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ORIGINAL ARTICLE

Food-caching chickadees do not exhibit directional bias when learning a spatial task

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Abstract

Animals frequently encounter situations in which they can choose to move either left or right. Consistent preferences to move a specific direction may be associated with lateralization, or the asymmetric structure and function of the brain and nervous system. Other lateralized behaviors commonly occur across taxa, possibly reflecting a selective advantage of cerebral specialization. Yet, lateralization and possible directional biases are rarely tested within an ecologically relevant context, such as movement, or while animals are making decisions on a larger scale. Here, we quantify to what extent wild food-caching mountain chickadees (*Poecile gambeli*) in their natural environment demonstrate consistent directional biases in movement when learning a spatial task. Directional bias was estimated from the direction (left or right) that birds moved around a square experimental apparatus while searching for a food reward at the beginning of the tasks, at which point birds had not yet fully learned the location of the food reward. Chickadees did not show a directional bias in movement at a population level. Individual variation in directional bias was significantly repeatable across years but did not significantly vary between two elevations and was not significantly associated with performance on either a spatial learning and memory task or a single spatial reversal learning task. Overall, our results show that chickadees did not show directional bias when deciding what direction to move during spatial cognitive tasks, suggesting that no consistent preference in movement direction may be advantageous when searching for food on a larger scale.

Significance statement

Many animals across a wide range of taxa will consistently prefer to use either their left or right side to complete certain types of tasks. Such asymmetric behaviors may be associated with asymmetries in brain structure and are well documented in birds. Yet, mountain chickadees did not show similar directional biases in their movement-based decision-making. Furthermore, biases in their movement were not associated with overall cognitive performance. These null results suggest that while strong left or right preferences may be beneficial in certain contexts, such biases might not be advantageous while foraging for food on a larger scale.

Keywords Spatial cognition · Lateralization · Directional bias · Foraging · Food-caching · Chickadee

Introduction

Animals regularly experience situations in which they must make decisions about where to go and what direction to move within their environments. Such decisions often involve choosing to move left or right from their starting position, such as when departing a perch, moving around an obstacle, or searching for food. However, it is unclear whether animals may have directional preferences that could bias these movement-related decisions. In other behaviors, there is widespread evidence across taxa that animals have

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directional preferences that are consistent for certain tasks or contexts (reviewed in Rogers et al. 2013). For example, some nonhuman primate species preferentially use their right hand while solving two-handed tasks (Hopkins et al. 2011), poecilid fish consistently turn to the left to avoid a predator and to the right to detour around a barrier (Bisazza et al. 1998b), and wild pigeons preferentially use one field of vision at a time to forage on different food types (Karenina and Giljov 2022). But little is known about how these directional biases might scale up to affect movement-related decisions. Individual biases in movement may be important to understand, particularly because many behavioral studies in both laboratory and field conditions record behaviors that are movement-based. For example, many spatial tasks require an individual to move around a circle (e.g., Croston et al. 2016; Gawel et al. 2019). If animals exhibited a significant preference to move left or right, such preferences might introduce a bias to estimates of learning performance involving circular search.

Many directional biases have been associated with lateralization or the asymmetric structure and function of the brain and nervous system (Rogers et al. 2013). Processes that are specialized to one side of the brain may result in control of that hemisphere over related behaviors (Rogers 2021). For example, in many vertebrates, the information collected by each eye is processed almost entirely by the opposite hemisphere (Rogers 2021). Thus, lateralization has been shown in visual-based behaviors that involve preferentially using one eye or field of vision to collect different types of information (Clayton and Krebs 1993, 1994; Jozet-Alves et al. 2012; Tommasi et al. 2000; Loconsole et al. 2021) or to collect information in different contexts (Robins and Rogers 2004; Ventolini et al. 2005; Zucca and Sovrano 2008; Shen et al. 2019). Directional biases in motor-based behaviors may also suggest lateralization, such as using one limb to hold food or manipulate objects (Bisazza et al. 1996; Magat and Brown 2009; Brown and Magat 2011; Hopkins et al. 2011; Zhao et al. 2012; Bell and Niven 2016; Isparta et al. 2020; Leaver et al. 2020) or consistently turning in one direction (Bisazza et al. 2000, 2001; Miler et al. 2017). However, although general trends have begun to develop (Rogers 2021), the literature is mixed about to what extent lateralized behaviors become fixed across populations (e.g., Hopkins 2006). More typically, individuals vary in both the direction (i.e., left or right) and strength (i.e., frequency of the behavior in the same direction) of the lateralization.

Many behavioral studies show that individuals with strongly lateralized behaviors perform better on cognitive tasks than more weakly lateralized individuals, including in associative learning (honeybees in Letzkus et al. 2008; larval antlions in Miler et al. 2017), visual discrimination (parrots in Magat and Brown 2009; wild American robins in Scharf et al. 2019; wild pigeons in Karenina and Giljov 2022), numeric counting (guppies in Dadda et al. 2015), problem solving (cats in Isparta et al. 2020), and dual tasks, such as predator vigilance while completing a foraging visual discrimination task (chicks in Rogers et al. 2004; marmosets in Piddington and Rogers 2013). However, other studies find negative (topminnows in Dadda et al. 2009; wild squirrels in Leaver et al. 2020) or null (pheasants in Whiteside et al. 2020) relationships between lateralized behaviors and cognitive abilities. These contrasting results suggest that there may be costs to lateralization (pheasants in Whiteside et al. 2018) or that non-cognitive factors might influence individual variation in directional biases (reviewed in Güntürkün et al. 2020; Rogers 2021). For instance, lateralized behaviors imply lateralized practice and learning with both perceptual and motor-based systems (reviewed in Güntürkün et al. 2020), potentially leading to improved motor control (locusts in Bell and Niven 2016) or decreased reaction times (fish in Dadda et al. 2010). Moreover, some lateralized behaviors appear task-dependent (parrots in Schiffner and Srinivasan 2013; Trinidadian guppies in Penry-Williams et al. 2022) and highly plastic, as individuals can adjust the strength of laterality based on the cognitive demands of the task (wild pigeons in Karenina and Giljov 2022) or predation risk (damselfish in Ferrari et al. 2017). Ultimately, it is important to understand how these directional biases might affect decision-making in animals, which has gotten less attention in the literature.

Here, we used data from three types of spatial cognitive tasks performed by wild food-caching mountain chickadees (*Poecile gambeli*) in their natural environment to assess whether chickadees show directional biases in movement while learning these spatial tasks. We also wanted to explore how possible directional biases might (a) be associated with differences in individual spatial cognitive performance, (b) vary with environmental conditions across two montane elevations, and (c) be repeatable across two consecutive years. All three cognitive tasks used the same 8-feeder array setup, with feeders positioned equidistantly on a square frame (Fig. 1). We estimated directional bias as birds searched for a single rewarding feeder at each array, using the order of feeders visited to estimate each bird's movement around the feeder array. Movement around a barrier has commonly been used to assess lateralization in fish through detour tasks (Bisazza et al. 1998a; Penry-Williams et al. 2022). Our method to estimate directional bias was similar: after visiting any unrewarding feeder at the array, birds could choose to move to equidistantly positioned feeders on the left or right to search another feeder for food (Fig. 1). Birds could clearly view both adjacent feeders from any given feeder perch. In this system, chickadees arrive at any feeder in the array and then move around the array to sample different feeders until they discover the single rewarding feeder (see Supplemental Video). Birds do not leave the array until they

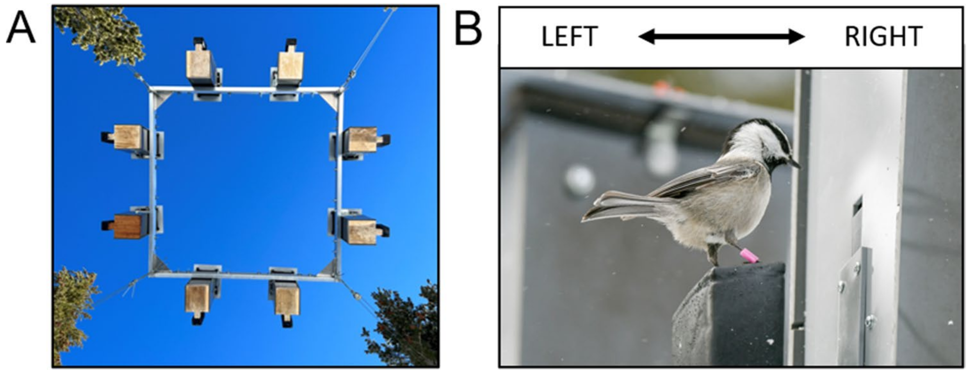

Fig. 1 (**A**) One of four feeder arrays, viewed from the ground looking upwards. Eight feeders (wooden squares) with protruding perches (black squares) are equidistantly arranged on a 122 × 122 cm square aluminum frame with 2 feeders per side. The array is elevated ca. 3 m in the air (depending on winter snow level) and is ca. > 3 m away from trees on all other sides. (**B**) A PIT-tagged bird (pink tag) lands on the perch of a feeder of an unrewarding feeder. The mechanized feeder door does not open, and the bird does not receive a food reward. The bird can choose to move left or right to visit another nearby feeder, indicated by arrows above the image

have obtained food at the rewarding feeder. Although the literature suggests that while lateralization and other directional biases could affect the learning process (reviewed in Rogers 2021), there is no evidence that the learning process could affect movement bias. However, we used a conservative approach and only analyzed data when the birds had not yet fully learned the location of the correct rewarding feeder and so had to search the feeder array to find the food reward. If any movement bias exists, it should be evident in continuous movement among feeders, and so we used the data with birds visiting at least 2 unrewarding feeders before finding a food reward, as this allowed us to examine the direction of 2 + moves (see Table 1 for terminology).

Cognition-based predictions

Chickadees are food-caching birds that rely on spatial learning and memory to hide and recover food caches during the winter. We would expect to find directional biases in behaviors related to spatial cognition in chickadees because the region of the brain related to spatial cognition in avian species (hippocampal formation) has specialized functional structures (Tommasi et al. 2003; Siegel et al. 2006) and spatial information is processed differently when collected from the left and right visual fields (Clayton and Krebs 1993; Tommasi et al. 2003). Furthermore, in food–caching species, there appears to be an asymmetrical transfer of spatial information from one side of the brain to the other (Clayton and Krebs 1993). If mountain chickadees have a directional bias while learning in a cognitive task, we would expect that over 50% of birds would show a movement bias in the same direction and that this bias would be repeatable within individuals between years. Furthermore, if these directional biases are associated with spatial cognitive ability, then we would expect that performance on spatial cognitive tasks should be associated with the strength of individual biases.

Environment-based predictions

We conducted this study in a montane system in which we have previously found significant cognitive, morphological, and behavioral differences between mountain chickadees from high (~ 2400 m) to low (~ 1900 m) elevations, as well as significant differences in winter environmental harshness and predictability across elevations (Branch and Pravosudov 2016; Croston et al. 2016, 2017; Kozlovsky et al. 2018; Pitera et al. 2018; Tello-Ramos et al. 2018; Sonnenberg et al. 2019; Benedict et al. 2021). Compared to birds at milder, lower elevations, birds at harsher, higher elevations experience a longer duration of snow cover (Kozlovsky et al. 2018) and less predictable foraging conditions, likely due to interruptions from frequent and variable winter storms (Pitera et al. 2018). Birds at high elevations also usually perform better on spatial learning and memory tasks (Croston et al. 2016) and appear less cognitively flexible (Tello-Ramos et al. 2018). If directional bias varies with environmental harshness or with the different demands of the environments at these two elevations, we expect to see a difference in the strength and possibly the direction of movement between birds from each elevation.

Sex-related predictions

During the cognitive tasks, birds participated willingly, and there was no strict control for how many birds could attempt to forage from the arrays at the same time. This raises the possibility that more dominant birds could have potentially displaced less dominant birds trying to visit certain feeders. Social dominance in chickadees and other Paridae species

Table 1 Definitions

Term	Definition or usage
Lateralization	Asymmetric structure and function of the brain and nervous system (Rogers et al. 2013). Also called cerebral or hemispheric specialization
Directional bias	Consistent behavior using one visual field, side of the body, limb, etc. Distinct from lateralization because directional biases might arise from various processes, not necessarily specialized structure or function in the brain that would indicate lateralization. Also called "directional preferences" or "asymmetric behavior"
Laterality	For this study, used to refer to asymmetry in behavior that is likely related to lateralization or has been shown to relate to lateralization. Whereas lateralization ultimately refers to the asymmetric neural structure or function between two sides of the brain and nervous system, laterality typically characterizes asymmetric behaviors or consistent directional biases that likely derive from lateralization
Strength of directional bias	Regardless of direction, the frequency or consistency of behaviors to one specific side (left or right). In this study, birds that move left and right relatively equally would have weak directional bias whereas birds that always move to the left or always move to the right would have a strong directional bias. Similar to the strength of laterality
Search behavior	When birds visit feeders at the feeder array at the beginning of a cognitive task, before learning the location of the rewarding feeder. If the bird makes at least 2 location errors in a trial, this suggests that the bird is searching for an unknown reward location
Trial	Begins when a bird visits a feeder at the array during a spatial cognitive task and ends when the bird finds the correct rewarding feeder and receives a food reward
Location errors	A visit to any unrewarding feeder location at the feeder arrays during spatial cognitive tasks
Spatial learning and memory performance	Performance on an associative learning task in which birds must learn one rewarding location out of 8 locations in a spatial array. Estimated using the mean number of location errors per trial in the first 20 trials of the spatial learning and memory task (Croston et al. 2016, 2017)
Single reversal learning performance	Performance on a reversal-learning task with a single reversal. After birds learn an association between a rewarding location and a food reward, the task is "reversed" by assigning birds to a different feeder in the feeder array and measuring learning performance for the new feeder. Estimated using the mean number of location errors per trial in the first 20 trials of the single reversal learning task (Croston et al. 2017)
Serial reversal learning task	Similar to the single reversal learning task, but after an initial learning period, the rewarding feeder location is alternated between two different locations every day

follows a linear hierarchy in which adult and juvenile males are socially dominant over females (e.g., Ekman 1989). Thus, if the direction that birds move around the arrays was affected by social dynamics rather than passive directional biases, then we would expect females to show weaker directional bias than males due to more frequent displacement by dominant birds. However, the study design limited the likelihood of displacement events: (1) birds were assigned to different rewarding feeders to distribute visits across all 8 feeders; (2) the study only analyzed instances when birds moved away from unrewarding feeders after making a location error, meaning that dominant birds would not gain a food reward by displacing birds from these unrewarding feeders and should not be motivated to do so; and (3) we estimated directional bias both as the initial direction that birds moved after first arriving at the feeder arrays and as the consistency in direction if birds moved twice in the same direction. The consistent directional bias should be more robust to displacement by other birds than an initial bias measured from one movement alone. It is unlikely that a bird would keep moving in the same direction following a displacement and that a bird would be consistently displaced at feeders that do not provide food. Finally, our previous work showed that social dominance status did not affect performance on these spatial cognitive tasks, measured as the number of location errors before visiting the rewarding feeder (Heinen et al. 2021).

Methods

Study system

All data were collected as part of a long-term study (2014—ongoing in 2022) of mountain chickadees at the Sagehen Experimental Forest in the Sierra Nevada mountains (Sagehen Creek Field Station, University of California Berkeley), located 10 km north of Truckee, CA, USA (Freas et al. 2012; Croston et al. 2016, 2017; Kozlovsky et al. 2018; Tello-Ramos et al. 2018). Within this system, there are substantial differences in winter conditions between higher (ca. 2400 m) and lower (ca. 1900 m) elevations: conditions at higher elevations are consistently harsher, characterized by lower ambient temperatures, longer duration of snow cover, more unpredictable interruptions in food availability due to more frequent and unpredictable snowfall, and more severe storms (Kozlovsky et al. 2018; Pitera et al. 2018). Annual banding efforts, nestbox breeding surveys, and cognition experiments were concentrated at the high and low-elevation sites to explore elevational differences since 2013. Birds were trapped at nestboxes during the summer or at established bird feeders using mistnets in the fall and winter. Trapped birds were banded with colored bands including a colored passive integrated transponder (PIT) tag with a unique alphanumeric ID (IB Technology, Leicestershire, UK). In this study system, sex (male or female) was determined from previous summer breeding survey records using physiological and behavioral evidence (e.g., brood patch or cloacal protuberance, song), if possible (Meigs et al. 1983; Pyle 1997).

Experimental apparatus

The data used in this study were collected in winter 2019–2020 and winter 2020–2021 as part of a long-term effort to test spatial cognitive performance in mountain chickadees. The data were collected using four spatial feeder arrays (two arrays per elevation, ca. 1.2 km apart) established in 2014 (Croston et al. 2016, 2017). Each array consisted of eight "smart" feeders mounted equidistantly to a square 1.2 m × 1.2 m aluminum frame raised ca. 3 m above the ground. Feeders were equipped with a radio frequency identification (RFID) data logger connected to a perch-mounted antenna that detected and logged the passive integrated transponders (i.e., PIT tags) of birds that landed at the feeders. Each feeder had a mechanized door to control access to a supply of black oil sunflower seeds (Croston et al. 2017; Tello-Ramos et al. 2018; Bridge et al. 2019). Feeders could be set to three different modes: (1) "open" mode, in which feeder doors were always open; (2) "all" mode, in which feeder doors were closed until any PIT-tagged bird triggered the door to open by landing on the feeder perch; and (3) "target" mode, which was similar to "all" mode except that each bird could only access food from one of the eight feeders, though all feeders recorded the time and PIT tag for all visits. "Open" and "all" modes were used to habituate birds to the feeders before annual cognitive tasks, whereas "target" mode was used during cognitive tasks to assign birds to one rewarding feeder each. Birds could forage during daylight hours, but feeders automatically turned off after sunset and turned back on before dawn, from ca. 20:00 to 06:00.

These 8-feeder arrays had not been used before to test directional biases or laterality but are well suited to measure such biases in movement. The feeder arrays allow animals freedom of movement while still forcing animals to make a left or right decision to move between feeders. When a chickadee arrives at any of the array feeders, it continues moving around the array to sample the feeders until it lands on the single rewarding feeder (see Supplemental Video). Birds usually do not leave the array until they find a rewarding feeder. When a bird lands on any feeder, it faces the feeder door which may open to provide access to food. If the door does not open (as in Fig. 1B), the bird usually moves left or right to the next closest feeder. In contrast, many other laboratory studies restrain animals to some degree, so animals can only use one eye or one leg for a given behavior. While those study designs collect invaluable data, our study design allows us to observe directional biases at a different scale, by observing the final decision of where the animal chooses to move given all options. Furthermore, the "smart" feeders are an advantage of our study design, as these feeders automatically recorded the visits of PIT-tagged birds and controlled individual access to the feeders. As such, our data were collected blindly with respect to individual performance and cognitive metrics. It was not possible to record data blindly with respect to elevation because our study was conducted in the field with different arrays at different elevations. Additionally, birds could participate in any number of trials during our study, each trial starting when a bird approached the array (i.e., visited any feeder) and ending when the bird visited the correct rewarding feeder and received a food reward (Table 1). Birds were likely motivated to participate in multiple trials because chickadees typically forage for seeds one at a time, leaving the array to consume or cache the seed after each successful visit (Croston et al. 2017; Tello-Ramos et al. 2018). As a food-caching species, chickadees cache rather than consume the majority of seeds obtained during trials; thus, motivation likely did not diminish during successive trials (e.g., Croston et al. 2016).

Estimating directional bias

Directional bias was estimated from the direction (left or right) that PIT-tagged birds moved around the feeder arrays, inferred from subsequent visits recorded automatically at smart feeders. Visits were not validated by visual observations to ensure that they represented real movements between feeders. But personal observations (by Pravosudov, Benedict) suggest that chickadees do not leave the array until they get a food reward from the correct feeder, and so they typically sample multiple unrewarding feeders in a row only leaving the array after they obtain food (Supplemental Video). Supporting these observations, the mean trial time for trials in which birds visited at least 2 unrewarding feeders was only 39.5 ± 73.9 s. Given the small amount of time it takes to complete a trial, it is unlikely that chickadees could frequently leave the feeder array to perch nearby (arrays were suspended in the air ca. 3 m high and were > 3 m away from nearby trees on all other sides). This suggests that visit data indicated movement at the beginning of each trial. Each trial was scored for two estimates of directional bias: (1) initial bias, or the direction the bird initially moved after starting a trial at an incorrect, unrewarding feeder, and (2) consistent bias, or the direction the bird consistently moved after its initial movement, if it moved in the same direction again after visiting the first unrewarding feeder and then after visiting the second unrewarding feeder (Fig. 2). Both types of directional bias were measured as "left" or "right" from the viewpoint of a bird at the feeder (Figs. 1 and 2).

A primary goal of the study was to describe the directionality of movement during the foraging-based search as birds were learning the location of the rewarding feeder in each task. To do this, we had to only use trials in which birds visited at least 2 unrewarding feeders before visiting the rewarding feeder (i.e., made at least 2 location errors) during "target" mode. The 2020–2021 sample size was reduced from 543,878 trials completed by 321 birds across all cognitive tasks to 16,712 trials completed by 316 birds across all tasks (Appendix Table 2). While this might seem like a dramatic reduction, 89.5% of the excluded trials showed near-perfect performance, in which we could not score direction because birds only visited the correct rewarding feeder before leaving the array. Using a minimum threshold of 2 or more location errors per trial was necessary because (1) these birds made at least 3 visits to feeders per trial and thus could be scored for consistent bias, which could only be estimated from at least 3 visits; (2) initial bias was always estimated when birds moved between 2 unrewarded locations (e.g., errors), avoiding any movement directly to the correct rewarding feeder from the adjacent feeder, which might reflect other processes; and (3) the exclusion ensured that birds were all at relatively the same point in the learning process when the data were collected, by eliminating the trials in which birds were performing perfectly or nearly perfectly (making 1 or no location errors). Making 2 or more location errors indicates that the birds had not yet fully learned the rewarding location, considering that, on average, chickadees typically make 1 or fewer errors after the first few trials (Croston et al. 2017; Sonnenberg et al. 2019; Tello-Ramos et al. 2018). Moreover, trials with only 1 location error are more ambiguous to analyze because while the single location error could be due to imperfect memory, it could also be due to birds waiting for the target feeder to be available. As our data were collected automatically, we do not know how frequently this might have occurred, but using a minimum of 2 location errors and including consistent bias estimates should reduce this in the dataset. It is important to note that the 2 + location error cutoff did not remove birds from the dataset based on learning ability, because all birds make errors at the beginning of the cognitive tasks while they learn. In addition, there is no evidence that the learning process can affect directional bias or laterality, whereas previous studies have suggested that directional bias could be expected to affect learning (Güntürkün et al. 2020; Rogers 2021).

To address our main study question, we needed to use all trials to analyze whether directional bias was apparent at the decision-making level, so that we could analyze behavior while birds were learning each task. Using all trials also provided the largest sample size and the most statistical power to detect any potential movement bias. But to explore whether directional bias might be present

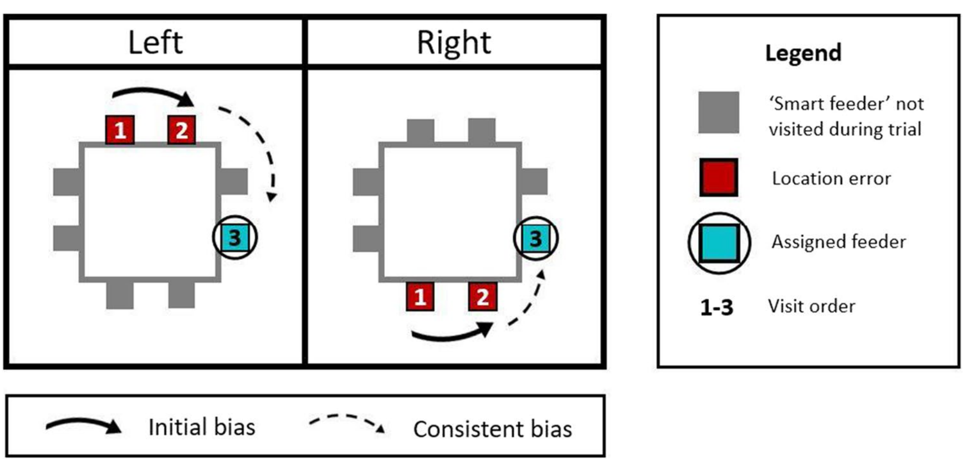

Fig. 2 Scoring trial direction. Trials were scored for initial bias based on the relative position of the second location error compared to the first location error: left and right. Similarly, consistent bias was scored for trials in which the position of the third visit was in the same direction as the initial bias. Trials were only scored if the feeder locations were within 2 feeders apart and if the bird made at least 2 location errors, indicating that birds had not yet learned the location of the rewarding feeder location. Note that this figure depicts a "top-down" depiction of the feeder arrays, in contrast to Fig. 1

before birds even started learning during each task, we also estimated initial and consistent bias using only the first trial of each task (i.e., after a new feeder location was assigned). During these “first trials,” birds had no knowledge of the correct feeder location and were about to begin the learning acquisition stage. In the serial reversal tasks, we included trials after every switch of the rewarding feeder. This subset comprised 26.7% of the all-trial initial bias dataset ($N = 269$) and 25.5% of the all-trial consistent bias dataset.

Additionally, we excluded any trials in which a bird crossed to the opposite side of the array during the first three visits per trial. This was largely because we could not determine the direction if the bird moved to the feeder directly across from it, and partially because moving across the entire array likely introduces more opportunity to be displaced by other birds and could be driven by other factors. To be conservative, we only used trials in which birds visited feeders that were no farther apart than 2 feeders. In 2020–2021, this excluded 1 bird and overall removed 2055 trials across all cognitive tasks from the initial dataset, and this excluded 3 birds and overall removed 5783 trials from the consistent bias dataset across all cognitive tasks (Appendix Table 2).

Quantifying directional bias

Directional bias (both initial and consistent) was quantified using a laterality index (LI, Eq. 1), using the total number of left-scored (L) and right-scored (R) trials per bird:

$$LI = \frac{L - R}{L + R} * 100 \quad (1)$$

The sign of the bias index (LI) represents the direction of the bias (negative numbers indicate “right,” and positive numbers indicate “left”), and the value represents the strength ($LI = \pm 100$ indicates that birds almost always moved in the same direction, and $LI = 0$ indicates that birds went left and right relatively equally). To estimate the strength of laterality regardless of direction, the absolute value of the bias index was used, ranging from weak to strong [0, 100] (Penry-Williams et al. 2022).

This index did not account for the total number of trials analyzed per bird, which ranged from 7 to 120 trials per bird across all cognitive tasks in the 2020–2021 initial bias dataset (mean = 48 ± 21 SD trials per bird) and from 6 to 65 trials per bird across all cognitive tasks in the 2020–2021 consistent bias dataset (mean = 27 ± 13 SD trials per bird). For 2019–2020, the datasets ranged from 6 to 221 trials per bird across all cognitive tasks for the initial bias dataset (mean = 34 ± 32 SD trials per bird) and from 6 to 44 trials per bird across all cognitive tasks in the consistent bias dataset (mean = 15 ± 7 trials per bird).

Directional data

To assess possible directional bias, we used data collected from spatial cognitive tasks during the winter season of 2020–2021: a spatial learning and memory task (January 13–17, 2021), a single reversal learning task (January 17–20, 2021), and two serial reversal learning tasks (January 20–26, 2021; February 10–26, 2021). Additionally, to estimate the repeatability of these bias scores, we used data collected from spatial cognitive tasks during the winter season of 2019–20: a spatial learning and memory task (high elevation: February 2–7, 2020; low elevation: January 20–24, 2020) and several single reversal learning tasks (high elevation: February 7–13, 2020; February 24–28, 2020; low elevation: January 24–29, 2020; and February 10–14, 2020). These 2019–2020 data were only used for the repeatability of directional biases within individuals between two consecutive years.

Spatial learning and memory task and single reversal learning task

Cognitive performance scores were used from two of the cognitive tasks conducted in 2020–2021 to explore how individual laterality scores might affect cognitive performance: spatial learning and memory ability and single spatial reversal learning ability. These two cognitive tasks were conducted consecutively following previously established protocols using the 8-feeder arrays (Croston et al. 2016, 2017; Tello-Ramos et al. 2018; Sonnenberg et al. 2019). Performance from the serial reversal tasks was not used because this performance was assessed through additional metrics that were not directly comparable to the other two cognitive tasks.

For the spatial learning and memory task (January 13–17, 2021), feeders were switched from “all” mode to “target” mode, restricting PIT-tagged birds from accessing all 8 feeders to just one. PIT-tagged birds were pseudorandomly assigned to rewarding feeders so that no bird was assigned to its most frequently visited feeder from “open” or “all” mode. The number of birds assigned to each feeder was relatively equal. Immediately following the spatial learning and memory task, the single spatial reversal learning task began by switching the rewarding feeder assignments for each bird to a new feeder on a different side of the square feeder array (January 17–20, 2021). To minimize social learning, birds that were assigned together to the same rewarding feeder in the spatial learning and memory task were individually assigned to separate rewarding feeders during the reversal task.

For both cognitive tasks, better performance was indicated by a lower number of mean location errors per trial in the first 20 trials. A trial started when a bird visited any feeder in the array and ended when the bird landed on the rewarding feeder and obtained food (Croston et al. 2017). Location errors were measured by the number of unrewarding feeders visited within a trial prior to visiting the rewarding feeder (Croston et al. 2017). Our previous work indicated that the mean performance across the first 20 trials provided a good point of comparison between individuals' cognitive performance, as it is directly related to survival (Sonnenberg et al. 2019).

Data exclusions

For statistical purposes, we only analyzed birds that had at least 6 scored trials across all the tasks in a given year, based on the minimum number of data points needed to find a significant deviation from a binomial distribution with equal probabilities of two outcomes. In 2020–2021, this excluded 11 and 18 birds from the initial and consistent bias datasets, respectively (Appendix Table 2). In 2019–20, this excluded 9 and 44 birds from the initial and consistent bias datasets, respectively. Furthermore, 2 birds were removed as outliers, one from each of the initial and consistent bias datasets for 2020–2021 (Appendix Table 2). For consistent bias data in 2020–2021, an additional 2819 trials across all birds and all cognitive tasks were excluded, in which birds moved in different directions for their first and second movements but otherwise would have been analyzed in the analysis. Similarly, for consistent bias data in 2019–2020, an additional 497 trials across all birds and all cognitive tasks were excluded, in which birds moved in different directions for their first and second movements but otherwise would have been analyzed in the analysis.

Statistical analysis

To determine whether overall behavior was more biased than compared to chance, we used one-sample two-tailed *t*-tests to compare the sample mean to a theoretical mean if there was no directional bias in the sample ($LI = 0$). This test was used for initial and consistent bias measured across all trials and for only the first trial subset.

To determine whether individual birds showed significantly more directional bias compared to the sample mean, we calculated individual z-scores using bias estimates from all trials (Hopkins 2006). We considered z-scores ≤ -1.96 relatively right-biased and z-scores $\geq +1.96$ relatively left-biased, because there is a 95% chance of randomly selecting a value between -1.96 and $+1.96$ (Wells 2003). Wald chi-squared goodness-of-fit tests indicated whether the number of relatively left, right, and unbiased trials across the entire sample differed significantly from chance. To determine whether different individuals demonstrated statistically significant directional bias, we conducted binomial tests for each individual using Bonferroni and Holm correction factors to reduce type I error by correcting for multiple comparisons. As this was a conservative method, we reported original and corrected *p*-values in the data uploaded to the Mendeley Data Repository (accessible at https://doi.org/10.17632/jsgv8jp8cp.2).

To analyze variation in directional bias, linear models (LM) were fitted using four response variables: an initial and consistent directional bias index for all trials and for the first trials of each task. For each of the bias index response variables, a separate LM was fitted for each predictor variable: elevation (categorical: high and low) and sex (categorical: male and female). This process was repeated with the strength alone of the bias index response variables.

To compare the initial and consistent bias indices measured across all trials, two LM were fitted with the consistent bias index and strength as response variables and two LM were fitted with the initial bias index and strength as predictor variables between the initial and consistent bias estimates.

Cognitive performance was estimated as the mean number of location errors per trial over the first 20 trials of the 2020–2021 spatial learning and memory task and the 2020–2021 single spatial reversal learning task following our previous work (Croston et al. 2017; Tello-Ramos et al. 2018; Sonnenberg et al. 2019; Heinen et al. 2021). Both metrics were used as response variables in LM with initial and consistent bias index and strength across all trials as predictors, and two other LM were fitted with initial and consistent bias strength (only) for the first trials. Fixed interaction effects of bias index estimates and elevation were also tested but were dropped from later analysis due to low explanatory value.

To estimate the individual repeatability of directional bias across all trials, a repeatability analysis (with likelihood ratio test) was conducted with LI estimates from the main 2020–2021 analysis and from 2019–2020. We also fit linear mixed effects models (LMER) with LI as the response variable to explore how directional bias across all trials varied between years in only the birds that were observed in both years. "Individual" was held as a random slope.

To assess whether the first 2 visits of each trial were equally likely to be to feeders on the same side of the array versus on different sides, we conducted a one-sample *t*-test with the proportion of "same-side" visits compared to a predicted mean value of 0.5.

The maximum sample size available was used for each analysis. Sample sizes varied because we did not have sex or cognitive data for every bird, as not all birds were sexed conclusively and as not all birds participated in both cognitive tasks, and we needed fewer minimum trials to calculate laterality (6 trials per year) than to calculate cognitive performance (20 trials per task).

Statistical software

Analyses were performed using R version 4.1.0 (R Core Team 2021). LM were performed using the base *stats* package. LMER were performed using *lme4* (Bates et al. 2015). Regression assumptions and goodness-of-fit were evaluated using *DHARMa* (Hartig 2020) and the analysis of variance (ANOVA) test using *car* (Fox and Weisberg 2019), reporting Wald chi-squared values. The *stats* package was also used to calculate *t*-tests, reporting *t*-values (t), sample means (μ), and 95% confidence intervals (CI); and binomial tests, reporting Bonferroni-corrected *p*-values to reduce type I error from multiple comparisons. The package *rptR* (Stoffel et al. 2017) was used to calculate repeatability (R), 95% CI, and goodness-of-fit based on a likelihood-ratio test, additionally reporting deviance (D). Plots were generated using *ggplot2* (Wickham 2016).

Results

Initial directional bias

In 2020–2021, 303 birds were scored for initial directional biases: 185 at high elevation and 118 at low elevation. Overall, chickadees showed a small significant initial bias to the left during search with a mean of 52% of left-scored trials across all cognitive tasks (mean $LI = 2.92$, one-sample *t*-test: $t = -36.05$, $df = 302$, $P < 0.001$, 95% $CI = [0.35, 5.49]$; $N = 303$; Fig. 3A). Similarly, when only the first trial of each task was analyzed separately, chickadees also showed a small significant initial bias to the left, with a mean of 53% of left-scored trials (mean $LI = 6.13$, one-sample *t*-test: $t = 3.23$, $df = 268$, $P = 0.001$, 95% $CI = [2.39, 9.87]$, $N = 269$). For all scored trials, approximately 21% of birds ($N = 65$) were relatively more biased compared to the population mean ($-1.96 \leq$ z-score ≥ 1.96), which was significantly fewer birds than would be expected by chance if the sample contained equal numbers of initial biased and unbiased birds ($\chi^2 = 98.78$, $df = 1$, $P < 0.001$; $N = 303$). However, only 4 individuals showed a statistically significant directional bias compared to a binomial distribution (all left-biased), after correcting for repeated measures using a Bonferroni correction factor.

There was no significant difference between elevations in initial directional bias across all scored trials (measured through the laterality index; LM: $F_{1,301} = 0.056$, $P = 0.81$; $N = 303$; Fig. 3A) or only across the first trials of each task (*LM*: $b = 0.06$, $SE = 3.93$, $F_{1,267} < 0.001$, $P < 0.001$). Of the birds with sex data in 2020–2021, there was no significant effect of sex on the initial directional bias across all trials (LM: $F_{1,128} = 0.58$, $P = 0.45$; $N = 130$) or in the first trials of each task (LM: $F_{1,114} = 0.02$, $P = 0.89$; $N = 116$).

Cognition and initial bias

Spatial learning and memory performance was not significantly associated with the initial bias index estimated from all scored trials with more than 2 location errors (LM: $b = -0.02$, $SE = 0.03$, $F_{1,229} = 0.74$, $P = 0.39$; $N = 231$; Fig. 4A), the strength alone of the initial bias estimated using all trials (LM: strength: $b = 0.004$, $SE = 0.03$, $F_{1,229} = 0.02$, $P = 0.89$; $N = 231$; Fig. 4C), or the strength alone of initial bias index estimated using only the first trial of each task (LM: $b = 0.02$, $SE = 0.03$, $F_{1,205} = 0.33$, $P = 0.56$, $N = 207$).

Single reversal-learning performance was also not significantly associated with the initial bias index estimated from all scored trials with 2 or more location errors (LM: $b = -0.03$, $SE = 0.02$, $F_{1,206} = 2.85$, $P = 0.09$; $N = 208$; Fig. 5A), the strength alone of initial bias estimated for all scored trials (LM: strength: $b = 0.03$, $SE = 0.02$, $F_{1,206} = 2.42$, $P = 0.12$; $N = 208$; Fig. 5C), or the strength alone of initial bias estimated from first trials only (LM: $b = -0.01$, $SE = 0.02$, $F_{1,189} = 0.29$, $P = 0.59$; $N = 191$).

Consistent directional bias

There were 294 birds scored for consistent directional bias in 2020–2021: 182 at high elevation and 112 at low elevation. The initial and consistent directional bias indices were tightly correlated (Pearson's product-moment correlation test—correlation coefficient = 0.84, 95% CI: [0.81, 0.87], $t = 26.76$, $df = 292$, $P < 0.001$; Fig. 6A). The strength of initial and consistent directional bias was also significantly correlated, but less tightly than for the full directional bias indices (Pearson's product-moment correlation test—correlation coefficient = 0.65, 95% CI: [0.58, 0.71], $t = 14.51$, $df = 292$, $P < 0.001$; Fig. 6B).

For consistent bias, birds showed a small but significant preference to move towards the left than towards the right, with a mean of 53% of trials to the left (mean $LI = 5.07$, one sample *t*-test: 95% CI [1.42, 8.71], $t = 2.47$, $df = 293$, $P = 0.01$; $N = 294$; Fig. 3B). In the first trial of each task, before birds could learn the new feeder location, birds also showed a small but significant bias to move left, with a mean of 55% left-scored trials (mean $LI = 9.65$, one sample *t*-test: $t = 3.44$, $df = 188$, $P < 0.001$, 95% $CI = [4.12, 15.18]$, $N = 189$). For all scored trials, approximately 22% of birds were relatively consistently biased ($N = 66$) compared to the sample mean ($-1.96 \leq$ z-score ≥ 1.96), which was significantly fewer birds than expected if the sample contained

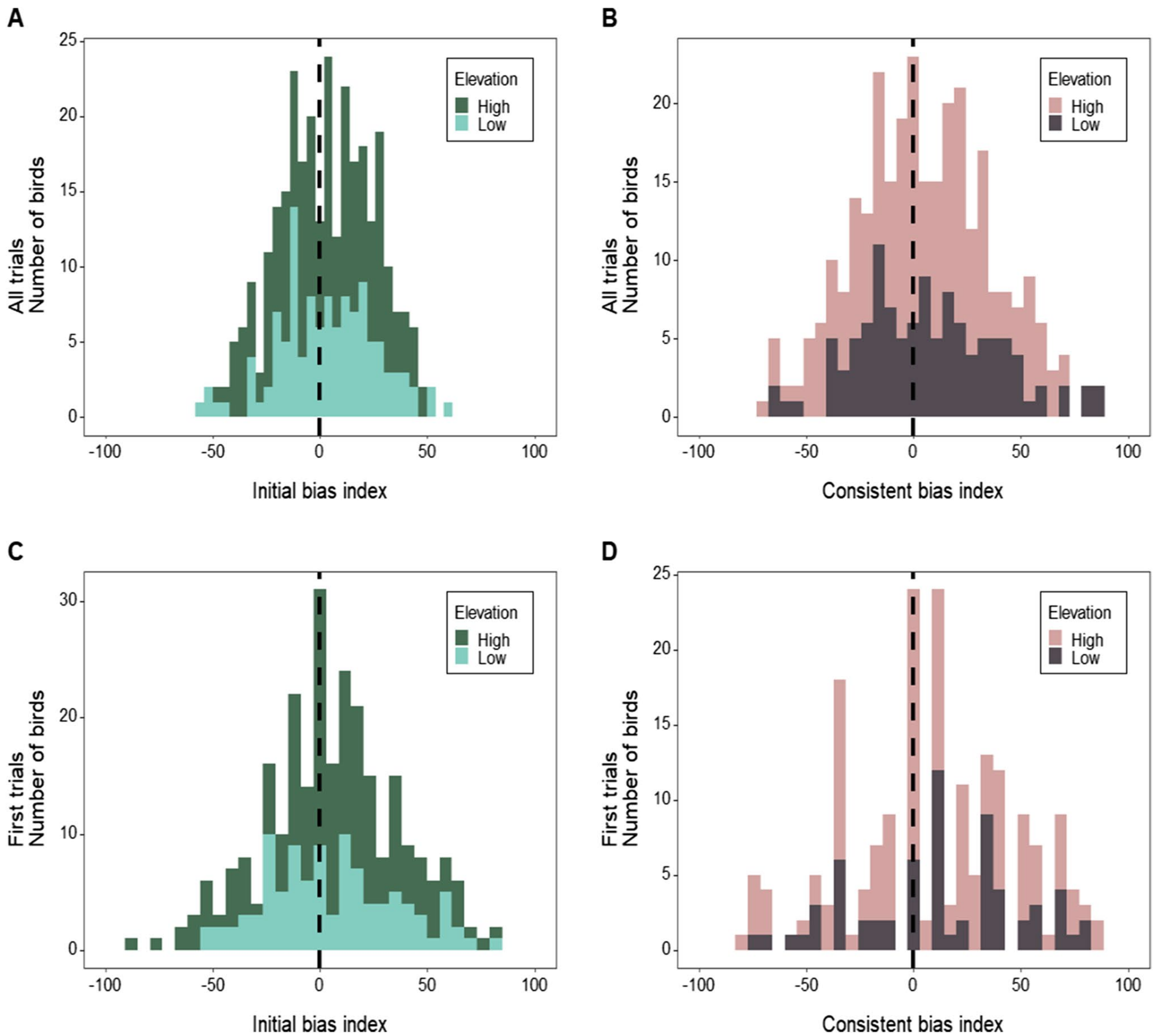

Fig. 3 Distribution of (**A**, **C**) initial and (**B**, **D**) consistent bias index scores for all trials in which birds visited at least 2 unrewarding feeders (e.g., 2 location errors) (**A**, **B**) and for the first trial of each task only (**C**, **D**) by elevation. The directional bias index ranges from fully right-biased ($LI = -100$) to fully left-biased ($LI = +100$) with middle values indicating unbiased ($LI = 0$, dashed line). Elevations are stacked (as in, low + high = total frequency). For (**A**), $N = 303$, for (**B**), $N = 294$, for (**C**) $N = 269$, and for (**D**) $N = 189$

equal numbers of consistent biased and unbiased birds ($\chi^2 = 89.27$, $df = 1$, p-value < 0.001; $N = 294$). Of the 66 relatively consistently biased birds, the majority were left-biased ($N = 43$). Only 3 birds were statistically significantly biased according to a binomial test, all left-biased (after correction for multiple tests using the Bonferroni correction factor).

Consistent directional bias across all trials with two or more location errors did not vary across elevations (LM: $b = 3.20$, $SE = 3.81$, $F_{1,292} = 0.70$, $P = 0.40$; $N = 294$; Fig. 3B) or between sexes (LM: $b = -6.45$, $SE = 6.24$, $F_{1,122} = 1.07$, $P = 0.30$; $N = 124$). Consistent directional bias in the first trial of each task also did not vary across elevations (LM: $b = 4.04$, $SE = 5.91$, $F_{1,187} = 0.47$, $P = 0.49$; $N = 189$) or between sexes (LM: $b = -4.53$, $SE = 8.82$, $F_{1,84} = 0.26$, $P = 0.61$; $N = 86$).

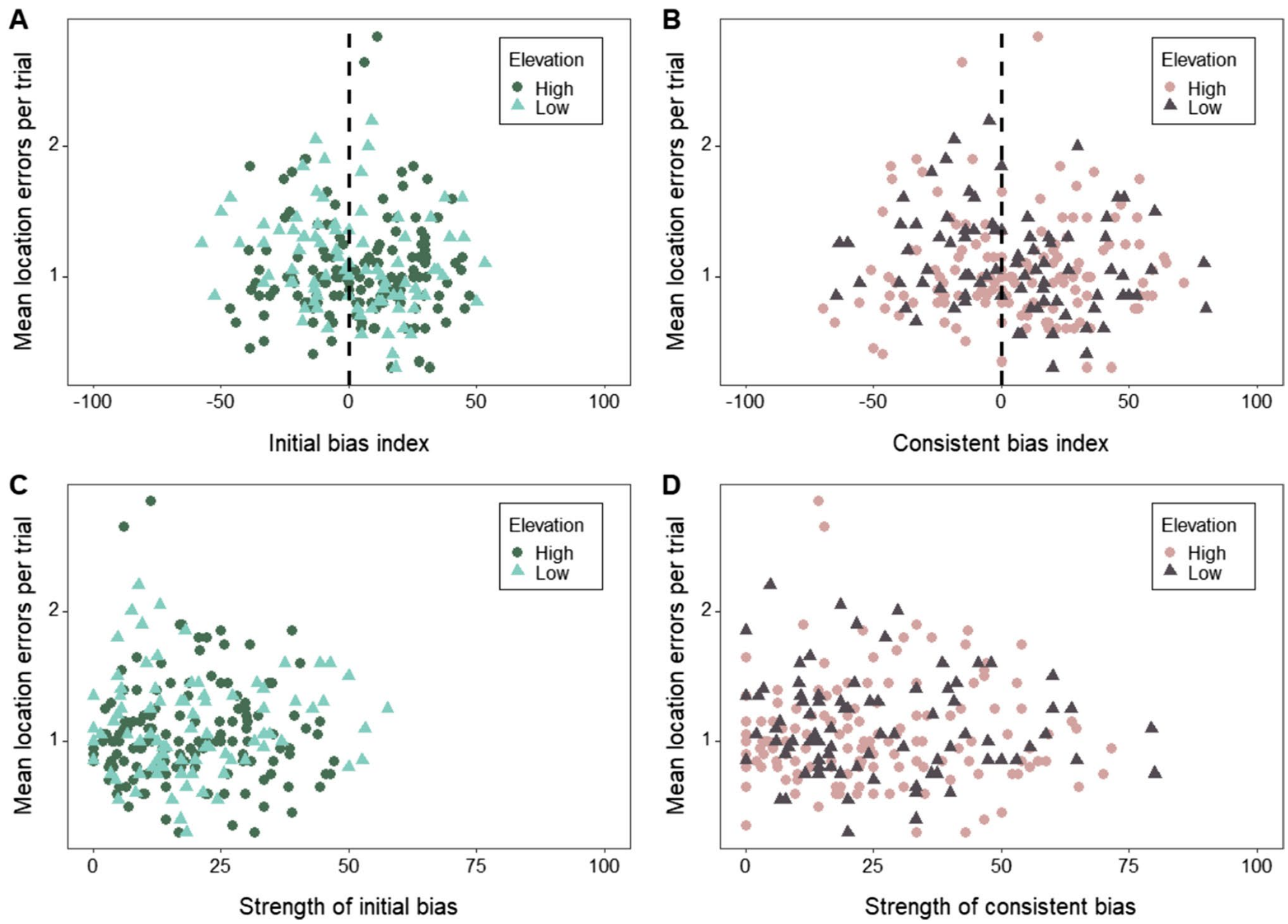

Fig. 4 Spatial learning and memory performance by (**A**) initial bias, (**B**) consistent bias, (**C**) strength of initial bias, and (**D**) strength of consistent bias. All bias estimates used all trials with at least 2 location errors. Cognitive performance measured in mean location errors per trial in the first 20 trials of the cognitive task, with smaller values indicative of better performance. Directional bias index ranges from fully right-biased ($LI = -100$) to fully left-biased ($LI = +100$) and the strength ranges from no bias (strength = 0) to fully biased (strength = + 100) regardless of direction. For (**A**) and (**C**), $N = 229$. For (**B**) and (**D**), $N = 225$

Cognition and consistent directional bias

Variation in spatial learning and memory performance was not significantly associated with differences in consistent directional biases for all scored trials with 2 or more location errors (LM: $b = -0.02$, $SE = 0.03$, $F_{1,223} = 0.34$, $P = 0.56$; $N = 225$; Fig. 4B), the strength alone of consistent biases for all scored trials (LM: $b = -0.01$, $SE = 0.03$, $F_{1,223} = 0.14$, $P = 0.71$; $N = 225$; Fig. 4D) or the strength alone of consistent biases for only the first trial of each task (LM: $b = -0.01$, $SE = 0.03$, $F_{1,156} = 0.12$, $P = 0.74$; $N = 158$).

Similarly, variation in single reversal learning performance was not associated with differences in consistent directional biases for all scored trials with at least 2 location errors (LM: $b = -0.02$, $SE = 0.\ 02$, $F_{1,202} = 1.63$, $P = 0.20$; $N = 204$; Fig. 5B), or the strength alone of consistent directional biases using all scored trials (LM: $b = 0.03$, $SE = 0.02$, $F_{1,202} = 2.91$, $P = 0.09$; $N = 204$; Fig. 5D) or the strength alone of consistent biases using only the first trial of each task (LM: $b = 0.03$, $SE = 0.02$, $F_{1,143} = 1.56$, $P = 0.21$; $N = 145$).

Repeatability of directional bias index

In 2019–2020, 206 birds were scored for initial directional bias and 170 for consistent bias. Between 2019–2020 and 2020–2021, directional bias scores were significantly repeatable at the individual level for both initial bias (repeatability: $R = 0.27$, 95% $CI = [0.10, 0.43]$, $D = 8.57$, $df = 1$, $P < 0.001$; $N = 385$) and consistent bias (repeatability: $R = 0.22$, 95% $CI = [0.04, 0.39]$, $D = 6.87$, $df = 1$, $P = 0.004$; $N = 366$). For only the subset of birds that were detected in both years,

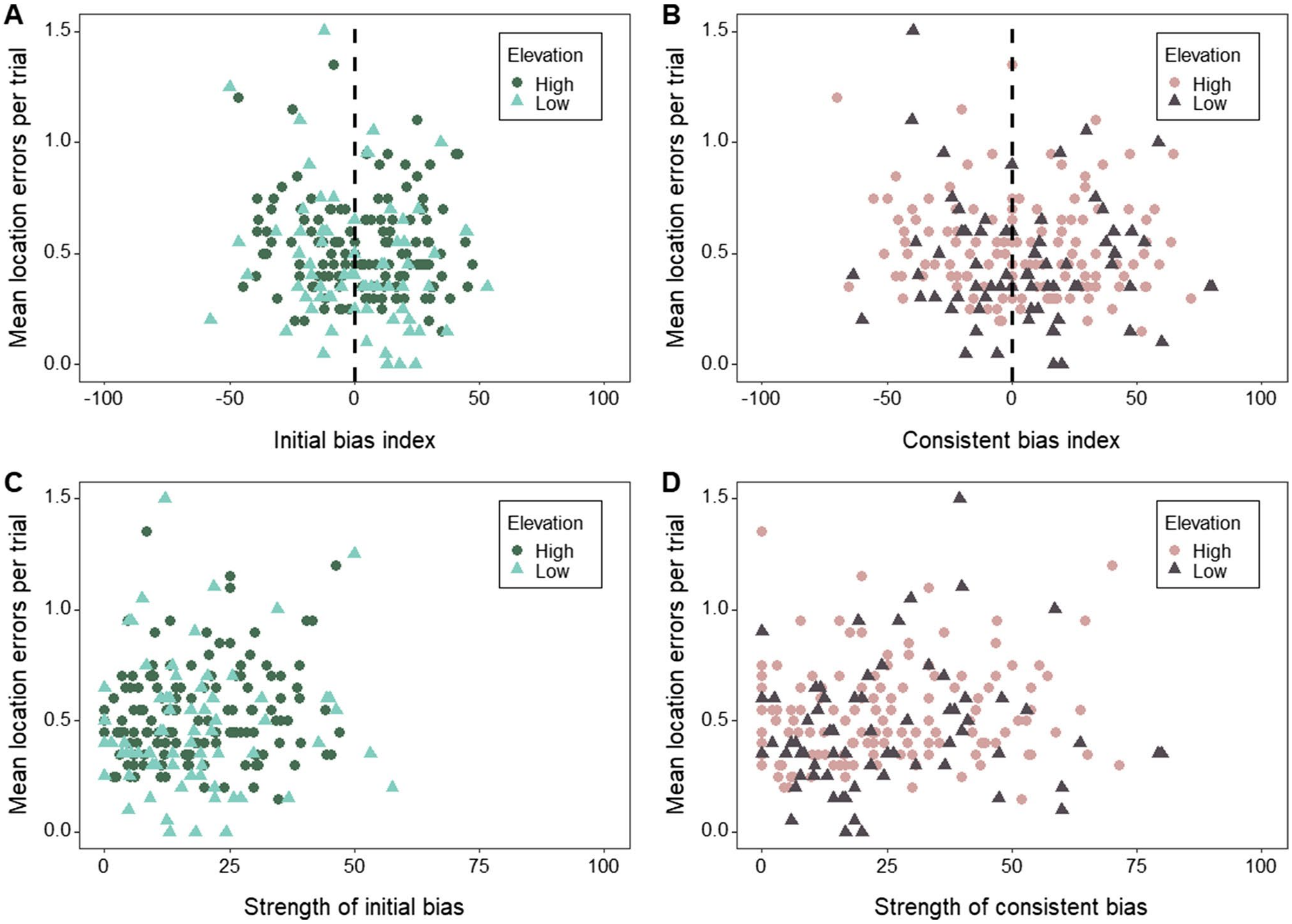

Fig. 5 Single reversal learning performance by (**A**) initial bias, (**B**) consistent bias, (**C**) the strength of initial bias, and (**D**) the strength of consistent bias. All bias estimates used all trials with at least 2 location errors. Cognitive performance measured in mean location errors per trial in the first 20 trials of the cognitive task, with smaller values indicative of better performance. Directional bias index ranges from fully right-biased ($LI = -100$) to fully left-biased ($LI = +100$) and the strength ranges from no bias (strength = 0) to fully biased (strength = +100) regardless of direction. Initial directional bias (greens) and consistent directional bias (red and purple) shown by high (green and red circles) and low (light green and purple triangles) elevation, even though elevation was not a focal variable. For (**A**) and (**C**), $N = 208$. For (**B**) and (**D**), $N = 204$

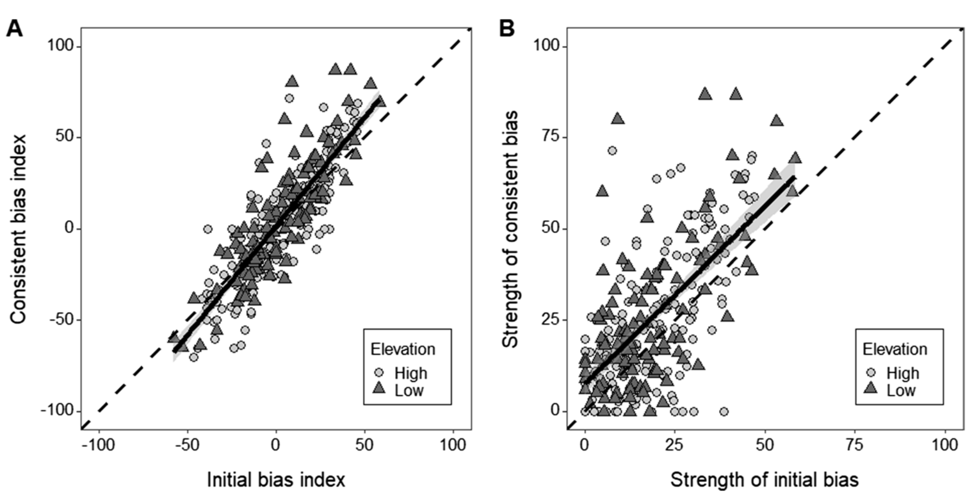

Fig. 6 Initial versus consistent bias (**A**) index and (**B**) strength. All bias estimates use all trials with at least 2 location errors. Black dashed line indicates a 1:1 relationship. Dark black line indicates linear regression line with 95% confidence interval (shaded areas). For (**A**), negative index values represent right bias and positive index values represent left bias. For (**B**), values range from weak or no directional bias (strength = 0) to fully left- or right-biased (strength = 100). $N = 294$

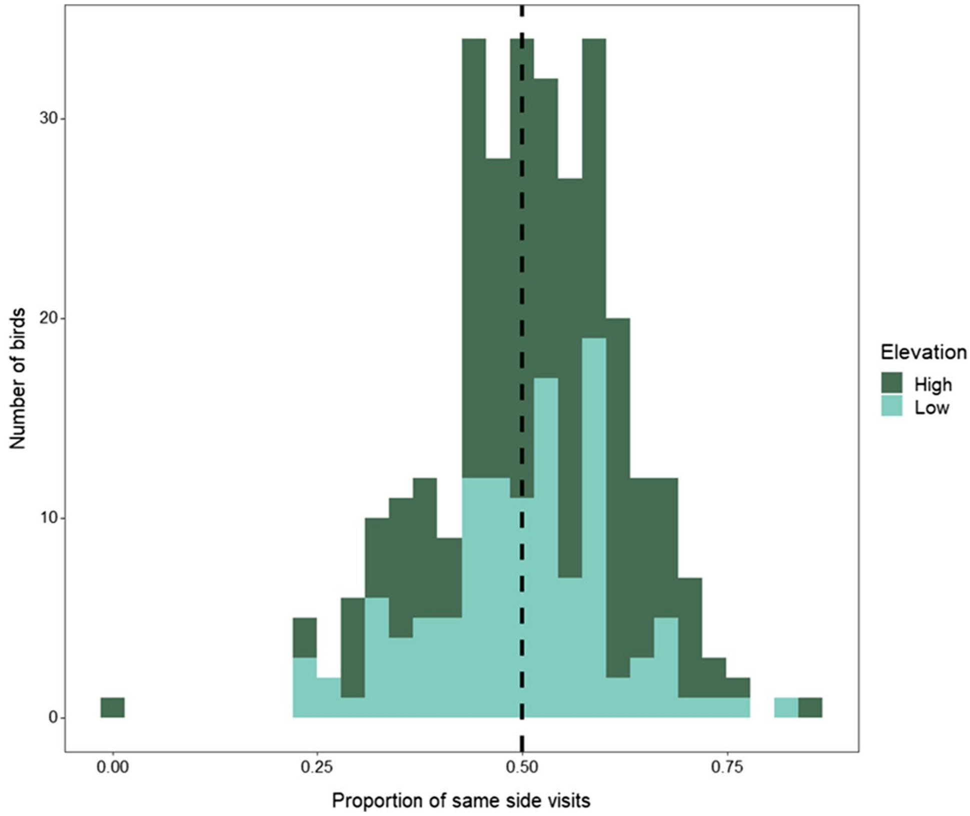

Fig. 7 Proportion of visits to a feeder on the same side of the feeder array as the previous feeder out of the total trials analyzed for initial directional bias. Shown by elevation. Dashed line indicates an equal proportion (0.50). $N = 303$

there was also no significant difference between years for initial (LMER: $b = -3.54$, $SE = 2.92$, $\chi 2 = 1.46$, $df = 1$, $P = 0.23$; $N = 124$) or consistent bias (LMER: $b = -2.03$, $SE = 3.74$, $\chi 2 = 0.38$, $df = 1$, $P = 0.54$; $N = 107$).

Same-side visits

Across all trials, birds were equally likely to visit the feeder on the same side of the array as a feeder on an adjacent side, with 51% of trials to the same side feeder (one-sample *t*-test: $t = 1.56$, $df = 302$, $P = 0.12$, 95% $CI = [0.50, 0.52]$, $N = 303$; Fig. 7).

Discussion

Overall, we found that chickadees did not show a directional bias in movement around the feeder arrays at either high or low elevation when learning a spatial task: although there was a small but significant bias to the left for both initial and consistent biases, this bias was small, and the majority of birds chose to move left and right relatively equally while searching for a food reward. Search behavior was targeted in the analysis by using trials in which birds visited at least 2 unrewarding feeders before visiting the rewarding feeder (e.g., 2 or more location errors), so that birds had not yet learned the location of the rewarding feeder during the spatial cognitive tasks. However, the results were the same even if we only used the first trial of each task before the birds even located the rewarding feeder. Few birds showed significant directional biases, although there was notable individual variation. There were no significant trends in directional bias scores between elevations or sexes, suggesting that movement around the arrays was not significantly influenced by environmental harshness or social dominance. Furthermore, neither variation in performance on the spatial learning and memory task nor in performance in the single spatial reversal learning task was significantly associated with differences in directional biases or in the strength of directional biases, regardless of whether we used all trials in which birds visited at least 2 unrewarding feeders or only the first trial for each task. These null results suggest that estimates of spatial cognitive performance measured using this experimental apparatus did not appear to be significantly biased by the direction birds chose to move around the feeder array. In addition, these results also show that the learning process does not affect the movement bias during all trials with 2 or more location errors.

The directional bias index was mildly and significantly repeatable for both initial and consistent bias. This suggests that although individuals were not strongly biased to the left or right, birds behaved similarly while searching for a food reward between two consecutive winter seasons, with most birds having no bias. This may suggest that birds have somewhat stable search strategies or general movement preferences at the feeder arrays, but that this behavior was not well-described by passive directional biases estimated using the bias index. Furthermore, there was no significant difference in directional bias estimates between two consecutive years, for birds detected during both years. This further suggests that the individual variation reported in our study did not appear to vary across the sample with annual conditions.

These null results suggest that chickadees do not demonstrate directional biases in movement during a search when learning a spatial task, and thus existing lateralization may not affect decision-making related to movement direction while searching the feeder arrays. However, as our study is observational and does not directly test for lateralization, we can only speculate, and it is also possible that any existing lateralization simply does not affect the decision process during movement while searching for food. Lateralization has been well documented in avian species (Rogers 2021), including in spatial cognition (Clayton and Krebs 1993; Güntürkün et al. 2020) and associated brain regions (Siegel et al. 2006; Jonckers et al. 2015). However, motor-based laterality may not directly correlate with lateralization; rather, it may only indicate that one hemisphere dominates the control of a given behavior (Rogers 2021). If neither hemisphere dominates a chickadee's decision to move left or right after approaching the feeder array, then lateralization may not be detected through movement around the array. Furthermore, lateralization appears to be task dependent (Schiffner and Srinivasan 2013; Karenina and Giljov 2022; Penry-Williams et al. 2022). Thus, it is possible that birds might not demonstrate laterality while searching for a food reward without additional cognitive demands, such as predator vigilance (Piddington and Rogers 2013). We did not have the data to explore this alternative.

Other noncognitive factors may have affected the direction chickadees chose to move around the feeder array, obscuring possible lateralization that may have been present. For example, socially subordinate birds may have been displaced from preferred feeders by more dominant birds, leading to more random directional bias. However, we find this unlikely because we found no significant difference in either initial or consistent directional bias between sexes for all trials and the first trials alone, despite known differences in social dominance between males and females in Parid species (Ekman 1989). This is consistent with our previous work, which shows that social dominance rank did not significantly affect learning (i.e., number of errors; Heinen et al. 2021). It is possible that we did not see effects of dominance or displacement because we only used trials with at least 2 consecutive location errors and only analyzed trials in which birds consistently moved in the same direction without being displaced. Such random disruption might have been more prominent in our dataset if we included trials with only one location error or if we included trials in which birds moved across the feeder array. In addition, it is unlikely that a bird would be displaced multiple times while consistently moving in the same direction across the feeders that do not provide food.

Our results do not support previous literature suggesting that more strongly lateralized individuals perform better on spatial cognitive tasks (Rogers 2021). However, our findings are not completely unexpected and contribute to a body of work reporting null or negative associations between behavioral asymmetries and cognitive performance (e.g., Whiteside et al. 2020). It is possible that our findings are simply due to the lack of lateralization in foraging-based search behavior in chickadees, and that by measuring a different behavior we might find the expected relationship between directional biases and cognitive ability. This might be likely if the cognitive processes and structures involved in foraging search behavior differ from those involved in spatial learning and memory. To assess this, further research involving several lateralized behaviors across multiple types of cognitive tasks (i.e., spatial learning, visual discrimination) would be needed. Another explanation could be that directional biases may be costly in natural conditions due to environmental factors. To speculate, a directional bias in movement could result in poorer foraging efficiency or less efficient cache retrieval. Such a directional bias may be more costly in harsh environments in which food may be less predictable or metabolic requirements may be higher, and thus could be less expressed in our study system. If this were the case, we might expect the directional bias to vary seasonally or vary across elevations that differ significantly in environmental harshness. However, we did not find the latter. And although we do not have the data to test for seasonal variation in directional biases, we have found that variation in spatial learning and memory has a strong genetic component (Branch et al. 2022). Finally, we speculate that lateralization in food-caching species may be constrained by other aspects of cognition that may be more advantageous, such as memory transfer or memory capacity. As food-caching species, chickadees rely on spatial learning and memory ability for cache retrieval, and this cognitive ability appears to be under selection in our birds (Sonnenberg et al. 2019). But the benefits of lateralization for food caching are still unclear and may depend on the ability to transfer information from one side of the brain to the other (Clayton and Krebs 1993). While more research is needed, this could explain why directional biases were not evident in the spatial cognitive tasks used in this study.

Finally, we must consider several limitations of the study and the possible effects on our interpretations. First, although feeders were relatively equidistant from each other, the square shape of the feeder array could bias birds to move more frequently between feeders on the same side of the square array. However, birds were equally likely to move to another feeder on the same side of the array as to a feeder on one of the adjacent sides after making a location error. Second, we used visit data to infer movements between feeders, but did not validate these data through behavioral observations. As such, we do not know how frequently birds might have left the arrays in between feeder visits, leading us to misinterpret those trials for left or right bias. However, we have often observed that birds do not leave the arrays until they have found a food reward, typically sampling feeders consecutively until they reach the correct feeder (see Supplemental Video). In addition, the mean time for trials with at least 2 location errors was small (< 1 min), suggesting that visits were in rapid succession. This suggests that birds likely did not move away from the array during these trials, considering that all nearby foliage was ca. 3 m away and so the only nearby perches that were not attached to the feeders were the wires that suspended each array. Plus, consistent bias estimates should be more robust to this type of bias than initial bias since consistent bias was calculated from two consecutive pairs of visit data. Yet, initial and consistent directional bias estimates yielded similar results in our analyses, suggesting that both bias estimates used visit data that represented actual movements around the feeder array. Third, in our study, we estimated directional bias while birds were learning a spatial task, specifically using trials with 2 or more location errors so that birds had not fully learned the feeder location yet. Thus, there is a possibility that learning itself might have affected the birds' movement rather than passive directional biases. We find this unlikely because if learning affected movement bias, then we would expect to see an association between cognitive ability and directional biases, which we do not. We also found similar results whether we used all qualifying trials from the cognitive tasks or only the first trials of each task, in which birds had not found the correct rewarding feeder yet and thus should be free from a possible confounding effect of learning. Finally, our study can only address movement bias, not lateralization. While directional bias might be very similar, the feeder perches allow birds to use either eye to look in either direction before deciding to move to another feeder perch. Thus, our setup is appropriate to evaluate how directional biases might affect decision-making during a cognitive task. But they should not be used to assume that these movements are driven by underlying lateralization at a neural level.

A major challenge in cognitive ecology is designing experiments that test specific cognitive traits and minimize the role of other non-cognitive factors. Assessing how directional biases might impact behavior during cognitive tasks is thus essential to interpret cognitive performance, especially when experiments are conducted in natural settings. Our null results from a large sample size suggest that mountain chickadees in our system did not demonstrate strong left or right preferences in movement while searching for a food reward, before learning the location of a rewarding feeder during spatial cognitive tasks. These results also show that individual variation in these directional biases did not affect performance on two cognitive tasks. Directional biases may still be present in our population and may be more apparent in other tasks or behaviors, but more research is needed to understand how such biases might be important for spatial cognitive tasks in natural contexts.

Overall, we did not detect significant directional biases in the foraging behavior of food-caching mountain chickadees across two consecutive winters in two different environments characterized by strong differences in winter conditions. Our results show that search behavior in food-caching chickadees did not appear to be biased by individual preferences in movement-related decision-making, which might allow a more efficient search during foraging.

Appendix

Table 2 Number of birds excluded during data preparation for 2020–2021 and 2019–2020 in order to create initial and consistent bias datasets

	Number of birds			
	2020–2021		2019–2020	
Reason for exclusion	Initial bias	Consistent bias	Initial bias	Consistent bias
Location errors < 2	5	5	4	4
Distance between feeders > 2	1	3	2	3
Trials scored left or right < 6	11	18	9	44
Outlier	1	1	0	0
Final dataset	303	294	206	170

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-022-03275-6.

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Author contribution LMB: conceptualization, methodology, investigation, formal analysis, writing—original draft preparation, writing—reviewing and editing, and visualization. VKH: methodology, investigation, data curation, and writing—reviewing and editing. BRS: investigation, and writing—reviewing and editing. AMP: investigation and writing—reviewing and editing. ESB: methodology and writing—reviewing and editing. VVP: conceptualization, methodology, investigation, writing—original draft preparation, writing—reviewing and editing, funding acquisition, project administration, and supervision.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Mendeley Data Repository, https://doi.org/10.17632/jsgv8jp8cp.2.

Declarations

Ethics approval The use of animals adhered to the guidelines set forth by the University of Nevada Reno Institutional Care and Use Committee (Protocol 00818, 00046, and 00603) and the California Department of Fish and Wildlife (Permit D-0011776516–4). Banding wild birds adhered to guidelines set forth by the U.S. Geological Survey Bird Banding Laboratory (Federal Bird Banding Permit 22878). To the best of our knowledge, no birds were harmed by the collection of these data, and birds were only handled for a few minutes during banding. We detected no negative effects of using PIT-tags and color bands during our study.

Conflict of interest The authors declare no competing interests.

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