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Effect of vertical complexity of rearing environment on the ontogeny of depth perception in laying hens

By

CLAIRE TAYLOR JONES

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

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in the

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of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2021

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Effect of vertical complexity of rearing environment on the ontogeny of depth perception in laying hens

ABSTRACT

Multi-tiered aviary systems provide laying hens with opportunities for species-specific behaviors, including vertical and horizontal movement. However, collisions and failed landings that occur during vertical movement can be associated with injuries, such as keel bone fractures. Previous studies have suggested that floor rearing of pullets with minimal access to elevated structures may negatively impact adult laying hens' ability to navigate vertical space. Unfortunately, it is not clear whether this is due to deficits in physical ability or if differences in rearing environment influence the development of adequate spatial cognition. The effect of rearing environment on the development of spatial cognition was investigated in egg-laying hens using a novel Y-maze task and a visual cliff task at 7-8, 15-16, and 29-30 weeks of age. Dekalb White pullets (n = 450) were reared in three different environments until 16 weeks of age: floor, single-tier aviary, and multi-tier aviary. At 16 weeks, all birds received a multi-tier aviary for the laying period. Distance perception was evaluated via a Y-maze task with a ratio of 1:3 or 1:1 difference in escape arm length and exit choice was recorded. To evaluate depth perception, hens were placed on a perch in the center of the visual cliff table, facing the perceptual cliff. Each bird was tested with three trials with random assignment of cliff depth at 15, 30, and 90 cm below the perch. Birds were given the option of escaping by jumping to a platform suspended over the visual cliff. Behaviors recorded included crossing the visual cliff and number of downward head orientations over the cliff edge. It was found that birds, regardless of age and rearing treatment, exited the Y-maze through the shorter (0.70, *P*

< .001) arm more than chance. An interaction effect of age and rearing treatment on crossing the visual cliff (Wald $x^2 = 12.734$, df = 4, $p = 0.013$) was found, with hens reared on the floor being less likely to cross the visual cliff at 8 and 16 weeks of age than 30 week old floor reared birds or multi and single treatment birds at 8 and 30 weeks old. These results suggest that floor reared birds do not have impaired depth perception, but do respond differently when faced with a vertical structure prior to being transitioned to adult housing. This has implications for pullet rearing and the importance of early access to vertical structures.

INTRODUCTION

Laying Hen Housing

Hens housed in conventional cage systems produce the majority of eggs worldwide, however in recent years many countries have shifted to alternative production systems, such as cage-free aviaries (Rondoni et al., 2020). Conventional cages house small groups of about 6-7 hens in cages with about 67-86 square inches of space per bird (United Egg Producers, 2017b). Cage-free aviaries, on the other hand, house hens in large flocks with approximately 144 square inches of space per hen (United Egg Producers, 2017a). The shift away from conventional cages is due to increasing consumer preference for cage-free eggs, resulting from consumers' concerns about the welfare of laying hens housed in conventional cages (Rondoni et al., 2020). Consumers perceive hens from cage-free systems to have enhanced welfare when compared to hens in conventional cages, despite many consumers being unaware of the meaning behind egg labels or the differences between different production systems (Rondoni et al., 2020). This public perception has also coincided with several states passing legislation that aims to phase out the use of conventional cages. California's Proposition 12 mandates that all eggs produced and sold in California are cage-free by 2022. Similar legislation has followed, including bills passed in Colorado (Egg-laying Hen Confinement Standards), Michigan (Senate Bill No. 174), Oregon (Senate Bill 1019), and Washington (Wholesome Eggs and Egg Products Act).

Cage-free systems have numerous welfare benefits when compared to conventional cages, including the opportunity to perform natural behaviors such as wing flapping, dust bathing, perching, and flying (Lay et al., 2011). However, cage-free systems also come with drawbacks. Adult hens housed in commercial aviaries are prone to injuries including keel bone

fractures, which may occur during collisions with tiers, perches, and other features of the aviary (Sandilands et al., 2009). There is a great need to determine why collisions are so common in cage-free aviary systems and what management solutions can be implemented to reduce injuries in cage-free flocks.

Young hens, or pullets, are housed in a rearing system for the first 15 to 18 weeks of life before being moved to their adult laying system. The complexity of the pullet rearing environment and early access to vertical space has been shown to play a role in reducing keel bone fracture prevalence in adult hens (Ali et al., 2019; reviewed by Janczak & Riber, 2015). Gunnarsson et al. (2000) proposed that rearing chicks on the floor, without access to perches or platforms, impairs the development of spatial cognition. One possibility is that this impairment to spatial cognition could contribute to failed landings and navigation of commercial aviaries, increasing the incidents of fractures.

Many studies since Gunnarsson et al. (2000) have continued to provide evidence that the complexity of rearing environment influences performance on spatial cognition tasks (Norman et al. 2019; Tahamtani et al., 2015; Wichman, 2007). However, none of these studies have specifically looked at the development of depth perception and its potential relation with early exposure to vertical space. A deficit in precision of depth perception could explain the occurrence of failed landings and falls, due to an inability to properly gauge the distance to fly or jump.

Depth Perception

The visual cliff has been used for the evaluation of depth perception and differential visual depth threshold (see Glossary) since its invention by Walk et al. in 1957. It utilizes two depths, a shallow side and a deep side, and can be adapted for a variety of species. The subject is

placed between the shallow and deep side, in the center of the table. The shallow side is typically level or about a few centimeters below the starting point of the subject, however, the bottom of the deep side is far below the subject. The deep side is covered with a sheet of plexiglass so the subject can perceive this depth but, unbeknownst to the subject, is not in danger of falling. Many designs employ the use of checkerboard patterns to provide visual perspective, allowing for an easy determination of depth. The behavior of the subject (i.e., move to the shallow side or deep side) is recorded to determine their ability to differentiate the depths and avoid "falling down" the perceptual precipice created by the deep side of the visual cliff.

The visual cliff is a widely used test of depth perception that has the advantage of involving a clear, straightforward choice: Does the animal move to the shallow or deep side? The test is not physically challenging for the subject and there is no training required. Therefore, many animals can be tested and there are no confounding variables of learning or physical ability. Despite these advantages, the visual cliff is not free of flaws. The plexiglass over the "deep side" of the cliff can provide tactile information about the presence of a barrier if the subject comes in contact with the surface. There is also a potential for reflection on the plexiglass, giving a visual indicator of an additional surface over the "deep side." If the subject can detect the plexiglass barrier through either visual or tactile information, the illusion of a cliff ceases and the test no longer compares the subjects' reaction to differential depths.

Using the visual cliff paradigm, chickens have been found to have excellent depth perception, preferring the shallow side of a visual cliff significantly more than the deep side from as early as one day of age (Walk & Gibson, 1961). Additionally, four day old chicks readily jump down a drop off of less than 10 inches to join their companions but hesitate if the drop is more than 16 inches (Thorndike, 1899). Chicks demonstrate a 2 inch threshold for differential

visual depth, meaning, chicks perceive a difference in depth only when the discrepancy is 2 or more inches (Walk et al., 1968).

Unlike humans, chickens do not require binocular vision, or stereopsis, to perceive depth. Chicks with monocular vision (one eye occluded) are able to perceive depth as well as their binocular counterparts, with both groups choosing the shallow over the deep side of the visual cliff significantly more (Schiffman & Walk, 1963). Although all birds have binocular vision, there is no evidence that birds other than certain birds of prey use stereopsis (see Glossary) to acquire information on relative depth (Tyrrell & Fernandez-Juricic, 2017). Instead, birds use monocular cues such as motion parallax and interposition (see Glossary) to judge relative depth (Tyrrell & Fernandez-Juricic, 2017).

Walk and Gibson (1961) provided evidence that the ability to perceive depth is innate in multiple species. However, certain factors during development, such as light and monocular deprivation, can cause impairments in depth perception (Timney, 1983; Walk & Bond, 1968). This raises an interesting question: can other differences in visual experience, such as reduced experience with height and depth, alter depth perception abilities?

Spatial Cognition

The relationship between spatial cognition and rearing environment in laying hens has been evaluated in previous studies using a variety of tests including the jump test, holeboard task, radial maze, and detour paradigm. In order to better understand these tests, the definition of spatial cognition must be addressed. Spatial cognition is defined as a multifaceted topic entailing the perception, processing, and interpretation of objects, space, and movement (Waller & Nadel, 2013). Spatial cognition encompasses many different aspects of visual perception such as spatial memory and navigation, determining the orientation of objects, and perceiving depth (Waller &

Nadel, 2013). Despite this diverse array of topics under this broad term, many researchers outside the field of cognition discuss spatial cognition without specifying which aspect they are investigating. By failing to properly define terms or control for a precise aspect of spatial cognition, researchers can inadvertently measure unintended variables and make inaccurate conclusions.

Over the last two decades there has been a great deal of interest in how the complexity of rearing environments may affect the development of spatial cognition skills/abilities of laying hens. Multiple tests have been used to evaluate different aspects of spatial cognition, including: jump test, holeboard test, radial maze, and detour task. Each test has targeted objectives aimed at specific cognitive processes, and comes with advantages and disadvantages.

Jump Test

Gunnarsson et al. (2000) developed a jump test to evaluate spatial cognition in laying hens, however the specific aspect of spatial cognition being explored was not addressed. The jump test involves placing a feeder on an elevated platform with or without a second, lower platform to assist in access. Pullets' latency to reach the food reward is measured to assess their ability to navigate elevated structures. Chicks were raised with either early access (1 day old) or late access (8 weeks old) to perches of varying heights. At 9 weeks old, all birds were placed on perches 2 to 4 times daily to encourage perch use. At 15 weeks all food in the home pen was placed on a 60 cm high tier, which the pullets could access from the ground or via nearby perches. At 16 weeks old, food deprived birds were presented with a feeder on an elevated tier in a testing pen. The height of this tier increased by 40 cm each trial up to 160 cm high and an intermediate tier was provided to aid in access for two of these trials. The time it took for the birds to reach the food after entering the testing pen was recorded and used to measure their

success and ease at reaching the elevated food reward. There was no difference in the time that it took birds from different rearing treatments to successfully reach the food on the 40 cm tier. However, there was a significant difference between the treatments when the difficulty of the task increased, with more successes and shorter latencies to access the food from the early access to perch group. The number of birds that successfully reached the tier decreased with increasing difficulty for both rearing treatments.

Norman et al. (2019) also conducted a jump test with chicks reared from hatching in either a control or enriched treatment. The control treatment had no elevated structures while the enriched treatment had eight wooden perches arranged in an A-frame structure and a ramp leading up to a platform with additional perches. Like the Gunnarsson et al. (2000) study, this test also utilized staggered tiers that could be used to reach a reward, however the tiers were opaque and companion chicks were used as a reward instead of food. Chicks were tested at 14-15 and 28-29 days of age by placing them in a compartment with familiar chicks from their home pen in a mesh holding container. For the first test the companion chicks were on a 20 cm high tier and there were no other tiers present. For the second test, the 20 cm tier remained, however the companion chicks were placed on an additional 40 cm high tier. The time taken and success of the experimental chick to reach the confined companion birds was recorded. Norman et al. (2019) found that there was a significant difference between age groups on success of reaching the reward, however there was no effect of rearing treatment. In addition, there were no significant differences in the latency to complete the jump test between treatment or age.

There are many possible reasons for why there were different results from these two studies. First of all, the difference in ages tested in these two studies could have played a role as Norman et al. (2019) found there was greater success for 28-29 day old chicks when compared to

14-15 day old chicks. Additionally, the use of opaque tiers prevented the chick from seeing the reward on the tier in Norman et al. (2019). It is possible that the inability to see the reward reduced the birds' motivation to jump to the raised platform. Most importantly, the different rewards used (i.e., food versus social proximity) could have also played a role in the different performances observed.

Regardless of the reasons for the difference in results, this test poses issues when used as an evaluation of spatial cognition. The aspect of spatial cognition being measured was not specified and, more importantly, this test confounds spatial cognition with physical ability. The task increases in difficulty to reach the reward with each trial. This would explain the decrease in number of birds in both rearing treatments that were able to successfully reach the tier as the difficulty increased. Also, providing perches to pullets increases leg muscle deposition in adult hens (Hester et al., 2013), which could explain the different performances among the birds of the two rearing treatments.

Gunnarsson et al. (2000) defended this test as a valid measure of cognition because there was "no obvious reason to believe that the physical effort required to jump from 40 to 80 cm was substantially different" (Gunnarsson et al., 2000, p. 226). Although jumping from the ground to the 40 cm tier may not be more physically taxing than reaching the 80 cm tier from the 40 cm tier, these were not the only heights presented to the birds. It would be reasonable to presume that reaching the 80 cm tier without the aid of an additional platform would be more difficult than reaching the 40 cm tier.

The jump test may be used to evaluate differences in physical ability to reach higher tiers, however this task cannot separate physical ability from spatial cognition (e.g., visual acuity,

depth perception, etc.). For these reasons, the jump test may not be an ideal test to determine if spatial cognition is impaired by lack of access to vertical space during rearing.

Holeboard Test

A holeboard test is used to evaluate spatial reference memory and spatial working memory (van der Staay et al., 2012). This test involves presenting a subject with a variety of baited (i.e., food reward) and unbaited holes arranged in a grid. An animal is placed in the arena and is given free choice to visit any or all of possible holes. However, revisiting a previously baited hole will not be reinforced, as the food reward will have already been consumed.

Working memory keeps a small amount of relevant information readily accessible while an animal is completing a task (Cowan, 2014). Spatial working memory can be evaluated by measuring how often the subjects revisit holes where they already consumed the reward or holes which have already found to be unbaited (van der Staay et al., 2012). This is measured by the ratio of rewarded visits to the number of visits to the baited holes (Tahamtani, 2015). Alternatively, reference memory is a form of long-term memory. Spatial reference memory can be examined using the holeboard apparatus by repeating the same array of baited and unbaited holes over the course of multiple trials to determine if the animals' rate of finding baited holes improves (van der Staay et al., 2012). This is determined using the ratio of the number of visits to baited holes to the number of visits to all holes (Tahamtani et al.,, 2015). This improvement implies the location of the food rewards is being retained in reference memory and is being successful retrieved during trials (van der Staay et al., 2012).

Tahamtani et al. (2015) investigated the impacts of rearing environment on navigation and spatial memory in laying hens using a holeboard task. Chicks were housed in cages for 4 weeks after which the aviary-reared birds were released into multi-tiered aviaries while the cage-

reared birds remained in cages until 16 weeks, when all birds were placed in group-housed furnished cages. The holeboard task consisted of nine chalk circles with a blue cup positioned in the center, concealing a food reward of live mealworms. The birds were trained to associate the cups with a food reward and only those that readily explored the test arena and found mealworms were selected.

Four phases were used for training and testing: uncued acquisition, cued acquisition, over-training, and reversal phase. Uncued training and testing involved baiting only three of the nine cups in the same configuration for all exposures without any specific cues for guidance. This acts as a baseline for the hens' ability to learn the location of the baited cups without any specific cues. Cued acquisition followed, where hens were trained and tested on the same configuration, however red boards were placed under the baited cups to serve as an extra cue for the location of the food reward. This presents new information that may improve the birds' ability to complete the task. For over-training, the cues were removed to reestablish their baseline performance. Finally, for the reversal phase, the hens were trained and tested on an alternate configuration of three baited and six unbaited cups. The reversal phase requires flexibility in reference memory, as the birds must override the previously learned configuration in favor of the new information.

It was found that cage-reared birds took longer to complete the holeboard task during the reversal phase than the aviary-reared birds, while the aviary birds had a better score for their working memory during the reversal phase when compared to cage-reared birds. This impairment of working memory during task reversal may be due to the decreased complexity found in caged systems when compared to aviary systems. Multi-tiered aviaries provide a greater amount of variability in terms of social interaction and location of resources than caged systems,

potentially aiding in the development of spatial learning and memory.

The holeboard task is an excellent test for evaluating spatial learning and memory, however this test is not relevant at determining if these birds are more capable of navigating vertical space, since it occurs on a flat surface, or geometric plane (see Glossary), and does not take into account vertical space. It offers insight on differences in the working memory of cage-reared and aviary-reared birds, however these results do not suggest that chicks reared with access to vertical space have an enhanced ability to avoid colliding with structures.

Radial Maze

A radial maze is a cognitive task that is designed to evaluate spatial working memory (Paul et al., 2009). It involves multiple walkways, or arms, radiating out from a central chamber. A food reward is located at the end of each arm and the subject is placed in the center. The subject is then allowed to freely choose to enter the arms of the maze until all food rewards have been found. The optimum strategy for finding the food rewards would be to enter each arm only once, as previously visited arms will no longer contain a food reward. In order to efficiently solve the maze, the subject must employ working memory to retain information about which arms have already been visited. The animal does this by noting cues, typically extra-maze cues, to determine which arms they have already entered (Paul et al., 2009). Since this maze requires adequate spatial memory, it has been employed as a technique to evaluate spatial cognition in fowl raised in environments of varying complexity.

Wichman (2007) used a radial maze to investigate spatial ability in 16 week old laying hens raised in three different rearing environments. All rearing treatments had access to perches at 20 and 40 cm of height but the control group had not additional enrichments. The floor enrichment group had the addition of wooden blocks while the hanging enrichment group had

hanging discs and bottles at beak height. At 16 weeks of age, once all birds had been regularly perching, the birds were tested on an eight arm radial maze. Each arm of the maze was baited at the end with a mealworm and birds were given 20 minutes to freely explore the maze.

In order to simulate the practice of moving hens from their rearing house to the laying house, all birds were moved to larger, more complex pens at 18 weeks of age. These new pens included perches at varying heights (20, 40, 50, 90 and 130 cm), which could be reached from the floor or by jumping from one perch to another. It was found that there was a relationship between performance on the radial maze and propensity to perch. Birds that used perches the most during the two hours period after being released into the new, complex pens required fewer visits to the arms of the radial maze in order to find all eight of the mealworms. Wichman (2007) suggested that based on these results, there is a relationship between two-dimensional spatial ability required for performance on the radial maze and three-dimensional skills required for perching. However, there were no significant differences between the treatment groups for onset of perching or performance on the radial maze. The author suggested that the low height of the perches might have allowed for easy access to vertical space between all treatments. Therefore, there may not have been enough variation between the perching behavior of the chicks to result in clear differences between treatments.

Whiteside et al. (2016) used an eight arm radial maze to investigate the impacts of floor rearing on spatial cognition and survivability of pheasants raised in captivity and released for hunting. Whiteside et al. (2016) reared one day old chicks until 7 weeks of age in three different environments: standard commercial rearing with no access to perches; access to natural hazel boughs perches; and access to artificial perches. There were no significant differences between the natural and artificial perch groups, so these conditions were combined for analysis.

At 6 weeks of age, 27 chicks were tested on an eight arm radial maze to assess their spatial working memory. At the center of the maze there was a circular starting compartment and at the end of each arm of the maze there was a barrier, which concealed a food reward. Orientation in the maze was possible using extra-maze cues as the walls of the testing room were all different in color. The birds were first habituated to the arena for four days and were tested on the task on the fourth day. To solve the maze correctly, the birds had to enter each arm only once and consume the food reward. If a bird entered an arm where they had already eaten the food reward, it was recorded as an error. Those that made the fewest errors were determined to have better spatial memory than those that made more errors.

Birds reared without access to perches made significantly more errors in their first eight choices than birds reared with access to perches. This suggests that access to perches at a young age improved the spatial memory of pheasants, potentially aiding in their survival post release. It is also of interest to note that birds reared with perches roosted at night during the two weeks post-release significantly more than birds reared without perches. These two studies demonstrate that performance on a two-dimensional test of spatial memory may impact future use of vertical space. This could have implications for hens' ability to recall the location of resources (i.e., long-term spatial memory) and ability to navigate complex environments as an adult (i.e., working spatial memory).

Detour Paradigm

In the detour paradigm, the most direct route to a goal is blocked; the animal must walk around a barrier to reach the goal (Kabadayi et al., 2018). For the initially visible goal detour task, the goal is visible at the starting point but then becomes occluded by opaque walls as the animal begins to move through the apparatus. When the goal is initially visible, spatial working

memory as well as route planning must occur in order to hold the location of the goal in memory and make choices about what route should be taken. Success on this task is often interpreted as the animal making a mental representation of the location of the non-visible goal and then using this representation to determine the best route (Kabadayi et al., 2018). Due to the use of route planning and the need to hold the location of the goal in working memory in order to successful solve this task, the detour paradigm has been frequently used to evaluate spatial working memory and route planning.

For Wichman (2007) and Norman et al. (2019), the detour apparatus consisted of an arena with a starting compartment in the center, across from a holding compartment. The starting compartment consisted of three walls, two opaque and one of wire mesh, so that the holding compartment was visible through the wire mesh. Opposite the wire mesh screen was an opening in the back of the compartment allowing access to the rest of the arena. The holding compartment consisting of a screen so that its contents could be seen by the individual in the starting compartment. The test chick was placed in the central starting compartment and two of its companions from it home pen were placed in the holding compartment. To solve this task, the chicks had to walk away from the familiar chicks, out of the central staring compartment, and around this compartment to reach their companions.

Wichman (2007) tested ninety 3 day old chicks on a detour test to evaluate their spatial ability. The young chicks had not yet developed perching behavior at the time they were tested. As with the radial arm experiment, all birds were raised in three different rearing environments: control, floor enrichment, and hanging enrichment. The time it took chicks to reach their companions was recorded or, if the chick was unable to successfully complete the task, the trial elapsed at 10 minutes.

Forty out of the ninety chicks successfully solved the detour task, however, no relationship was found between onset of perching behavior and the performance of the chicks on the detour test. This result suggests that the detour test is not a good predictor of early perching behavior in pullets. Additionally, there was no significant effect of rearing environment complexity on the success of chicks in the detour task.

Norman et al. (2019) also employed a detour task in order to assess if early access to elevated structures affects spatial working memory and route planning in laying hens. They raised chicks from one day of age in either a control or enriched treatment. The control treatment had no elevated structures and the enriched treatment had eight wooden perches arranged in an A-frame structure. In addition to the perches the enriched treatment also had a ramp leading up a platform placed on the second rung of perches.

Norman et al (2019) tested 48 chicks from both rearing treatments on a detour task at either 14-15 days of age or 28-29 days of age. The test chick was placed in the starting compartment and two of its companions from their home pen were placed in the holding compartment. If the chick left the start box and began walking around the compartment, but then reentered the center of the arena, this was considered an orientation error. Chicks were given a maximum of 5 minutes to complete the task. Out of the 96 birds tested, 67 (33 from enriched, 34 from control) completed the detour task. There was no significant difference between treatment groups for the number of orientation errors, however the latency to complete the task was significantly shorter for the enriched as compared to the control chicks.

Morris (2009) also used a detour task to assess birds from two different rearing treatments on their spatial reasoning (see Glossary). For this study, the unenriched condition included shavings, a hanging feeder, and a nipple line drinker. The enriched treatment included

two perches, three live plants for cover, a dustbathing box, two hanging party decorations, and hidden mealworms. At 10 weeks of age 42 birds (21 from each treatment) were tested on a detour task. The target consisted of three familiar birds from their home pen. The familiar birds were confined to a holding pen in the top left corner of the arena. A barrier was located at the other end of the arena and was made of a mesh screen with an opening on the far-left side, across from the target. The test bird was placed in a starting location behind the barrier and was given 3 to 5 minutes to acclimate in a mesh enclosure. Once the enclosure was lifted, the birds were given 5 minutes to reach their companions. The latency of each test bird to navigate around the barrier and come within 0.5 m of the target was recorded.

No significant differences between treatment groups in time taken to reach companions were found. These results contradict the finding from Norman et al. (2019) that birds with accesses to vertical structures at a young age did have a shorter latency to complete the detour task than birds without access to vertical structures. It is possible that the difference in these results could be due to the different rearing environments and detour design of these two experiments. The design of the detour task in Morris (2009) differed from the design of Norman et al. (2019) and Wichman (2007). Instead of the chick walking away from the companion chicks to reach the goal, the Morris (2009) design required the chicks to walk towards the companions with them in view at all times through a mesh screen. The birds were unable to take the most direct route, a diagonal path across the arena, but were not required to walk away from the target at any point to successfully solve the task. Due to this, the Morris (2009) detour task does differ from the classic detour task used by Wichman (2007) and Norman et al. (2019).

However, the inconsistent results between Wichman (2007) and Norman et al. (2019) should be taken into account when interpreting the impact of rearing environment on

performance in a detour task. Due to the varying results across these studies, it is difficult to make definitive conclusions. However, the lack of relationship found by Wichman (2007) between early success on the detour task and onset of perching behavior suggests this task may not be relevant to use of vertical space.

Gaps

There is growing evidence in the literature that vertical complexity of the rearing environment of pullets can impact spatial abilities of domestic laying hens. However, tasks such as the holeboard test, radial maze, and detour task only test spatial navigation and memory (see Glossary) and this is done on a single geometric plane, the ground. Adult hens utilize three-dimensional space and therefore their spatial cognitive abilities should also be investigated in relation to depth. While these tasks evaluate spatial memory in hens, a useful skill for finding resources in the aviary, they are not appropriate for assessing visual perception as it relates to navigating around structures in the aviary. In contrast, the jump test does use three-dimensional space and does not directly test spatial memory, making it more relevant to the use of vertical space in commercial aviaries. However, due to the increasing physical difficulty of this test, visual perception and physical skill cannot be separated.

There is a great need to study the depth perception of laying hens in relation to complexity of rearing treatment by using both a floor test and a test that utilizes three-dimensional space. This methodology would allow for the comparison of performance on both two-dimensional and a three-dimensional test, providing greater evidence for the translation of floor tests of spatial cognition to the use of vertical space. It is also important to investigate depth perception as this aspect of spatial cognition has not been specifically evaluated in relation to rearing environment. Adequate perception of depth seems to be vital for gauging the distance to

fly or jump between perches and platforms in an aviary. Without proper depth perception, collisions and falls would be likely to occur due to misjudgment of distances.

Objectives

The aim of this study is to gain a better understanding of how rearing environment impacts the cognitive and spatial development of laying hens by examining the development of depth perception. Laying hens reared in three environments of differing vertical complexity (floor, single-tier, and multi-tier) were tested on a Y-maze and visual cliff task to evaluate depth perception acuity. Both tasks utilize hens' motivation to escape after being caught and handled, by offering them the option to exit the experimental apparatuses. Previous studies have used food or social rewards in spatial cognition tasks. In this study, variance in responses due to appetite or sociality was removed by utilizing the hens' motivation to escape. The Y-maze uses arms of varying lengths to determine if the bird can assess distance and choose the shortest path to escape. The visual cliff was used to evaluate perception of depth as well as method of crossing the visual cliff. In order to account for the disadvantages associated with the traditional visual cliff test, the plexiglass was illuminated and the table was covered with a canopy to reduce reflection. Additionally, a perch was added in the center of the table, 15 cm above the plexiglass. This prevented tactile information from pecking the glass and the birds' feet, further concealing the plexiglass. These tests offer greater insight into the topic of spatial cognitive development by examining a previously overlooked, yet relevant aspect of spatial cognition: depth perception.

MATERIALS AND METHODS

Animals and Housing

One thousand and eight female Dekalb White chicks were obtained from a commercial hatchery at one day of age and were housed at the Hopkins Avian Facility at the University of California, Davis, USA. Chicks were randomly assigned to one of three rearing treatments for 16 weeks: floor, single-tier, or multi-tier. All pens were 3 x 3 m with wood shavings over concrete flooring, automatic water lines with 12 nipple drinkers, and two 30lb plastic round feeders. The floor treatment (FLOOR) included three floor perches (210 cm long x 10 cm high) but no other access to vertical space. The single treatment (SINGLE) comprised of a single-tiered structure (1 m long x 0.6 m wide x 0.6 m high), one elevated perch (0.3 m high), one floor perch (10 cm high), and a ramp (1 m long at a 40° angle) to the platform. The multi treatment (MULTI) consisted of a two-tiered structure (lower tier: 1 m long x 0.3 m wide x 0.6 m high; upper tier: 1m long x 0.3 m wide x 1.2 m high), three elevated perches (0.3 m, 1 m, and 1.2 m high), one floor perch (210 cm long x 10 cm high), and a ramp (2 m long at a 40° angle) to the top platform. Platforms were made of black DURA-SLAT® (Addison, Texas, USA) poultry and kennel flooring; ramps were constructed out of McNICHOLS® (Tampa, Florida, USA) wire gridwall; and all perches were made of metal pipe (5 cm diameter).

At 16 weeks of age all birds were removed from their rearing pen and the rearing systems were replaced with an adult multi-tiered system. This system included three tiers (0.6 m, 1.2 m, and 1.8 m high) with white DURA-SLAT® (Addison, Texas, USA) poultry and kennel flooring and a nest box ("Best Nest Box", 1.2 m long) on the lowest tier. Additionally, five one-meter long perches were positioned at various heights (0.5 m, 1 m, and 1.7 m high) on the system.

Data Collection

At each of three time points (7-8 weeks, 15-16 weeks, and 29-30 weeks) a sample of 150 Dekalb White hens were tested (n=450). Due to camera malfunctions, video data from three birds (a SINGLE pullet at 8 weeks, a SINGLE hen at 30 weeks, and a FLOOR hen at 30 weeks) were excluded from the visual cliff data set. An equal number of birds were randomly selected from each of three rearing treatments. All birds were tested on the Y-maze task the first week and the visual cliff the second week of testing. Birds were caught from their home pen just prior to testing by corralling them with a metal catch pen. All trials were recorded using a Sony Action Camera and specific behaviors were coded from the video data using Noldus Observer XT (version 13).

Y-Maze

The Y-maze was constructed out of 1 m high wooden boards and included a starting chamber and two arms (Figure 1). The starting chamber was 40 cm long and 60 cm wide, with a guillotine door. The two arms of the maze were 60 cm wide and were joined at a 50^{o} vertex. The arms were adjustable in length so that each arm could be either 30 cm or 90 cm long. The maze was covered by soft, plastic black mesh, preventing the birds from flying out. Both arms were open at the end, allowing the bird the choice to escape into an arena via one of the arms.

Chalk lines were drawn where the entrance met each arm of the maze to indicate that the bird had entered an arm of the maze. Additional lines were also drawn at 30 cm from the entrance to mark the point the bird had chosen that side of the maze. Birds were tested with two consecutive trials where the length of each arm varied so that the birds are presented with a 1:3 ratio (90 and 30 cm) and a 1:1 ratio (90 and 90 cm).

The Y-maze was randomly configured into one of three different orientations: equal length arms or unequal with either the right or left arm being shorter in length. The equal configuration had a 1:1 ratio (90 cm arms), while the unequal configuration consisted of a 1:3 ratio (one 90 cm and one 30 cm arm). A bird was randomly selected, caught using a catch pen, and assigned an ID number. The bird was then placed into the entrance of the maze facing the vertex of the two arms of the maze. When the bird was placed, a timer was started and the guillotine door of the entrance was closed behind them. If the subject had not exited the maze after two minutes had elapsed, the guillotine door was lifted and then immediately closed to encourage movement of the bird out of the maze. Trials ended after the bird had successfully crossed one of the 30 cm lines or after 2.5 minutes had elapsed. Trials were then repeated once more with the same individual so that each bird was presented with both the equal and the unequal configuration. Time spent in each arm, exit choice, and frequency of flying within or out of maze was recorded (see Table 1). The exit choice was considered correct if, when presented with arms of different lengths, the bird chose the shortest path to the arena (the 30 cm arm).

Visual Cliff

Two visual cliff tables were used. A small table (61 cm wide x 122 cm long) was used for the 8-week-old pullets while a larger table (76 cm wide x 152 cm long) was used for the 16 and 30-week-old birds (Figure 2). This allowed for the proportions of the table to remain consistent as the birds grew. The tables consisted of a piece of plexiglass atop a wooden frame, covered by a white tarp canopy which was open at the two narrow ends of the table. The canopy enclosed the table so there were only two open ends as well as prevented glare on the glass from the overhead lights. Half of the table was considered the “shallow side” and had a fixed board covered in checkerboard material just beneath the glass. The opposite side of the table was

considered the “deep side” and had a movable, checkerboard shelf that could be adjusted to be just below, 15 cm below, or 75 cm below the glass. When the shelf was adjusted to be 15 or 75 cm below the glass, the deep side of the table provided the illusion of depth without the risk of the bird falling. A strip of LED lights was just below and perpendicular to the glass on the deep side, in order to illuminate the glass and reduce reflection. A perch was secured in the center of the table between the division of the deep and shallow sides and 15 cm above the glass. The placement of the perch allowed the birds to sit in the center of the table and prevented the bird from receiving any tactile information about the presence of glass on the deep side; the bird could not extend its toes over the edge of the cliff or bend forward enough to peck the glass. A platform made from poultry flooring was suspended over the deep side of the cliff at 30 cm wide and 20 cm from the perch for the small table and 37.5 cm wide and 25 cm from the perch for the large table. The bird had the option of escaping through the open ends of the canopy by either jumping from the perch to the platform or by walking across the shallow side.

Each bird was presented with all three depths below the perch (15, 30, and 90 cm) in a randomized order. The depth of the cliff was adjusted prior to the start of the trial. Before each trial involving perceived depth (30 and 90 cm) both sides of the plexiglass was wiped down with a microfiber cloth to remove all dust and debris. The experimenter stood by the opening of the shallow side and placed the bird on perch facing the deep side of the table. The research assistant started the timer and the experimenter released their hands as soon as the bird had grasped the perch with both feet simultaneously. The experimenter remained standing at the shallow side of the table until one minute had elapsed or the bird had exited through one of the ends of the table. If after one minute the bird had not exited the apparatus, the experimenter extended their right arm towards the perch with fingers outstretched to encourage them to exit through the deep side

of the table. The trial ended after the bird had exited or after 1.5 minutes had elapsed. This procedure was repeated until the bird had completed three trials and had been presented with all three depths. Latency to jump to the platform, type of jump, and frequency of looking down, or orienting the head down, were recorded (see Table 2).

Reliability

In order to verify that behaviors were coded consistently, inter- and intra-coder agreement was calculated using Cohen's κ. Observations were considered to be in agreement if the behaviors recorded were identical and within 2 s of each other. It was found that there was very strong agreement ($\kappa > 0.88$) for intra-coder reliability with each research assistant re-coding 10 videos. For inter-coder reliability, all observers coded 5% of the videos and it was also found that they had very strong agreement ($\kappa > 0.86$).

Statistical analysis

Each bird was considered the experimental unit, and data were assessed for normality prior to analysis. All statistical analyses were conducted in IBM® SPSS® Statistics 27.0 (Armonk, NY: IBM Corp) with a significance level set to $P < 0.05$. Data are presented as the mean (±standard deviation). A binomial logistic regression and a binomial test were run on choice to exit through the short or long arm for the unequal Y-maze configurations as well as the choice to exit left or right for the equal Y-maze configurations. The main and interaction effects of age and rearing treatment on the latency to exit the Y-maze was analyzed using a factorial ANOVA with pen as a random effect. Birds that did not exit the maze were assigned the maximum amount of time for the trial (150 s).

A generalized estimating equation was performed to investigate the main and interaction effects of age, depth and rearing treatment, as well as the random effect of pen, on crossing the

visual cliff (Y/N) and type of movement (Step/Jump) used to cross the cliff. A factorial ANOVA was used to assess the main and interaction effects of age, depth, and rearing treatment, as well as the random effect of pen on the frequency of looking down over the cliff edge while on perch and the latency to cross the visual cliff. Birds that did not cross the cliff were assigned the maximum amount of time for the trial (90 s).

RESULTS

Y-Maze

There were no main effects of age or rearing treatment, nor an interaction, on the choice of Y-maze arm. Out of the 450 birds tested, 345 chose to exit the unequal maze configuration through one of the two arms. Regardless of treatment or age, birds exited the unequal maze configuration using the short side more than would be predicted by chance (0.70, $p < 0.001$) (Figure 3). Preference for arm length did not reflect a location bias as there were no main or interaction effects of rearing treatment or age on exiting through a particular arm. The birds exited the equal maze configuration as predicted by chance (0.53, $p = 0.432$).

A main effect of age on the latency to exit the maze was found ($F_{(2, 889)} = 39.212$, $p < 0.001$). Post hoc comparisons using the Tukey HSD test indicated that the mean latency for the 7 week age group (81.47 s ± 51.72 s) was significantly shorter than the 15 (117.3 s ± 44.51 s, $p < 0.001$) and 29 week birds (92.77 s ± 55.29 s, $p = 0.018$). Finally, the 15 week old birds (117.3 s ± 44.51 s) took longer to exit the maze than 7 (81.47 s ± 51.72 s, $p < 0.001$) and 29 week olds (92.77 s ± 55.29 s, $p < 0.001$) (Figure 4).

Visual Cliff

All birds were more likely to cross (Wald $x^2 = 31.909$, df = 2, $p < 0.001$) at the 15 cm depth (0.28 ± 0.47) than the 30 cm (0.38 ± 0.49) and 90 cm (0.41 ± 0.49) depths (Figure 5). Rearing treatment also had a main effect (Wald $x^2 = 12.881$, df = 2, $p = 0.003$) on crossing the visual cliff. FLOOR birds (0.53 ± 0.5) were less likely to cross the visual cliff than SINGLE (0.65 ± 0.48) and MULTI (0.69 ± 0.46) birds. Additionally, age had a main effect (Wald $x^2 = 77.562$, df = 2, $p < 0.001$) with pullets at 16 weeks (0.4 ± 0.49) being less likely to cross than

birds at 8 (0.7 ± 0.46) and 30 weeks (0.78 ± 0.41). An interaction effect of age and rearing treatment on crossing the visual cliff (Wald $x^2 = 12.734$, df = 4, $p = 0.013$) was found with hens reared on the floor at 8 and 16 weeks of age being less likely to cross the visual cliff than 30 week old FLOOR birds or MULTI and SINGLE birds at 8 and 30 weeks old (Figure 5).

The latency to cross the visual cliff ($F_{(1, 438)} = 21.968$, $p < 0.001$) showed that birds crossed faster at the 15 cm depth (49.17 s ± 34.75 s) than the 30 cm (55.57 s ± 34.59 s) and 90 cm depths (57.19 s ± 34.64 s). Age had a main effect ($F_{(2, 438)} = 35.654$, $p < 0.001$) on latency to cross the visual cliff. Birds at 16 weeks (67.8 s ± 31.31 s) took significantly longer than 8 week old (43.19 s ± 36.13 s) and 30 week old (50.83 s ± 31.67) birds (Figure 6). Additionally, a main effect of rearing treatment ($F_{(2, 438)} = 6.106$, $p = 0.002$) was found, with FLOOR birds (60.04 s ± 33.74 s) having longer latencies to cross the visual cliff than SINGLE (51.25 s ± 35.21 s) and MULTI (50.66 s, ± 34.38 s) birds. Finally, an interaction effect of age and rearing treatment was found ($F_{(4, 438)} = 3.972$, $p = 0.004$) with FLOOR birds at 8 weeks having a significantly longer latency than SINGLE and MULTI birds of the same age.

No main effects or interactions between age, depth, and rearing treatment on cross type (Step/Jump) were found. However, the birds that crossed the visual cliff stepped significantly more than 50% of the time (0.82, $p < 0.001$).

There was a main effect of depth ($F_{(1, 438)} = 62.362$, $p < .001$), rearing treatment ($F_{(2,438)} = 4.646$, $p = 0.010$), and age ($F_{(2,438)} = 15.782$, $p < 0.001$) on frequency of looking down. The FLOOR birds (1.95 ± 1.83) looked down more often than the MULTI (M = 1.62, ± 1.74, $p = 0.024$) and SINGLE (1.54 ± 1.68, $p = 0.004$) birds. Birds at 16 weeks of age (2.14 ± 1.91) looked down significantly more when compared to 8 weeks (1.60 ± 1.85, $p < 0.001$) and 30 weeks (1.37 ± 1.41, $p < 0.001$). Interactions were found between depth and age ($F_{(2, 438)} = 3.856$, $p = 0.022$)

with 16 week old pullets looking down more at 90 cm than 8 and 30 week old birds (Figure 7). There was also an interaction between depth and rearing treatment ($F_{(2, 438)} = 5.175$, $p = 0.006$) with FLOOR and MULTI birds looking down more at 90 cm than the 15 and 30 cm depths.

DISCUSSION

In order to better understand how access to vertical structures during the pullet phase impacts the development of spatial cognition in hens, this study utilized two novel depth perception tasks: Y-maze and visual cliff test. SINGLE and MULTI birds, as well as their FLOOR reared counterparts, were shown to exit the Y-maze through the short arm significantly more frequently than what would be expected by chance. These results demonstrate that the birds were able to choose the shorter escape route when presented with the choice between 30 and 90 cm. This suggests that, regardless of rearing system, birds were able to differentiate between these two distances on a floor based task.

FLOOR birds also crossed the visual cliff at the same frequency as SINGLE and MULTI reared at each cliff depth. If FLOOR birds did not adequately perceive depth, it could be expected that they would cross the cliff at the same frequency regardless of the height. However, it was found that all birds, regardless of rearing treatment, were more likely to cross at 15 cm depth than the 30 cm and 90 cm depths. Additionally, all birds had a significantly shorter latency when crossing at the 15 cm depth when compared to the greater depths. These results demonstrate that the birds were responding differently to variations in depth, suggesting that FLOOR birds do not have a deficit in the ability to perceive depth when compared to SINGLE and MULTI birds. This is an intriguing addition to the current evidence that access to vertical space at a young age improves spatial memory and navigation in hens (Norman et al., 2019; Tahamtani et al., 2015; Whiteside et al., 2016). Although these aspects of spatial cognition are affected by access to perches and platforms during development, depth perception appears to be unaffected.

This study provides evidence that the development of accurate depth perception does not require specific visual experience, such as exposure to depth and height, during rearing. This is supported by studies with one-day old chicks (Walk & Gibson, 1961) and four-day old chicks without exposure to vertical structures (Thorndike, 1899) which found these animals already possessed the ability to perceive depth. Gibson and Walk (1961) preformed a cross species comparison of depth perception abilities by testing infant rat pups, kittens, chicks, lambs, goat kids, and piglets on a visual cliff. They found that all animals tested, including animals that were less than one day of age, demonstrated avoidance of the deep side of the visual cliff, akin to that of their adult counterparts. Gibson and Walk (1961) concluded that depth perception abilities are most likely innate, however did not exclude the chance that learning may play a role to some degree.

The theory that depth perception is an innate skill was challenged by Tallarico and Ferrell (1964) when they raised chicks on either the deep side or the shallow side of a visual cliff for the first 30-40 hours of life. When placed in the center of a visual cliff with both deep side and shallow sides present, chicks reared in the deep side environment were significantly more likely to cross to the deep side of the cliff than those reared on the shallow side. Tallarico and Ferrell (1964) used this as evidence that there is not innate avoidance of a drop off or cliff. However, chicks reared in the deep side environment may have learned that the cliff was not a real threat and that there was a barrier preventing them from falling. Instead, evidence from previous research as well as the current study suggests that depth perception is an innate ability and rearing chicks on the floor does not prevent the proper development of depth perception.

Despite the same ability to perceive depth between the three treatments, differences in frequency of crossing the visual cliff was observed between the FLOOR, SINGLE, and MULTI

birds. The FLOOR birds were significantly less likely to cross the visual cliff than the other two treatments at 8 and 16 weeks of age. This difference dissipated at week 30, when all birds, regardless of rearing treatment, crossed the visual cliff at the same rate. This suggests that something inhibited the FLOOR birds from crossing the visual cliff at a younger age when housed in the treatment pen and that this reversed after the FLOOR birds were exposed to their vertically complex adult housing after 16 weeks.

One possibility is that MULTI and SINGLE birds may have been more comfortable with the platform as a landing surface due to their experience with poultry flooring in their home pens. However, the appearance of the poultry flooring in the visual cliff and rearing pen systems was different in color and size. The color of the poultry flooring in the MULTI and SINGLE rearing treatment pens was black as opposed to the white poultry flooring used for the visual cliff. Also, the platform was a much smaller landing surface than the large platforms used in the MULTI and SINGLE rearing systems as well as the adult multi-tier aviary system.

Another possibility is that FLOOR birds were less likely to cross due to lack of experience with tall vertical structures such as perches and platforms above 30 cm in height. This lack of experience with vertical structures may prevent hens reared on the floor from readily crossing between perches and platforms when first introduced to a more vertically complex environment. FLOOR birds at 8 weeks of age also showed significantly longer latencies to cross the visual cliff than SINGLE and MULTI birds at 8 weeks of age. This further supports our suggestion that FLOOR reared birds were tentative when using vertical structures prior to being transitioned into their adult housing. However, it is interesting that FLOOR birds at 16 weeks did not have significantly longer latency than the other rearing treatments. Meaning that FLOOR

pullets at 16 weeks of age were less hesitant than their 8 week old counterparts to utilize vertical structures.

Across all treatments, birds at 16 weeks of age were less likely to cross the visual cliff than at 8 or 30 weeks old. Additionally, all birds exhibited longer latencies to exit the Y-maze and cross the visual cliff at this intermediate time point when compared to the other time points. The visual cliff was smaller at the 8 week time point and was increased in size by 25% for the 16 and 30 week time points. The size of the visual cliff was increased after the first time point due to the increased size of the birds. It is important to note that the frequency of crossing and latencies to cross were not significantly different between the 8 week and 30 week old birds despite the table being the same size at 30 weeks as it was at 16 weeks. Also, the birds did not cross the cliff at 16 weeks of age significantly differently than at the other time points, demonstrating that they did not need to jump or fly to reach the platform. Therefore, it is unlikely that the size difference of the visual cliff contributed to the avoidance of crossing and longer latencies observed at the 16 week time point. It would be expected that if the increase in size of the visual cliff increased the physical difficulty of the task, the birds would have no longer been able to consistently step from the perch to the platform for the visual cliff. Furthermore, an increase in latency to exit the Y-maze was also observed at 15 weeks of age, even though the Y-maze remained the same size for all three time points.

We suggest that the birds may have been less likely to exit the Y-maze and cross the visual cliff at the intermediate time points due to increased fearfulness. Tonic immobility is a catatonic-like state induced by a short period of physical restraint, typically after placing a chicken on their back and briefly holding them in place (Jones, 1986). Tonic immobility has been found to relate to fearfulness, with longer periods of tonic immobility being associated with

other fear-inducing situations such as novel environment or loud noise (Jones, 1986). Previous studies on the development of fear in chickens have found that tonic immobility is longer at the onset of sexual maturity (Campo & Carnicer, 1993; Ghareeb et al., 2008). Campo & Carnicer (1993) and Ghareeb et al. (2008) tested laying hens at a range of ages (1-32 weeks and 3-35 weeks, respectively) on a tonic immobility task. Campo and Carnicer (1993) found that tonic immobility increased until its maximum duration at 16 weeks of age, before declining and then slowly increasing after 24 weeks. Similarly, Ghareeb et al. (2008) found that tonic immobility reached its maximum duration at 20 weeks of age before decreasing in duration for each subsequent age tested. These results suggest that fearfulness in laying hens is elevated around the onset of lay.

The differences we observed in latency to complete the depth perception tests are potentially due to increased age-related fearfulness. Heightened levels of fear in hens may result in decreased exploratory behavior in a novel environment (reviewed by Forkman et al., 2007). Increased age-related fearfulness resulting in decreased exploratory behavior would explain the longer latencies we observed on the Y-maze and visual cliff task during the intermediate time point as well as the decreased frequency in crossing the visual cliff at this time point. However, this is only speculation as we did not measure general fearfulness of the birds. Exploring this relationship in future studies is needed to better understand the relationship between age-related fearfulness, exploratory behavior, and latency to complete cognitive tasks.

It is important for further studies to investigate the impacts of increased age-related fearfulness on the transition into a novel housing system. The onset of sexual maturity is both the time period when laying hens are transferred to their adult housing systems and the time at which age-related fearfulness may be elevated. The potential effects of this fearfulness on exploratory

behaviors in a novel environment and willingness to use novel structures at this age could be crucial to better understanding that transition from rearing to adult housing in laying hens.

At 16 week old birds looked down significantly more over the visual cliff than 8 and 30 week old birds. Additionally, FLOOR birds looked down significantly more often than MULTI and SINGLE birds across all time points. Looking time is a measure used in pre-verbal infants and non-human animals to evaluate attentional bias, with an increase in looking time often being associated with novelty, vigilance directed at a threat, or violation of expectations (Winters et al., 2015). In the present study, looking frequency instead of duration was used due to the tendency of the hens to orient their head down and then back up very quickly and frequently. This increased frequency in looking down over the visual cliff could be attributed to the depth violating their expectations or attentional bias towards the novelty of a cliff. Depth would be novel and potentially unexpected for FLOOR birds who had never previously encountered a cliff, perch over 30 cm high, or platform. As previously discussed, it is likely the birds were experiencing greater fearfulness at the intermediate time points. Due to this and previous studies using looking time as a measure of hyper-vigilance and attention (DeLoache & LoBue, 2009; Mandalaywala et al., 2014), the increased frequency of looking down at this time point may have been related to increased fearfulness, attention, and caution towards the cliff.

Implications

By investigating how hens perceive their environment we can make more informed decisions about improved rearing practices. Our results do not suggest that floor rearing of pullets has an impact on the development of proper depth perception, however differences in behavior were observed. The FLOOR birds were more hesitant to cross from a perch to a platform over the visual cliff, with longer latencies and decreased likelihood of crossing when

compared to SINGLE and MULTI birds. This suggests that floor reared birds are more hesitant to utilize vertical structures just prior to being transitioned into their adult housing. This could have implications for the welfare of floor reared birds transitioned into multi-tiered aviaries.

Hens are highly motivated to use nest boxes and may show signs of frustration when access to these recourses are inhibited, suggesting the importance of nest boxes for hens and their welfare (reviewed by Cronin & Hemsworth, 2012; Hemsworth & Edwards, 2020). Hesitation and avoidance of using the system could prevent hens from taking full advantage of the benefits provided in multi-tiered aviaries, such as nest boxes. This is supported by previous findings that, when compared to aviary-reared birds, floor reared birds are more likely to lay floor eggs and less likely to utilize resources located on raised platforms (Colson et al., 2005; Colson et al., 2008; Gunnarsson, 1999). In order to best prepare pullets for multi-tiered aviaries, birds should be provisioned at a young age with vertical structures, such as perches, ramps, and platforms.

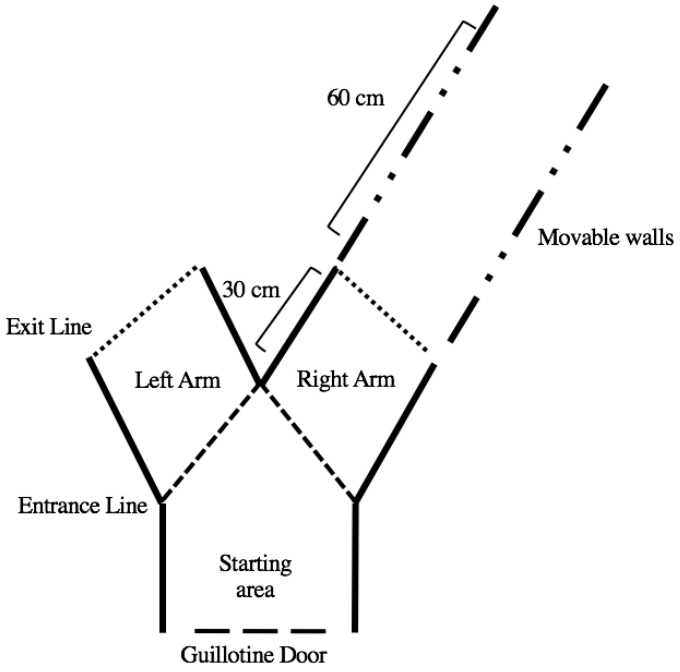
Conclusion

It is clear from previous studies that aspects of spatial cognition, including spatial memory and navigation, are impacted by early experience with vertical structures (Norman et al., 2019; Tahamtani et al., 2015; Whiteside et al., 2016). However, the current study presents evidence that a crucial aspect of spatial cognition needed for gauging flight and landing distance, depth perception, is not affected by differences in vertical complexity of the rearing environment. The results of this study show that pullets reared in more vertically complex rearing systems cross from a perch to a platform more readily than those reared on the floor, however this discrepancy disappeared by the final time point. This suggests that floor reared birds are more hesitant to cross from a perch to a platform and that this effect is reversed after exposure to a vertically complex environment. Future research is needed to determine how long

it takes for the reversal to occur and the degree to which floor reared birds will avoid using or transitioning between vertical structures. This hesitation and avoidance of vertical structures could have implications for improved rearing practices for pullets destined for multi-tiered aviary systems.

FIGURES

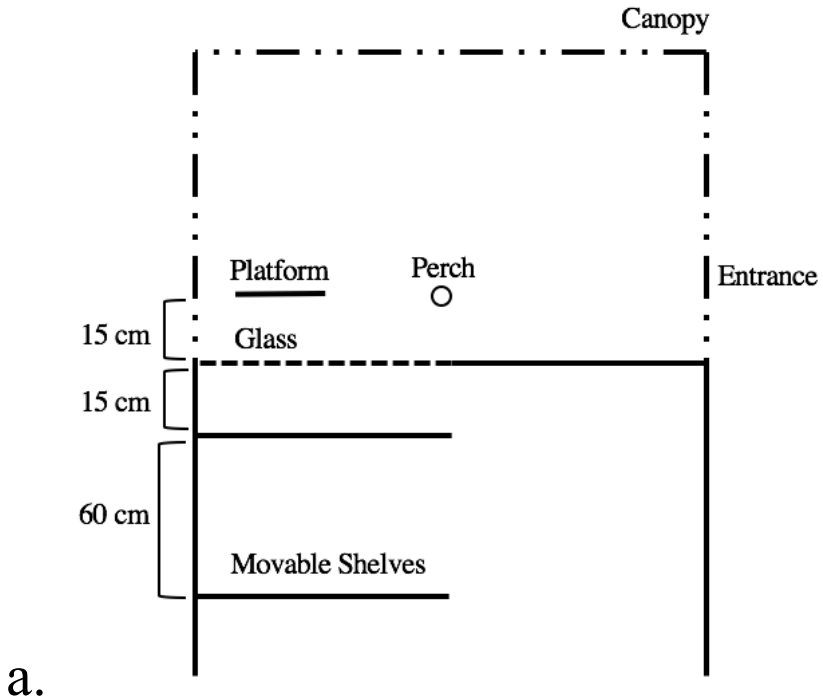
Figure 1. (a) Diagram of Y-maze apparatus from above. (b) Pullet facing the short arm of the Y-maze in an unequal configuration.



a.

b.

Figure 2. (a) Diagram of visual cliff apparatus from the sagittal plane. (b) Photograph of pullet on perch overlooking the 90 cm cliff depth.



a.

b.

Figure 3. Frequency of exiting the Y-maze through the short versus long arm broken down by rearing treatment at all ages (n = 345). There were no main or interaction effects of age or rearing treatment on exit arm choice, with all birds choosing the shorter arm significantly more often (0.70, $p < 0.001$) than the longer arm.

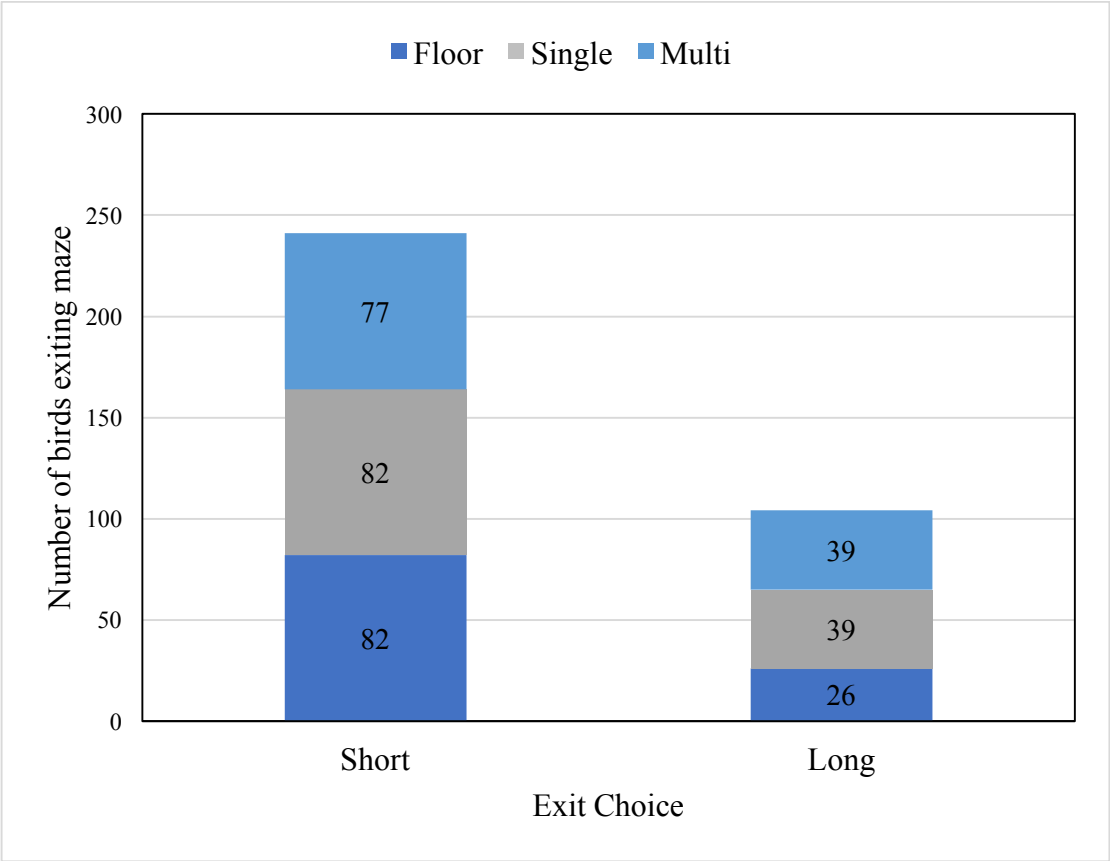


Figure 4. Latency to exit Y-maze separated by rearing treatment. A main effect of age on the latency to exit the maze ($F_{(2, 889)} = 39.212$, $p < 0.001$) was found with 15 week old birds (117.3 s ± 44.51 s) taking longer to exit the maze than at 7 (81.47 s ± 51.72 s, $p < 0.001$) and 29 weeks (92.77 s ± 55.29 s, $p < 0.001$).

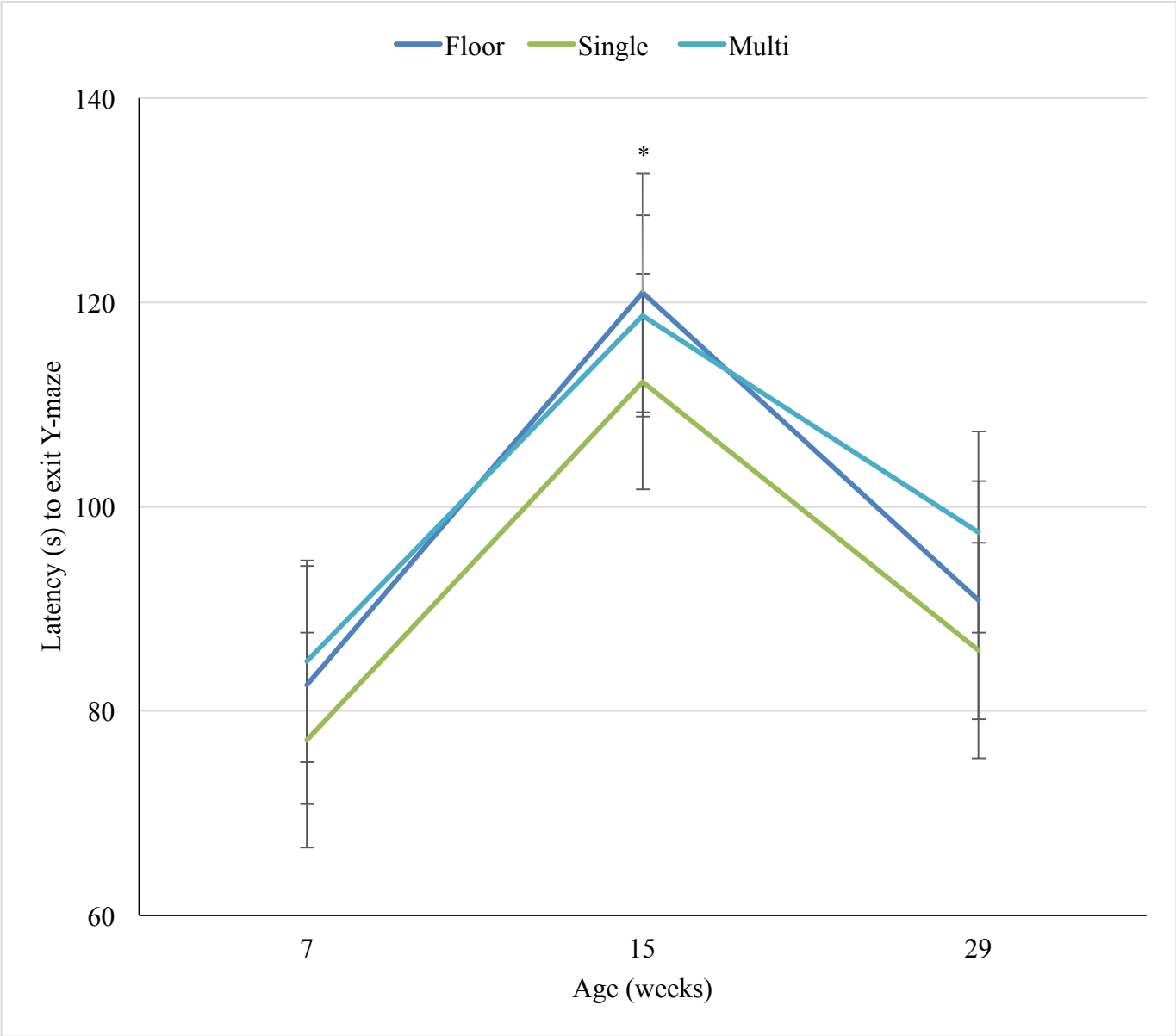


Figure 5. Frequency of crossing visual cliff at (a) 15 cm, (b) 30 cm, and (c) 90 cm for each rearing treatment and age. There were main effects of depth (Wald $x^2 = 31.909$, df = 2, $p < 0.001$), rearing treatment (Wald $x^2 = 12.881$, df = 2, $p = 0.003$), and age (Wald $x^2 = 77.562$, df = 2, $p < 0.001$) on crossing the visual cliff. All birds being significantly more likely to cross at 15 cm depth (M = 0.28, SE = 0.036) than the 30 cm (M = 0.38, SE = 0.025) and 90 cm (M = 0.41, SD = 0.025) depths. FLOOR birds (0.53 ± 0.5) were less likely to cross the visual cliff than SINGLE (0.65 ± 0.48) and MULTI (0.69 ± 0.46) birds and 16 week old birds (0.4 ± 0.49) were less likely to cross than birds at 8 (0.7 ± 0.46) and 30 weeks (0.78 ± .41).

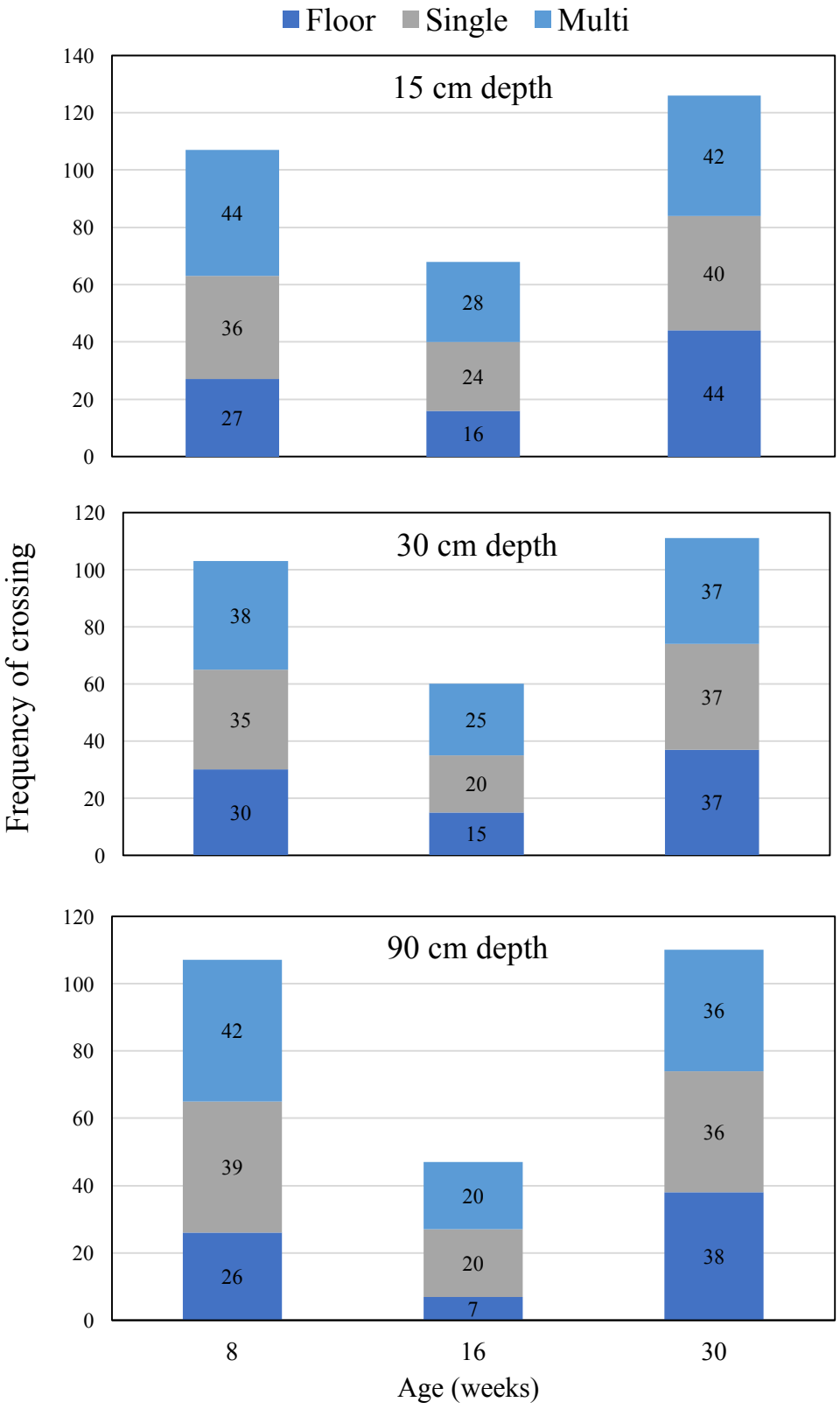

Floor
Single
Multi
15 cm depth
30 cm depth
90 cm depth
Frequency of crossing
Age (weeks)
8
16
30
0
20
40
60
80
100
120
140
27
36
44
16
24
28
44
40
42
30
35
38
15
20
25
37
37
37
26
39
42
7
20
20
38
36
36

Figure 6. Latency to cross the visual cliff by age and rearing treatment. Age had a main effect ($F_{(2, 438)} = 35.654$, $p < 0.001$) on latency to cross the visual cliff with birds at 16 weeks (67.8 s ± 31.31 s) taking significantly longer than 8 (43.19 s ± 36.13 s) and 30 week old (50.83 s ± 31.67) birds

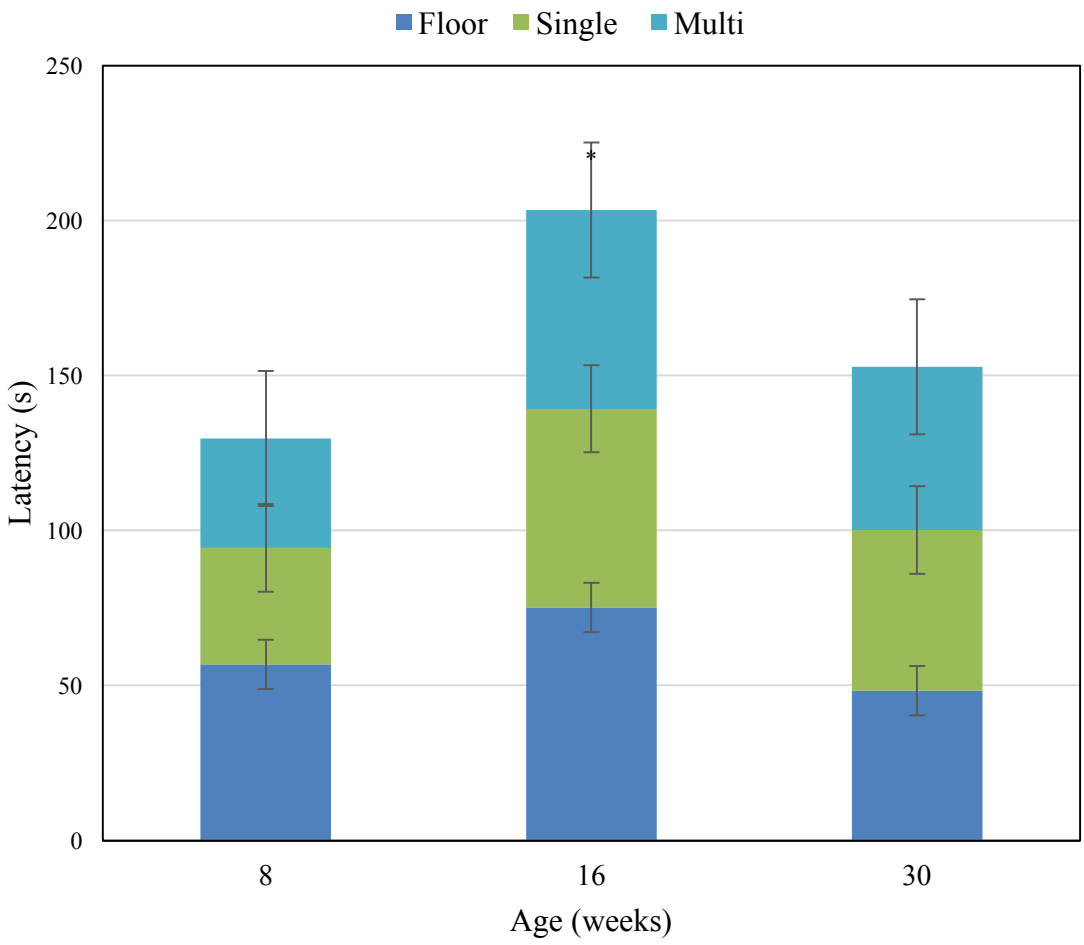
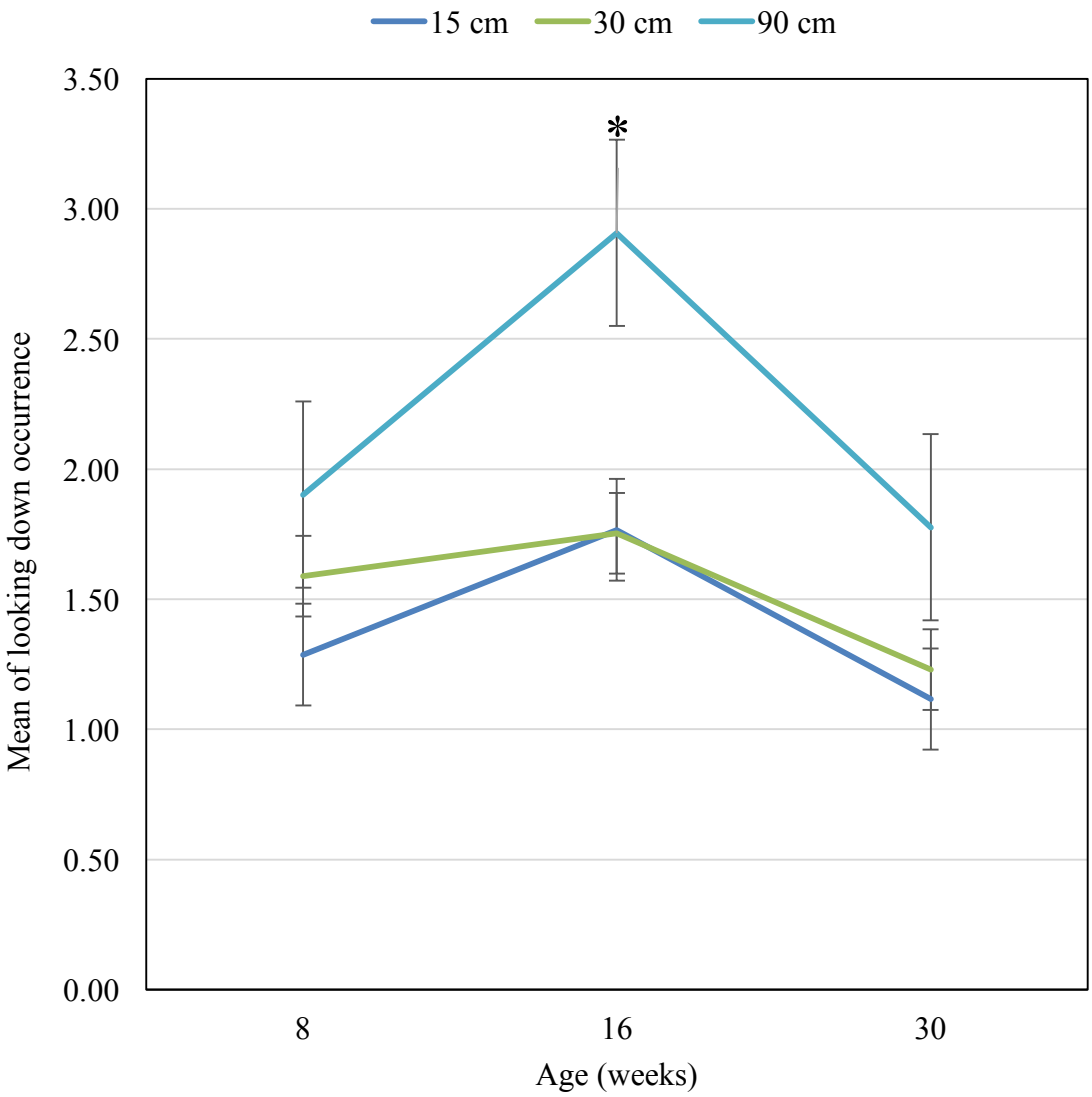


Figure 7. Mean frequency of looking down over the visual cliff by depth and age for all rearing treatments. Interactions were found between depth and age ($F(2, 438) = 3.856$, $p = 0.022$) with 16 week old birds looking down over the cliff edge more often at the 90 cm depth than 8 and 30 week old birds.



TABLES

Table 1. Ethogram used to score the behavior observed in the Y-maze.

Behavior	***Description***
Entrance	Guillotine door closes and the bird is in entrance area
Left Side	Head, neck, and body cross the entrance line on the left side
Right Side	Head, neck, and body cross the entrance line on the right side
Fly Out	Bird crosses exit line while flying
Fly Up	Both feet leave the ground while wings are flapping
Left Line	Head, neck, and body of bird crosses the exit line on the left side
Right Line	Head, neck, and body of bird crosses the exit line on the right side

Table 2. Ethogram used to score the behavior observed on the visual cliff.

Behavior	***Description***
On Shallow	One or both feet touch the shallow side of the table
On Perch	The pads of both feet touch perch simultaneously
On Platform	Both feet have contact with the platform
Face Shallow	The bird is on the perch and turns so that their toes are facing the shallow side of the table
Look Down	While the bird is on the perch and facing the deep side, the tip of their beak points down over the deep side and is perpendicular to the plexiglass
Step Across	While crossing the cliff, one foot remains on perch while other foot reaches platform
Jump Across	While crossing the cliff, both feet leave perch and have no contact with any part of the visual cliff table

GLOSSARY

Term	***Definition***
Depth Perception	The ability of an individual to perceive the relative distance of objects
Differential Visual Depth Threshold	Shortest distance at which an individual can perceive a difference in depth
Geometric Plane	A flat, two-dimensional surface
Interposition	A monocular depth cue used when two objects are in the same line of sight but the closer object partially obstructs the farther object.
Motion Parallax	A monocular depth cue utilizing the relative velocities of objects observed by a moving individual. Objects moving at a constant speed will appear to move faster if they are closer to the observer than if the object was farther away.
Spatial Memory	The storage and retrieval of information relevant to recalling the location of an object or route planning.
Spatial Reasoning	The capacity to visualize and comprehend the properties of three-dimensional objects even with limited information.
Stereopsis	Perception of depth due to the discrepancy in visual input coming from both eyes; binocular vision

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