ability. Finally, because of their proficiency at exploiting anthropogenic resources, as well as their status as a zoonotic disease vector, raccoons are commonly involved in conflict with humans and confronted by a variety of lethal and non-lethal management strategies not experienced by some other urban species (Gehrt, 2004; Hadidian et al., 2010). Thus, investigation of raccoon cognition and behavior has the potential to uncover greater insights into the traits that facilitate persistence in novel and urban environments.

Here, we investigated the behavior and cognitive flexibility of wild, free-ranging raccoons in an urban environment using an automated testing device adapted for field use. Although there are many ways to study behavioral flexibility, flexibility in cognition is traditionally assessed using a test known as reversal learning (Mackintosh et al., 1968). In the reversal-learning paradigm, a previously learned reward association is reversed, and the ability of the animal to correctly respond to the new reward contingency by changing its behavior remains a widely accepted measure of cognitive, and thereby behavioral, flexibility (Audet and Lefebvre, 2017; Izquierdo et al., 2017). In this study, we used a spatial reversal-learning task, in which raccoons were required to discriminate between right versus left stimuli to receive an automated food reward. We assessed cognitive flexibility by quantifying the number of errors raccoons made during their reversals and identified several potential predictors of performance. Following increasingly common research methods employed in studies of free-ranging birds (e.g. Aplin et al., 2015; Bridge et al., 2019; Cauchoix et al., 2017), we used radio frequency identification (RFID) to identify individual raccoons within our study population and to collect repeated measures of each individual's performance across a series of multiple, successive reversal events (i.e. serial reversal learning). In addition to assessing cognitive flexibility, our automated testing device represents a novel, anthropogenically derived source of food. Thus, we also consider our study to be an analogue that demonstrates how wild raccoons living in an urban landscape respond to novel but potentially risky opportunities in their environment.

Our aims were to evaluate how individual traits and testing conditions affected the behavior and cognitive performance of wild raccoons within our urban study population. We did this in two ways. First, we tested how individual biological and behavioral traits predicted habituation to the automated testing device, learning to operate the device and performance in reversal learning. Although our study was generally exploratory owing to its novelty, we did expect to find individual variation in the response of raccoons to testing based on the perceived risks and rewards associated with the testing device. Specifically, we predicted increased habituation and use of the device by raccoons that were in poorer body condition (i.e. in greater nutritional deficit), younger (i.e. more naïve), bolder (i.e. more aggressive and risk prone) and more social (i.e. more likely to be influenced by the presence of conspecifics). We also

expected to find differences in the reversal-learning performance of juveniles and adults potentially stemming from differences in developmental effects (e.g. juvenile cognitive flexibility is underdeveloped in comparison with adults) or simply their general interest in the task (e.g. juveniles tend to show increased interest and thereby success) (Kayser et al., 2021). Second, we compared the performance of wild raccoons tested in natural conditions with the performance of wild raccoons previously tested in captive conditions (Stanton et al., 2021). We expected that raccoons tested in natural conditions would have to contend with interruptions and competing demands on their time and attention not experienced by raccoons tested in captive conditions (e.g. vigilance, social interactions) (Benson-Amram et al., 2013), and therefore predicted that wild raccoons tested in natural conditions would perform worse in comparison with wild raccoons tested in captivity.

MATERIALS AND METHODS

Study subjects and site

This research was conducted with wild raccoons [*Procyon lotor* (Linnaeus 1758)] living in the city of Laramie, Wyoming, USA. Located in southeastern Wyoming, Laramie sits on a high elevation prairie (2200 m) between the Laramie Range and the Medicine Bow Mountains. It encompasses approximately 29 km², is home to approximately 32,000 people, and generally experiences long, cold winters and short, mild summers (US Census Bureau, 2021; Western Regional Climate Center, 2016). We characterize this population of raccoons as urban based on the density of humans living in Laramie (approximately 1711 per square mile; US Census Bureau, 2021) as well as the presence of built structural components (e.g. houses, buildings, roads) and other anthropogenic entities (e.g. garbage, pets), albeit at a smaller scale than other major US cities (Moll et al., 2019).

To identify free-ranging raccoons, we conducted annual trapping at locations within Laramie where we had confirmed raccoon presence via trail camera footage. Trapping took place from August 2015 until September 2019 during warmer months when the overnight lows were above 32°F (typically during August and September, but trapping was opportunistic and varied annually from May to October). Tomahawk live traps (81.28×25.4×25.4 cm, length×width×height) baited with wet cat food were set at dusk and checked between the hours of 03:00 and 05:00 h the following morning. We transported captured raccoons in the Tomahawk traps from their capture location to the University of Wyoming's Red Buttes Environmental Laboratory for processing, located 13 km south of Laramie and approximately 15 min from any of our trapping sites. We continued trapping at each site until we captured either no raccoons or only trapped recaptured individuals for multiple (3–7) nights at that location during a trapping year.

At the Red Buttes Environmental Laboratory, we immobilized raccoons in the trap with an intramuscular injection of Telazol® (100 mg ml⁻¹) using a 9–11 mg kg⁻¹ dose, depending on the size of the individual. Once a raccoon was successfully immobilized, we removed the raccoon from its trap for processing. We determined the sex and approximate age of each raccoon by inspecting their teeth, genitals, reproductive status and body size. Specifically, individuals were classified as an adult if their teeth showed signs of wear, they weighed at least 5 kg and they showed signs of having bred previously and/or during the current season (e.g. teats large and dark in color, testicles descended, fur missing from testicles) (Grau et al., 1970; Pitt et al., 2008). We also weighed each raccoon and measured its body length from the tip of its nose to the base of its tail. We then

performed a linear regression of these two measures to calculate a body condition score (BCS) for each individual (i.e. residuals from a regression of body mass and body length) (Schulte-Hostedde et al., 2005). We injected every raccoon with a subcutaneous passive integrated transponder (PIT) tag between the raccoon's shoulder blades, which allowed us to identify every individual via RFID methods. Once the raccoons were fully recovered from the effects of Telazol®, we offered each raccoon food and water before transport back to its capture site for release.

Behavioral assessments

To assess individual variation in traits that might influence participation or performance in cognitive testing, we performed standardized assessment of raccoon behavior at several points during capture and processing in the 2016–2019 trapping years. These assessments were adapted from similar studies of mammals that measured docility as a reaction to humans during trapping and handling, which is generally considered to be related to other personality traits such as aggression and boldness (Petelle et al., 2013; Réale et al., 2000). The production of aggressive vocalizations (emittance of growls, snarls, snorts, hisses and barks; Sieber, 1984), movement (the raccoon shifting its body in the trap) and contact with humans (the raccoon sticks its arm out of the trap and makes contact with trap handler) were recorded during human–raccoon encounters at four distinct phases: capture (when raccoons were collected from the field and transported to Red Buttes), immobilization (when raccoons were approached and injected with Telazol®), feeding (when raccoons were presented with food and water after recovery from immobilization) and release (when raccoons were transported back to their capture location and released). An observer recorded the production of these three behaviors at multiple standardized points during each of the four phases (Table S1), and the humans interacting with the raccoons at each phase (i.e. trap handlers) were instructed to confirm the production of each behavior. Thus, all behaviors were agreed upon by handlers and observers. The frequency of behaviors was aggregated into three categories: behavior never produced (0), behavior produced in less than half of the observations made (1), or behavior produced in more than half of the observations made (2). If an individual was recaptured multiple times in a single year, it was released, and therefore each individual was only assessed once during each trapping year. Given that raccoon personality has yet to be empirically assessed, we based our predictions on what is known about docility in other mammalian species (e.g. Petelle et al., 2013; Réale et al., 2000), and therefore defined less docile raccoons as individuals that vocalized, moved and made physical contact with humans more frequently than more docile individuals.

Trappability

The readiness of an animal to be trapped is also often associated with boldness (Carter et al., 2012; Garamszegi et al., 2009; Réale et al., 2000). Therefore, we also calculated a trappability score for each individual captured during 2016–2019 by counting the number of times an individual was captured at a single site within a trapping year and dividing it by the total number of nights that traps were open at that site during a trapping year (Réale et al., 2000). If an individual was captured at more than one site during a trapping year (uncommon, *N*=11 raccoons over five trapping years), then its trapping score was averaged between the different sites. Raccoons that were not trapped during a trapping year but were confirmed alive and present at a trapping site (via PIT tag detection after trapping efforts finished) were assigned a score of 0 for years when

they were not trapped. It is possible that some animals were not captured on certain nights because all traps were occupied by other individuals; however, such trapping saturation was uncommon and occurred only on a minority of nights from 2016 to 2019 (9% of trapping nights across years). In addition, we typically set several traps at a site for over 3 weeks, providing ample opportunity for capture.

RFID-enabled operant conditioning device

We tested raccoons using an automated operant conditioning device similar to a classic ‘Skinner box’ (Skinner, 1938). The device was made of plywood (84.5×57.8×80.0 cm, length×width×height) and contained a large copper antenna that was placed at an ~45 deg angle within the walls of the device. The antenna was attached to a long-range RFID reader (Biomark® IS1001 Reader), which detected the presence of PIT tags within its reading range (i.e. tags within the device or approximately 30 cm outside of the device) and was used to deliver tests specific to an individual while keeping a record of that individual’s progress across time. The device had two LED buttons that could be pressed to indicate a selection. Pushing on the correct button (positive stimulus) would automatically trigger the release of a small amount of dry dog kibble (reward) from a food-dispensing chute located midway between the buttons. In contrast, pushing the incorrect button (negative stimulus) would emit a low-pitched sound accompanied by a brief (2 s) timeout in which the LED lights shut off and the device became unresponsive to additional button pushes (Movie 1). We restricted the timeout period to 2 s so that we could avoid abandonment of a trial, which was previously observed in captivity when longer timeout periods were used (Stanton et al., 2021). The device was powered by a 12 V sealed, rechargeable battery and we used a Raspberry Pi computer board to control the RFID antenna, LED button interface, motor control and data-logging requirements. We placed one or two devices at each study site depending on the amount of space available and the anticipated volume of raccoons at each site, which we based on trapping success specific to that site. When two devices were used simultaneously, raccoons were assigned to only one of the boxes, such that only one box would respond to the raccoon’s PIT tag. During initial testing of the boxes, we experienced minor malfunctions and had to remove the devices from the field for repair. Also, raccoons would occasionally remove the buttons from the testing interface, which we were able to repair in the field the morning following a testing session. Data collected during times of malfunction, button destruction or heavy social interference were not included in the dataset ($N=9$ trials).

Reversal-learning protocol

Trials were conducted during October–November 2018 and May–October 2019 at four study sites spaced 1–3.5 km apart (mean=1.84 km). All four study sites, which also served as trapping sites, were located on the west side of Laramie close to a greenbelt and the Laramie River (Supplementary Materials and Methods). Raccoons were presented with a two-choice spatial paradigm similar to the methods of Cauchoix et al. (2017) and Stanton et al. (2021). Our testing protocol was fully automated and therefore did not require active experimenter involvement. The devices were switched on at dusk and switched off the following morning, so that testing occurred at night, when raccoons are typically most active. To motivate participation and habituate the raccoons to the lights and sounds of the device, raccoons would receive up to three rewards for simply approaching the box every night (Movie 2). This provided raccoons with an opportunity to learn that the devices were

a source of food and did not pose a risk. We placed inaccessible food within the LED buttons to encourage the raccoons to explore the buttons and shape the button-pushing behavior (Morand-Ferron et al., 2015b). During the first few nights that the boxes were in the field, we also placed a small amount of high-value food rewards (e.g. sardines, peanut butter) within and around the device to attract raccoons to the device and again aid in shaping the button-pushing behavior.

Each raccoon began with a training phase in which they were rewarded for pushing on either button (e.g. on the left or right side) 11 times. This allowed raccoons to begin to form an association between pushing the buttons and receiving a food reward. Whichever side the raccoon pushed more frequently during this training phase was considered to be its preferred side and is why we chose an odd number of training pushes (i.e. 11 pushes). Once a raccoon completed its training phase by pushing on the buttons 11 times, it was automatically advanced into trials where it was rewarded only for correct selections and received a brief timeout for incorrect selections. The initial correct response was determined by the raccoon’s preferred side so that raccoons were reinforced for pushing the button they selected more frequently during their training phase. We employed a 90% learning criterion in which raccoons were required to make nine correct selections out of 10 consecutive selections before a reversal was initiated. When the raccoon met the 90% criterion, the device automatically reversed the reward contingency so that the opposite button (i.e. right or left side) became the new correct selection. The number of incorrect selections (i.e. number of errors) was counted for each reversal event. To ensure that raccoons in the area had an opportunity to encounter the boxes and complete serial reversals, we conducted trials for 12–20 nights (mean=17) at a study site before moving the devices to a new study site in the interest of time and increasing our sample size. We found this to be a sufficient amount of testing time at a site because it was uncommon for new individuals to visit a site after 10 days of testing and most active participants could complete multiple serial reversals during this time. Raccoons were not limited in the minimum or maximum number of reversals that they could complete during this time, and our goal was to obtain as many reversals as possible for every individual.

Captive trials

We recently conducted a similar reversal-learning study with wild-caught raccoons at the USDA National Wildlife Research Center (NWRC) in Fort Collins, Colorado, USA (Stanton et al., 2021). Raccoons ($N=11$ adults) were trapped in the Laporte, CO, area as a part of ongoing USDA research and had been in captivity for 16–21 months prior to the start of cognitive testing. Thus, we were interested in comparing the results of these wild-caught raccoons tested under captive, controlled conditions (i.e. ‘captive raccoons’) with those of the wild, free-ranging raccoons tested under natural conditions in Laramie. Captive raccoons were presented with the same two-choice spatial paradigm as in the present study and they were required to meet a 90% learning criterion. There are a few differences between protocols inherent to each testing condition, which include presence/absence of conspecifics, the ages of participants, and the timing and delivery of trials (see Supplementary Materials and Methods). Additional details regarding the captive study can be found in Stanton et al. (2021).

Statistical analyses

All analyses were performed using R (<https://www.r-project.org/>). *Post hoc* comparative analyses of factors in generalized linear

models (GLMs) and generalized linear mixed models (GLMMs) were performed using contrast tests with the package *emmeans* (<https://CRAN.R-project.org/package=emmeans>). Model selection was performed using Akaike's information criterion corrected for small sample sized (AICc) with the package *MuMIn* (<https://CRAN.R-project.org/package=MuMIn>). Data were transformed to achieve normality where necessary and model summary tables are provided in the supplementary material (Tables S1–S5). Unlike testing conducted in laboratory and captive settings, our sample size, and thus power for analyses, was based on opportunistic visitation and participation by free-ranging individuals.

Biological and behavioral traits

We assessed whether age and sex (independent variables) were related to BCS, trappability and docility (dependent variables) using GLMs. We also wanted to know how these traits may have influenced participation in our study. However, raccoons were not always trapped the same year they were tested, and we therefore only included biological variables that were repeatable across trapping years. If a raccoon was not captured during the year it was tested ($N=16$), we assigned an 'age at testing' based on our knowledge of its most recent age: if a raccoon had been trapped as an adult, it was always considered an adult thereafter. If the raccoon was trapped as a juvenile and 2 years had passed since it was trapped, we re-classified that individual as an adult at the time of testing; if not, it was still considered a juvenile.

Docility was first measured by calculating the repeatability of behaviors (i.e. vocalization, movement and contact) within a single trapping event (i.e. across each of the four testing phases: capture, immobilization, feeding and release) and, when applicable, across trapping events (i.e. recaptures across trapping years). When calculating the repeatability of behaviors across trapping events, behavioral scores were aggregated across the four trapping phases so that an individual had a single score for each behavior for a trapping year. Thus, within a testing phase, an individual could score either a 0, 1 or 2 for each behavior in each phase, and when aggregated for a year, the individual could receive 0–8 for each behavior. We created GLMMs that included the behavior of interest (i.e. vocalization, movement or contact) as our response variable and either testing phase or trapping year as a fixed effect with raccoon ID as a random effect. We used these models to measure repeatability of each behavior by calculating intraclass correlation coefficients (ICCs) (Nakagawa and Schielzeth, 2010). Typically, ICC scores above 0.8 are considered to indicate high repeatability (Koo and Li, 2016). We also assessed repeatability in trappability using a similar GLMM with trappability score as our response variable.

Response to testing devices

Although the testing devices functioned as a source of food for the raccoons, the devices contained both visual (LED lights) and auditory stimuli (motor sound activated when reward was dispensed) that may have been perceived as risky by raccoons. Therefore, we wanted to understand whether and why raccoons exhibited different responses to the devices. We categorized all raccoons as having habituated (1) or not habituated (0) to the devices. Raccoons considered to have habituated to the device came to the device on multiple nights and ate their (three) free nightly rewards (i.e. readily fed from the boxes) and/or they learned the association between the buttons and the food reward (i.e. participated in reversal-learning trials; Movie 1). Raccoons that did not habituate to the device were those that approached the device and ran away as soon as the motor turned on, and either never returned or returned at some point but ran away again

(i.e. never readily fed from the boxes; Movie 3). It is possible that some animals encountered the devices but were not close enough to be detected by the RFID antenna and were therefore not included in this study.

We expected that those raccoons that were bolder (i.e. less docile, more trappable) and/or were in a lower body condition would be more likely to habituate to the testing device. Because reactions to novelty and risk can be affected by the presence of conspecifics (Boogert et al., 2018; Moretti et al., 2015; Stöwe et al., 2006), we also expected that raccoons that first encountered the device alone may have been more likely to startle and ultimately not habituate to the device in comparison with those that encountered the device while with one or more conspecifics. To assess this possibility, we reviewed video footage of each raccoon's first encounter with the device and categorized each individual as being either alone (1) or not alone (0) when they first approached the device as our measure of sociality. We then examined whether and how our variables of interest predicted whether animals habituated to the devices (0,1) using binomial GLMs. We included the sex, age at time of testing and sociality of raccoons as fixed effects in our model, as well as any additional variables of interest that showed high repeatability (i.e. $ICC > 0.8$). Because we expected that some of our measures might covary, we calculated generalized variance inflation factors (GVIFs) for all terms included in our final models (Fox and Monette, 1992).

Lastly, we examined whether and how our variables of interest predicted whether habituated individuals successfully learned to use the devices. We considered raccoons to have learned to use the testing device if they completed their training phase, as this required the raccoons to produce the learned behavior multiple times (i.e. push on the buttons 11 times). We did this by sub-setting our dataset to include only those individuals that habituated to the device. We expected that although all of these raccoons habituated to the devices, some individuals may have been more willing to enter and spend time in the device, which would facilitate better learning of the task. We also suspected that there may be some sex- or age-based differences in learning ability. We therefore categorized every individual to have learned (1) or not learned (0) how to use the devices. We then used GVIFs and binomial GLMs to test whether the fixed effects of sex, age at testing and repeatable measures of docility (e.g. vocalization, movement, contact) predicted a raccoon's success at learning to use the device.

Reversal learning in free-ranging raccoons

Using the same statistical methods outlined in Stanton et al. (2021), we measured performance in reversal learning by calculating the number of errors an individual made before reaching criterion (i.e. before completing a reversal). We first used GLMMs to test how reversal number, age, sex and prolonged breaks in testing time (i.e. the reversal started on one testing night but was completed on a separate, subsequent testing night; 0, 1) affected the number of errors a raccoon made with raccoon ID as a random effect. We then built separate GLMs for each individual with reversal number and correct side as fixed effects and number of errors as the response variable. This allowed us to determine whether individuals showed improvement by demonstrating a decreasing trend in number of errors made across reversals, and whether individuals had a side bias (i.e. significant effect of side assigned as the positive stimulus) that would affect performance.

Natural versus captive conditions

Finally, we compared the performance of our wild, free-ranging raccoons in natural conditions (described above) with that of the

wild, captive raccoons tested in Stanton et al. (2021). We asked whether performance differed in natural versus captive conditions by testing the interaction between experimental condition and reversal number on the number of errors made using a GLMM, and included both random slopes and intercepts for raccoon ID. To investigate potential influence of age on reversal learning, we built a second GLMM of the same structure but removed juveniles from the dataset, which allowed us to compare adults from both natural and captive conditions. It is important to note that because our protocols differed in captive and natural conditions, we remain conservative in our inferences stemming from these comparisons.

Ethical note

All trapping and experimental methods were approved under the University of Wyoming Institutional Animal Care and Use Committee (most recent protocol no. 20180813SB00321-02) and the Wyoming Game and Fish Department (Chapter 33 Permit ID: 1019), and are in accordance with the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes, 2016). Research with captive raccoons was approved by the USDA National Wildlife Research Center Institute for Animal Care and Use Committee (QA-2825).

RESULTS

Biological and behavioral traits

From 2015 to 2019, we captured and marked 204 raccoons in Laramie: 25 adult females, 33 adult males, 67 juvenile females and 79 juvenile males. We found that juvenile raccoons were in poorer body condition compared with adults, and that female raccoons were in better body condition than males (Gaussian GLM: age: odds ratio=0.91, $P<0.001$; sex: odds ratio=0.94, $P=0.01$) (Table S2). *Post hoc* analyses revealed that the sex-based difference in BCS was driven by age, such that adult females differed from adult males (contrast test: $z=-2.598$, $P=0.03$), but juvenile females did not differ from juvenile males (contrast test: $z=-0.070$, $P=0.99$). We did not find any age- or sex-based differences in trappability (quasibinomial GLM: age: odds ratio=0.85, $P=0.347$; sex: odds ratio=1.17, $P=0.391$) (Table S2). The majority of raccoons were only captured/recaptured within a single trapping year; however, 25 individuals were captured in more than one trapping year between 2016 and 2019.

We collected 186 complete behavioral assessments from 165 individuals captured between 2016 and 2019. These 186 behavioral assessments were used to test repeatability of behaviors across the four testing phases. Testing phase had a significant effect on the expression of vocalizations and movement behavior within a trapping event (Table S3), indicating that the type of interaction with humans likely elicited different behavioral responses from the raccoons. Our model for contact with humans across testing phases was not supported, as contact with humans occurred very infrequently. We found moderate repeatability in vocalization (ICC=0.56) but no repeatability in movement (ICC=0.05) across testing phases. We collected two behavioral assessments for 20 of the 25 raccoons that were recaptured across trapping years, and we used these 40 assessments to calculate repeatability in vocalization, movement, and contact with humans across trapping years. Trapping year did not have an effect on any of the behaviors of interest (Table S3). We found high repeatability in vocalization (ICC=0.95), but no repeatability in movement (ICC=0.17) or contact (ICC=0.23). Therefore, we collected a single, aggregated vocalization score (0–7; interquartile range=3) taken from an individual's first behavioral assessment as an indicator of docility

for that individual. Using this score, we did not detect any age- or sex-based difference in docility (cumulative link model: age: odds ratio=1.37, $P=0.463$; sex: odds ratio=2.14, $P=0.102$) (Table S2).

We were able to calculate trappability scores for 31 individuals that were either captured or confirmed alive at a site following trapping efforts in 2016–2019, and used data from these 31 individuals to calculate repeatability in trappability. Trapping year had a significant, negative effect on trappability (GLMM: odds ratio=0.97, $P=0.001$) (Table S3), and we did not find repeatability in trappability (ICC=0.05). Further exploratory analyses of trappability, including the effects of age and sex, revealed that trappability decreased significantly from the first trapping year to the second trapping year (*post hoc* contrast test: $t=-3.408$, $P=0.0029$), but not from the second trapping year to the third (*post hoc* contrast test: $t=-1.403$, $P=0.3013$). This pattern was demonstrated by both juveniles and adults (*post hoc* contrast test: adults first observation to second observation: $t=-3.408$, $P=0.0044$; juveniles first observation to second observation: $t=-3.408$, $P=0.0043$), indicating that raccoons likely learned to avoid traps after their first trapping year, regardless of age (Fig. 1).

Response to testing devices

Our testing devices detected a total of 40 raccoons (14 adult females, 5 adult males, 12 juvenile females, 9 juvenile males), as well as four striped skunks (*Mephitis mephitis*; see Supplementary Materials and Methods for details), in 2018 and 2019. Of these 40 raccoons, 21 first encountered the device alone whereas 19 first encountered the device with one or more conspecifics. We found that juveniles were less likely to be alone upon their first encounter of the device compared with adults (binomial GLM: odds ratio=0.137, $P=0.03$) (Table S2). One raccoon (juvenile female) only encountered the device once on an early testing night when the device was malfunctioning, and was therefore removed from the dataset. Of the 39 remaining raccoons, 27 habituated to the device and 12 did not habituate to the device. Two raccoons lacked complete behavioral assessment data from when they were captured, and therefore data from 37 raccoons were used to evaluate predictors of habituation (Fig. 2). Because BCS and trappability were not repeatable and we

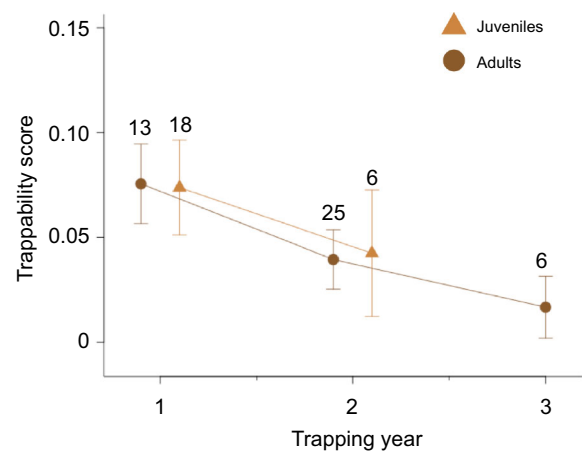


Fig. 1. Trappability scores for raccoons across trapping years (0=less trappable, 1=more trappable). GLMs indicated that both adults and juveniles decreased in trappability from the first year they were trapped to the second year they were either trapped or not trapped but confirmed alive and on site via RFID detection. Numbers indicate sample size for each group. Raccoons over 2 years old are considered to be adults, which is why there are no data points for juveniles in trapping year 3 above.

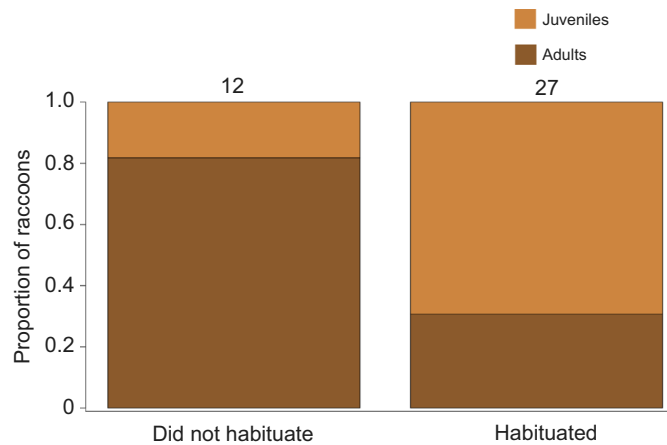


Fig. 2. Proportion of raccoons that did not habituate (left) or habituated (right) to the testing device. Numbers represent sample size for each group. GLMs indicated that juveniles were more likely to habituate to the device than adults.

lacked current scores for several of our participants, we included only age, sex, sociality and docility (i.e. first vocalization score) as predictors of habituation. None of the terms indicated covariance ($GVI\text{Fs} < 3$) and we used the dredge function and AICc model selection in MuMIn (<https://CRAN.R-project.org/package=MuMIn>) to compare all possible combinations of age, sex, sociality and docility. We performed full model averaging of two top models that were within 2 AICc values (Burnham and Anderson, 2002). Results from our averaged model indicated that juveniles were more likely to habituate to the device than adults, and that there was no influence of sex, sociality or docility (Table 1). Using the same statistical procedure with age, sex and docility as fixed effects ($GVI\text{Fs} < 3$), we found no effect of age or sex on learning to use the device. However, we found individuals with higher docility scores (i.e. lower vocalization scores) were more likely to learn to use the device than those with lower docility scores (i.e. higher vocalization scores) (Table 1).

Reversal learning in free-ranging raccoons

Of the 27 raccoons that habituated to the device, 19 learned the association between pushing on the buttons and receiving a food reward and 17 completed serial reversals. However, we encountered a high amount of social interference, characterized by multiple

raccoons attempting to enter the testing device at one time, resulting in erratic pushing of the buttons. Unfortunately, this meant that reversal-learning data were unreliable for seven of the 17 successful raccoons (five juveniles, two adults), leaving us with reversal-learning data for 10 raccoons.

Raccoons completed an average of 13 reversals (range: 3–38 reversals) and tended to make fewer errors across time (GLMM: odds ratio=0.98, $P < 0.0001$) (Table 1). We also found that juveniles tended to make more errors than adults (GLMM: odds ratio=1.27, $P = 0.01$), and that an overnight break in testing resulted in a higher number of errors for that reversal compared with reversals that were completed within a single testing night (GLMM: odds ratio=1.18, $P = 0.02$) (Table 1). Three raccoons showed significant improvement across serial reversals, and an additional four raccoons also showed a trend towards improvement characterized by a negative slope (Table 2; Table S4). In addition, it appeared that four raccoons likely had a right-side bias (Table 2).

Natural versus captive conditions

Raccoons tested in captivity generally completed more reversals (mean=27, range=4–32) than raccoons tested in natural conditions (mean=13, range=3–28). Comparisons of performance between the two groups (Fig. 3) suggested that raccoons did not differ in the number of errors being made initially (i.e. did not differ in intercept), but did demonstrate a difference in the number of errors being made across reversal events (i.e. differed in slope) (Table S5). Specifically, raccoons tested in captivity decreased the numbers of errors being made at a faster rate across reversals (GLMM: odds ratio=1.023, $P = 0.004$), and this remained constant when juveniles were removed from the dataset (GLMM: odds ratio=1.029, $P = 0.02$).

DISCUSSION

Animals living in anthropogenic landscapes are faced with both risks and rewards associated with humans (Schell et al., 2021). One strategy believed to facilitate adaptation to anthropogenic landscapes is use of cognition, which can allow animals to behave flexibly and thereby exploit or avoid various opportunities and threats (Sol et al., 2013). Here, we delivered a classic test of cognitive flexibility to a wild population of raccoons – a successful urban exploiter known for its adaptability and cognitive aptitude. Although most raccoons habituated to the testing device and many learned to complete serial reversals, we found that biological and behavioral factors may have influenced participation. Furthermore,

Table 1. Full model-averaged coefficients assessing the effect of predictor variables on habituation to the device, learning to push buttons and performance in wild reversal-learning trials

	Predictor	Estimate	s.e.	Confidence interval	Relative importance
Habituation					
Binomial GLM					
N=37 raccoons					
Averaged $R^2=0.30$					
	Age: juvenile	2.355	0.901	0.525, 4.184*	1.0
	Docility	0.049	0.144	-0.294, 0.631	0.29
Learning					
Binomial GLM					
N=26 raccoons					
Averaged $R^2=0.50$					
	Docility	-0.886	0.338	-1.584, -0.189*	1.0
	Sex: male	-0.250	0.739	-3.352, 1.527	0.27
Performance					
Poisson GLMM					
N=146 reversals					
(by 10 raccoons)					
Averaged $R^2=0.36$					
	Reversal no.	-0.022	0.005	-0.031, -0.013*	1.0
	Age: juvenile	0.242	0.096	0.053, 0.431*	1.0
	Break: yes	0.167	0.069	0.031, 0.303*	1.0

*Confidence intervals that do not overlap 0.

Note: variables that were not retained in the top models are not included in the table. Reference points for Age: juvenile, Sex: male and Break: yes are Age: adult, Sex: female and Break: no, respectively.

Table 2. Individual traits and performance in reversal learning of raccoons tested in natural and captive testing conditions

	Name	Sex	Age	Number of reversals	Side bias	Reversal improvement*
Natural	Allspice	F	Juvenile	16	Right	Yes
	Caper	F	Juvenile	6	No	Yes
	Chia	F	Juvenile	27	No	Yes
	Chive	F	Adult	17	No	Yes
	Coco	F	Adult	28	No	Yes
	Okefenoke	M	Juvenile	23	No	Yes
	Primrose	F	Juvenile	8	Right	No
	Tarragon	F	Juvenile	8	Right	No
	Tombigbee	M	Adult	10	Right	No
	Voyageurs	M	Juvenile	3	NA	Yes
Captive	Astrid	F	Adult	13	No	Yes
	Castor	M	Adult	31	Left	Yes
	Luna	F	Adult	4	NA	No
	Oberon	M	Adult	28	No	Yes
	Pollux	M	Adult	32	No	Yes
	Rigel	M	Adult	20	Left	Yes
	Sirius	M	Adult	23	No	Yes
	Vega	F	Adult	32	No	Yes

M, male; F, female; NA, could not be calculated owing to data limitations. *Improvement determined by slopes, *P*-values and confidence intervals (Table S4). Note: captive data are reproduced from Stanton et al. (2021).

we encountered some extraneous challenges associated with testing in natural conditions that likely influenced performance in reversal learning as well.

Response to testing and participation

First, we were interested in how motivation to participate may vary among sexes and age classes. We found that juvenile raccoons tended to be in poorer body condition than adults, and adult females tended to be in better body condition than adult males. This pattern is not surprising; males and females in sexually dimorphic species, such as raccoons, have different energetic demands (Isaac, 2005), and the difference in body condition between age classes is typically greater than within age classes in raccoons (Gehrt, 2003). Adult male and female raccoons also differ in their degree of sociality: adult males tend to associate more with other adult males, whereas adult females have more ephemeral associations but are often accompanied by kits (i.e. young juveniles) during the summer (Prange et al., 2011). Although we have observed similar associations in our Laramie population, we have also observed that as kits begin to gain independence in late summer through autumn, they often travel and forage with other kits (R. Fanelli, L.A.S., D. McDonald and S.B.-A., unpublished data). These associations among kits likely explain why we found that juveniles were less likely to be alone upon their first encounter with the testing device. We therefore believe that energetic demands and sociality, both of which appear higher in juveniles in our population, could explain why juveniles were more likely to habituate to the testing devices than adults. In addition, juveniles are less experienced and often more exploratory than adults, given a greater need to gather information about their environment in early life (Greenberg and Mettke-Hofmann, 2001; Reader, 2015). Thus, it is possible that their naïveté and greater exploratory tendencies also facilitated habituation. However, juveniles were not more trappable than adults, indicating that they can learn to avoid adverse situations. Therefore, our observations suggest that juvenile and adult raccoons will discriminate between risky and profitable situations, but that the behavioral and biological traits typical of juveniles may increase the likelihood of exploitation of profitable opportunities.

In accordance with previous research (e.g. Debeffe et al., 2015; Petelle et al., 2013), we found that individual variation in docility

was highly repeatable across trapping years. We also found that raccoons that demonstrated greater docility towards humans were more likely to learn how the testing device operated. This pattern might be explained by a relationship between emotional reactivity and cognitive ability (e.g. emotional reactivity hypothesis; Hare and Tomasello, 2005), which may be particularly relevant in urban environments. For example, the increased frequency of human–wildlife interactions via urbanization may be imposing selection pressures similar to that of domestication, whereby animals become less aggressive and responsive towards some environmental stimuli (e.g. via altered stress responses) (Geffroy et al., 2015, 2020a). A large body of literature suggests that the process of domestication also yields changes in cognitive ability, including, in some cases, improved learning and flexibility in behavior (e.g. Hare, 2017; Hare et al., 2002; Lewejohann et al., 2010; but see Range and Marshall-Pescini, 2022). Similarly, growing evidence suggests that shy, less aggressive and less active animals represent a reactive phenotype characterized by greater flexibility, as well as low speed–high accuracy trade-offs in cognition (Bray et al., 2015; Koolhaas et al., 2010; Sih and Del Giudice, 2012). Reactive phenotypes may be favored in urban environments, whereby individuals that are able to cope with the presence of humans and novel stimuli will be more likely to persist via increased learning and flexibility (Geffroy et al., 2020b). Therefore, future research on raccoons and other urban species would likely benefit from specific investigation of the link between docility towards humans and cognitive ability, as we suspect this may influence important aspects of wildlife behavior, including domestication and nuisance-prone activities (see below).

Although the presence of conspecifics did not directly predict habituation to the device in our analyses, social factors certainly played a prominent role in this study. We observed an unexpectedly high amount of social interference during trials. At times, raccoons demonstrated aggression towards one another, resulting in displacement from the testing device. At other times, raccoons were more tolerant of one another and shared access to the device, which resulted in the erratic pushing of buttons and ultimately led to the removal of data for seven individuals. Removal of these data was necessary to ensure the quality and interpretation of cognitive performance; however, we recognize that our reversal-learning

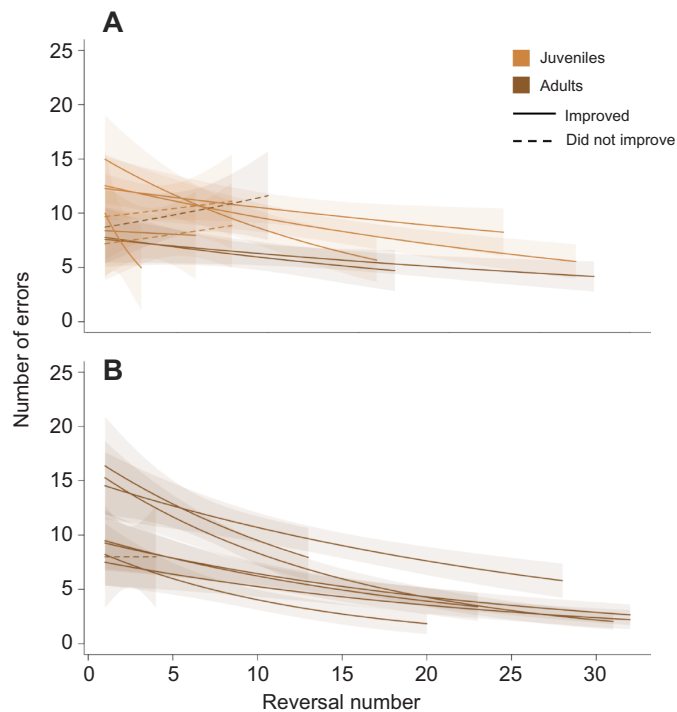


Fig. 3. Learning curves (\pm s.e.) for each study participant based on generalized linear models of individual performance. Raccoons were tested in (A) natural and (B) captive conditions. Individuals in both conditions generally demonstrated learning of this task; however, there is variation in performance. Solid lines represent individuals that showed improvement by making fewer errors across reversals, whereas dashed lines indicate that the individual did not show improvement across reversals. AICc model selection indicated that adults made fewer errors compared with juveniles in natural conditions. Although our results suggest that wild raccoons tested in captive conditions (B) outperformed wild raccoons tested in natural environments (A), it should be noted that protocols differed slightly between the two environments.

dataset may be biased towards more asocial individuals, or perhaps those that were better able to maintain access to the device (i.e. more dominant individuals that are less likely to be displaced). Although group composition and sociality may indeed influence the development and evolution of cognition (e.g. Aplin and Morand-Ferron, 2017; Ashton et al., 2018; Dunbar and Shultz, 2007), it is currently difficult to speculate on whether and how the differences in sociality observed in our study reflect actual differences in cognitive ability. Further research is currently needed to understand not only how raccoon sociality relates to their cognition in general, but also how raccoon social behavior and cognition may be changing as a result of the increasing densities and aggregations of raccoons in urban environments (Hauver et al., 2013; Schuttler et al., 2015; Wehtje and Gompper, 2011).

Reversal-learning performance

With regard to performance in reversal learning, we found that raccoons tested in natural conditions showed a general decrease in the number of errors being made across serial reversals, but that this was sensitive to prolonged gaps in testing (as is typical in studies of reversal learning; e.g. Mackintosh et al., 1968). Furthermore, our analyses suggest that juveniles made a higher number of errors than adults, which may be due to developmental effects (Kayser et al., 2021) or perhaps the ability of juveniles to maintain undisturbed access to the device (Hauver et al., 2013). It should be

noted, however, that many juveniles completed a high number of reversals and showed improvement over time. In addition, juvenile raccoons have mastered tasks requiring learning and have demonstrated flexible problem-solving abilities (e.g. Stanton et al., 2017). Thus, given our limited sample size, this difference in performance between juveniles and adults should be interpreted with caution.

We also found that wild raccoons tested in natural conditions completed fewer reversals and improved at a slower rate than wild raccoons tested in captive conditions. This pattern persisted even after reversal-learning data for juveniles were removed, indicating that this divergence was unlikely to have stemmed from potential differences between juveniles and adults. Unlike raccoons tested in captivity, raccoons tested in natural conditions had a greater need for vigilance and were observed frequently exiting the testing device to scan their surroundings (Movie 1). Moreover, captive raccoons received concentrated, undisturbed testing sessions, whereas testing in natural conditions was less structured and more susceptible to perturbations. Such distractions have been implicated in other cognitive studies comparing captive versus wild populations of carnivores (e.g. Benson-Amram et al., 2013), and likely contributed to greater performance by raccoons in captive conditions. Nevertheless, raccoons in both groups demonstrated rapid acquisition of this task and an ability to form and reverse learned associations, which is indicative of cognitive flexibility.

Cognition in the field

Our study presents several implications for the study of cognition in the field, as well as our more general understanding of nuisance animal cognition. First, in our efforts to adapt a classic cognitive paradigm (i.e. reversal learning) for assessment of large-bodied, terrestrial organisms, we highlight some of the challenges that persist when testing such non-traditional species in the field. For example, it appears that, unlike other studies focused on birds (e.g. Cauchoix et al., 2017), the lack of control inherent to field testing (e.g. prolonged gaps in testing, multiple individuals present) influenced our ability to collect data on reversal-learning performance for all raccoons tested. Furthermore, we point out that social interference may be especially difficult to overcome in anthropogenic landscapes, where increased gregariousness may be more common in certain taxa. Therefore, researchers interested in similar investigations must be prepared to circumvent heightened vigilance and social interference, perhaps by adjusting learning criteria, shortening testing periods and/or creating automated devices that only allow admittance of one individual at a time (e.g. Muns et al., 2018).

Second, we identified several biological and behavioral traits that may contribute to individual variation in participation and performance in our study. Representation of all four age–sex classes within our dataset paired with the individual variation we encountered during behavioral assessments and cognitive testing (e.g. vocalization range=0–7; interquartile range=3) suggests that our trapping and identification efforts are not limited to one specific behavioral phenotype, despite raccoons learning to avoid being trapped over time. This is essential not only for reducing sampling biases, but also for understanding individual variation in cognitive ability (Boogert et al., 2018; Webster and Rutz, 2020). Given that docility was repeatable and had implications for raccoon learning ability in our study, we believe that vocalization is a promising behavioral measure for use in future studies on individual variation in raccoons. Furthermore, vocalizations could be used to assess

interpopulation and intrapopulation docility towards humans, which may reveal how anthropogenic features, including human behavior, drive local adaptation and the possible domestication of urban wildlife.

Lastly, we suggest that a greater understanding of individual variation in species perceived as a nuisance will bolster our ability to mitigate human–wildlife conflict. Simple forms of learning, such as habituation and sensitization, as well as flexibility in behavior, often undermine non-lethal mitigation strategies such as playbacks, effigies and exclusion structures (Barrett et al., 2019; Blumstein, 2016). Therefore, identifying the biological and behavioral traits that influence learning, flexibility and risk-taking is essential for coexistence in expanding anthropogenic landscapes. Based on our results, managers and members of the public may need to be particularly aware of learning in juvenile raccoons, and therefore might consider focusing humane mitigation efforts (e.g. aversive conditioning) during times when juveniles are first venturing out of dens. Although our sample size is limited, we present evidence that raccoons quickly learn to avoid risky or adverse situations (e.g. trapping), but are able to exploit benign, profitable opportunities (e.g. feeding from an automated device). This discrimination ability and selectivity in risk-taking is likely to aid their successful adaptation to anthropogenic areas, but whether such learned risk-perception and flexibility is ubiquitous across raccoon populations or other urban species is an open question. Finally, based on our observations of wild raccoons, we see the potential for greater integration of animal personality research into studies of nuisance animal cognition. For example, problem individuals are often cited for their boldness because of a willingness to engage with novelty and incur risks (Barrett et al., 2019). Although bolder, proactive individuals may indeed be prone to more obvious forms of conflict (e.g. approaching humans), it is possible that shyer, reactive individuals are prone to less obvious forms of conflict that require greater associative learning or flexibility in behavior (e.g. raiding chicken coops). In this way, lethal removal of proactive individuals may be artificially selecting for reactive and particularly capable individuals that ultimately represent a greater management challenge (Schell et al., 2021).

Conclusions

In summary, our investigation has identified several environmental, individual and social factors that influence raccoon behavior and performance in cognitive testing. These results provide important insights into the ecology of raccoons, as well as our ability to study the contemporary evolution of cognition and behavior. We hope that this study will serve as an important step in expanding tests of cognition to non-traditional species and further research on the cognition of, and coexistence with, urban wildlife.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.A.S., S.B.-A.; Methodology: L.A.S., E.S.B., J.H., S.B.-A.; Software: E.S.B., J.H.; Validation: L.A.S., E.S.B., J.H.; Formal analysis: L.A.S.; Investigation: L.A.S., J.H.; Resources: L.A.S., E.S.B., S.B.-A.; Data curation: L.A.S., E.S.B., J.H.; Writing - original draft: L.A.S.; Writing - review & editing: L.A.S., E.S.B., J.H., S.B.-A.; Visualization: L.A.S.; Supervision: S.B.-A.; Project administration: L.A.S.; Funding acquisition: L.A.S., S.B.-A.

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Data availability

Data, R code used for analyses and Python code used to create the testing protocol can be found at: osf.io/ef53n.

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Supplementary Materials and Methods. Additional methods and results.

Animal trapping and detection

We set up camera traps in locations where we expected raccoons to be present (e.g. proximity to water and vegetation) or where Laramie residents had reported seeing raccoons in the past. This included city parks and public spaces, as well as private property (e.g. residential backyards, local businesses) that we gained permission to access. We subsequently trapped sites with confirmed raccoon presence using Tomahawk live traps (81.28×25.4×25.4 cm, length×width×height) baited with wet cat food. Our general goal was to trap all resident raccoons in Laramie, including juveniles born each year, however priority was given to trapping sites on the West side of Laramie where we believe the highest density of raccoons to be and therefore planned to conduct our cognitive experiments.

Trapping and testing was conducted annually between the months of May and October. In 2018, trapping occurred before testing began, whereas in 2019 testing occurred before and after trapping. In addition, because we did not have access to one of our study sites in 2018, it had not been trapped since 2017. The time between trapping and testing, therefore, varied among individuals tested at different sites in different years. Specifically, some individuals first encountered the testing device shortly after trapping ended (min.=6 days), whereas others experienced a much longer duration between trapping and testing (max.=655). We suspected that active trapping efforts could have made raccoons in the area to be more risk-adverse, and therefore variation in the time between trapping and testing could have influenced whether raccoons habituated to the device or not. When plotted, however, no clear pattern was found between habituation (0, 1) and the time since trapping (days). Thus, we are confident that our trapping protocol did not affect habituation to the testing device.

Note regarding striped skunks

Skunks were opportunistically trapped and marked under the same permits used for our research on raccoons (University of Wyoming Institutional Animal Care and Use Committee: no. 20180813SB00321-02; Wyoming Game and Fish Department Chapter 33 Permit ID: 1019). Thus, free-ranging, PIT-tagged skunks within our study sites ($n=14$) could be detected by the testing device and participate in testing. We detected a total for four skunks during the experiment, one of whom learned to operate the testing device and completed serial reversals. Although reversal learning data from skunks is not included in the present study, our observations of free-ranging skunks during field testing, paired with our testing of skunks in captive conditions (Stanton et al., 2021), suggests that the methodology outlined here can be used with multiple, mesocarnivore species in both natural and captive testing conditions.

Notable differences in captive versus natural conditions

Wild raccoons tested in captivity were all classified as adults at the time of trapping. They were tested once a night while alone in their home enclosure, and testing sessions lasted for 30 to 90 min. Individuals varied in their prior experience with the cognitive testing device, which likely influenced the number of errors being made initially but not across time (discussed in Stanton et al., 2021). Free-ranging raccoons, which constituted a mix of adults and juveniles, were able to start and stop testing at any point during a testing night, and they may have been subject to interruptions and/or social interference during testing. For these reasons we identified reversal events that were split between testing nights for free-ranging raccoons (e.g. started a reversal during one testing night but completed the reversal on a subsequent testing night) so that we could identify the effect of prolonged gaps in time (i.e. breaks) on performance. We also did not include data from natural conditions where high social interference may have affected the selections being made within the device. Similarly, if an individual experienced high social interference in the majority of their testing nights they were not included in the dataset ($n=7$). Lastly, captive testing was delivered in blocks of 10 selections, such that an individual was required to achieve nine out of 10 selections correct within a block. If not, then a new block of 10 selections was initiated. However, selections in wild conditions were not grouped into blocks and we instead used a sliding window format, in which the raccoon was required to make nine correct selections within 10 consecutive selections.

Table S1. Standardized points during the four phases of trapping when observations of raccoon behavior (i.e. vocalizations, movement, contact with humans) were made

Phase and observation points
Phase 1 – Capture <ul style="list-style-type: none">• Behavior when human first sees animal in trap• Behavior when human first stands next to trap at capture site• Behavior when human lifts trap cover and flags trap at capture site• Behavior when human moves trap from capture site to truck• Behavior when human moves trap from truck to injection site• Behavior when human removes trap cover
Phase 2 – Immobilization <ul style="list-style-type: none">• Behavior when human approaches trap for injection• Behavior when human probes raccoon with capped needle prior to injection• Behavior when human injects raccoon with needle
Phase 3 – Feeding <ul style="list-style-type: none">• Behavior when human first sees animal in trap after recovery• Behavior when human approaches trap and inserts cage divider• Behavior to human opening trap and placing food and water in cage, shut door, and remove cage divider
Phase 4 – Release <ul style="list-style-type: none">• Behavior when human first sees animal in trap• Behavior when human first stands next to trap• Behavior when human moves trap from recovery site to truck• Behavior when human moves trap from truck to release site• Behavior when human puts trap on ground prior to opening door• Behavior when human opens trap door at release site

Table S2. Model summary for the effects of age and sex on several biological and behavioral traits

	Type	N	R ²	P-value	lower CI	upper CI	Beta	t-value
Model – Body condition score								
BCS ~ Age*Sex	GLM (Gaussian)	204	0.13					
Age-juvenile				1.90e-06	-0.133497969	-0.05730414	-0.09540	-4.908
Sex-male				0.0101	-0.100235853	-0.01403327	-0.05713	-2.598
Age-juvenile:Sex-male				0.0316	0.005316034	0.10703337	0.05617	2.165
Model – Trappability								
Trappability score ~ Age*Sex	GLM (quasibinomial)	182	0.03					
Age-juvenile				0.347	-0.4993908	0.1837688	-0.1640	-0.943
Sex-male				0.391	-0.1993357	0.5252940	0.1587	0.860
Age-juvenile:Sex-male				0.615	-0.3280522	0.5481791	0.1125	0.504
Model – Docility								
Vocalization ~ Age*Sex	CLM (ordered)	178	NA					
Age-juvenile				0.4628	-0.5166814	1.153186	0.3117	0.734
Sex-male				0.1022	-0.1476393	1.687459	0.7627	1.634
Age-juvenile:Sex-male				0.0809	-2.1263523	0.117982	-0.9977	-1.746
<i>Hosmer-Lemeshow goodness-of-fit test: P=0.36</i>								
Model – Sociality								
Alone ~ Age*Sex	GLM (binomial)	40	0.17					
Age-juvenile				0.0258	-3.9005200	-0.3305036	-1.9924	-2.229
Sex-male				0.4254	-3.1526623	1.4290430	-0.8938	-0.797
Age-juvenile:Sex-male				0.5405	-2.0496705	3.7828333	0.8938	0.612

Table S3. Repeatability across trapping phases and years

Across trapping phases (i.e. within a trapping event):	<i>P</i> -value	lower CI	upper CI	Beta	<i>z</i> -value
Model (Cumulative Link Mixed Model fitted with the Laplace approximation)					
Vocalization Score ~ Testing Phase + (1 Animal ID)					
Phase2_Immobilization	2e-16	1.966033	3.164286	2.5652	8.392
Phase3_Feeding	5.71e-10	1.270956	2.446464	1.8587	6.198
Phase4_Release	2.80e-08	1.046816	2.188757	1.6178	5.553
ICC=Intercept ² / (Intercept ² + Residual ²): 3.733853 ² / (3.733853 ² + 3.289868 ²) = 0.5629606					
Movement Score ~ Testing Phase + (1 Animal ID)					
Phase2_Immobilization	0.000793	-1.2683350	-0.33291619	-0.8006	-3.355
Phase3_Feeding	0.066440	-0.8684571	0.02849198	-0.4200	-1.835
Phase4_Release	1.24e-08	0.8571170	1.75698505	1.3071	5.694
ICC=Intercept ² / (Intercept ² + Residual ²): 0.7397941 ² / (0.7397941 ² + 3.289868 ²) = 0.0481328					
Across trapping years (i.e. across trapping events):	<i>P</i> -value	lower CI	upper CI	Beta	<i>z</i> -value
Model (Cumulative Link Mixed Model fitted with the Laplace approximation)					
Vocalization Score ~ Trapping year + (1 Animal ID)					
Trapping2	0.489	-0.8754376	1.8313787	0.4780	0.692
ICC=Intercept ² / (Intercept ² + Residual ²): 15.68937 ² / (15.68937 ² + 3.289868 ²) = 0.9578829					
Movement Score ~ Trapping year + (1 Animal ID)					
Trapping2	0.879	-1.2323855	1.0548095	-0.08879	-0.152
ICC=Intercept ² / (Intercept ² + Residual ²): 0.4322022 ² / (0.4322022 ² + 3.289868 ²) = 0.01696623					
Contact Score ~ Trapping year + (1 Animal ID)					
Trapping2	0.51	-1.7733538	3.569532	0.8981	0.659
ICC=Intercept ² / (Intercept ² + Residual ²): 1.800755 ² / (1.800755 ² + 3.289868 ²) = 0.2305368					
	<i>P</i> -value	lower CI	upper CI	Beta	<i>t</i> -value
Model (Linear mixed model fit by REML)					
Trappability score ~ Trapping year + (1 Animal ID)					
Trapping2	0.000599	-0.05237997	-0.01660210	-0.034491	-3.836
ICC=Intercept ² / (Intercept ² + Residual ²): 0.0002763574 ² / (0.0002763574 ² + 0.001253317 ²) = 0.04636625					
Trappability score ~ Trapping year + Age*Sex + (1 Animal ID)					
Trapping2	0.00139	-0.05341176	-0.01491103	-0.034275	-3.429
Trapping3	0.00198	-0.09272151	-0.02331963	-0.058523	-3.246
Age-juvenile	0.82481	-0.02764886	0.02225475	-0.002856	-0.222
Sex-male	0.71031	-0.02095081	0.03007033	0.004928	0.374
Age-juvenile:Sex-male	0.64057	-0.03364403	0.05249216	0.010242	0.469

Table S4. Results of individual models from natural conditions and captive conditions (reproduced from Stanton et al., 2021). Note that cells lacking data indicate models that could not be calculated due to data limitations.

AID	Species	Model (Poisson GLM)	P-value	lower CI	upper CI	Beta	d.f.	z-value	R ²
<i>Natural Condition</i>									
Allspice	Raccoon	Error ~ Reversal Number	0.000313	-0.1002505	-0.02979731	-0.06469	14	-3.604	0.14
Allspice	Raccoon	Error ~ Reversal Number + Side	0.012448	-0.74827235	-0.09326059	-0.41669	13	-2.499	0.20
Caper	Raccoon	Error ~ Reversal Number	0.90	-0.175318	0.1539761	-0.01050	4	-0.125	0.005
Caper	Raccoon	Error ~ Reversal Number + Side	0.92	-0.6532777	0.5501225	-0.03077	4	-0.101	0.004
Chia	Raccoon	Error ~ Reversal Number	0.000259	0.2503498	0.26641278	-0.03135	25	-3.653	0.22
Chia	Raccoon	Error ~ Reversal Number + Side	0.953099	-0.2503498	0.26641278	0.007743	24	0.059	0.22
Chive	Raccoon	Error ~ Reversal Number	0.119	-0.07121936	0.007951245	-0.03140	15	-1.558	0.05
Chive	Raccoon	Error ~ Reversal Number + Side	0.573	-0.27407842	0.50241236	0.11127	14	0.564	0.05
Coco	Raccoon	Error ~ Reversal Number	0.0248	-0.0416705	-0.002889005	-0.022172	26	-2.244	0.07
Coco	Raccoon	Error ~ Reversal Number + Side	0.9322	-0.32435160	0.297415588	-0.013461	25	-0.085	0.07
Okefenoke	Raccoon	Error ~ Reversal Number	0.0681	-0.03761509	0.00129984	-0.018097	21	-1.824	0.03
Okefenoke	Raccoon	Error ~ Reversal Number + Side	0.1368	-0.06126938	0.45832968	0.196937	20	1.488	0.05
Primrose	Raccoon	Error ~ Reversal Number	0.586	-0.07725152	0.1375734	0.02979	6	0.545	0.02
Primrose	Raccoon	Error ~ Reversal Number + Side	0.021	0.12797689	1.294357	0.68135	5	2.307	0.4
Tarragon	Raccoon	Error ~ Reversal Number	0.684	-0.07447412	0.1138773	0.01951	6	0.407	0.006
Tarragon	Raccoon	Error ~ Reversal Number + Side	0.000319	-1.63090947	-0.4988743	-1.03183	5	-3.600	.59
Tombigbee	Raccoon	Error ~ Reversal Number	0.359	-0.03609974	0.1003113	0.03186	8	0.917	0.007
Tombigbee	Raccoon	Error ~ Reversal Number + Side	3.63e-11	-4.08943445	-2.2470621	-3.04527	7	-6.619	0.90
Voyageurs	Raccoon	Error ~ Reversal Number	0.196	-0.8969634	0.1698147	-0.3477	1	-1.293	0.64
Voyageurs	Raccoon	Error ~ Reversal Number + Side	-	-	-	-	-	-	-
<i>Captive Condition</i>									
Vega	Raccoon	Error ~ Reversal Number	3.76E-05	-0.05833	-0.02084	-0.03936	30	-4.12153	0.320028
Vega	Raccoon	Error ~ Reversal Number + Side	0.097243	-0.05013	0.622547	0.283916	29	0.097243	0.370285
Pollux	Raccoon	Error ~ Reversal Number	3.24E-06	-0.05743	-0.02348	-0.04027	30	-4.65468	0.324268

AID	Species	Model (Poisson GLM)	P-value	lower CI	upper CI	Beta	d.f.	z-value	R ²
Pollux	Raccoon	Error ~ Reversal Number + Side	0.383205	-0.43662	0.1698	-0.13465	29	0.383205	0.33515
Rigel	Raccoon	Error ~ Reversal Number	7.52E-05	-0.11835	-0.04034	-0.0786	18	-3.95933	0.363819
Rigel	Raccoon	Error ~ Reversal Number + Side	0.004783	0.193569	1.059276	0.620657	17	0.004783	0.541853
Sirius	Raccoon	Error ~ Reversal Number	0.00045	-0.07276	-0.02075	-0.04648	21	-3.50888	0.309757
Sirius	Raccoon	Error ~ Reversal Number + Side	0.183774	-0.10804	0.583329	0.233878	20	0.183774	0.353527
Oberon	Raccoon	Error ~ Reversal Number	1.15E-05	-0.04929	-0.0189	-0.034	26	-4.38788	0.261329
Oberon	Raccoon	Error ~ Reversal Number + Side	0.586995	-0.17394	0.310216	0.067008	25	0.586995	0.265255
Astrid	Raccoon	Error ~ Reversal Number	0.006162	-0.10381	-0.01741	-0.06029	11	-2.73905	0.221065
Astrid	Raccoon	Error ~ Reversal Number + Side	0.250413	-0.13142	0.517668	0.189896	10	0.250413	0.259792
Castor	Raccoon	Error ~ Reversal Number	7.43E-15	-0.08426	-0.05042	-0.06707	29	-7.77688	0.392246
Castor	Raccoon	Error ~ Reversal Number + Side	0.001128	0.18844	0.751212	0.466755	28	0.001128	0.456132
Luna	Raccoon	Error ~ Reversal Number	-	-	-	-	-	-	-
Luna	Raccoon	Error ~ Reversal Number + Side	-	-	-	-	-	-	-

Table S5. Summary table for captive versus natural testing conditions

Model (Poisson GLMM w/ log link)	P-value	lower CI	upper CI	Beta	z-value
<i>All individuals</i>					
Errors~Reversal*Condition+(1 + Test Animal ID)					
$R^2=0.67, d.f.=322$					
(Intercept)	< 2e-16	2.206773424	2.60344750	2.410069	25.184
Reversal	< 2e-16	-0.060474603	-0.03791021	-0.048339	-9.307
ConditionNatural	0.46246	-0.362887765	0.17420840	-0.095314	-0.735
Reversal:ConditionNatural	0.00437	0.006659671	0.04111761	0.022674	2.850
<i>Adults only</i>					
Errors~Reversal*Condition+(1 + Test Animal ID)					
$R^2=0.71, d.f.=231$					
(Intercept)	<2e-16	2.190584725	2.61736705	2.411200	24.202
Test	<2e-16	-0.062001121	-0.03782622	-0.048766	-8.960
ConditionNatural	0.1740	-0.676581780	0.15644441	-0.266346	-1.359
Test:ConditionNatural	0.0195	0.004615396	0.05764457	0.028737	2.336



Movie 1. Video footage from a testing session. As seen in the video, a raccoon is eating dog kibble (i.e., the food reward) inside of the testing device and then moves outside of the device to investigate his surroundings. He then moves back inside of the device and pushes on the button to his left, which is the incorrect choice. In response, a low-pitched sound is played as the lights in both LED buttons shut off and the device becomes unresponsive for two seconds. After the two second time-out period has passed, the raccoon makes the correct selection by pushing on the button to his right side and a small amount of dog kibble is released.



Movie 2. Video footage illustrating the process of habituation to the testing device. As the raccoon approaches the testing device, her PIT tag is detected, and the device automatically releases a food reward. The raccoon reacts by swiftly moving away from the device, but after a moment she returns to collect the food reward. Upon detection of her PIT tag again, the device releases a second food reward. This time the raccoon reacts by flinching at the sound of the device activating, but does not move away from the device as she did before. Finally, the device releases a third reward and although the raccoon flinches again, it appears less than her previous reaction.



Movie 3. An example of a raccoon that never habituates to the testing device. The raccoon is seen approaching the device for the first (and only) time. When his PIT tag is detected, the device activates and releases a food reward. In response, the raccoon runs away and is not detected again by a testing device for the remainder of the study.ud