

INTEGRATING TECHNOLOGY AND ANIMAL WELFARE: SPACE AND RESOURCE
USE OF INDIVIDUAL NON-CAGE LAYING HENS

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ABSTRACT

INTEGRATING TECHNOLOGY AND ANIMAL WELFARE: SPACE AND RESOURCE USE OF INDIVIDUAL NON-CAGE LAYING HENS

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Little is known about how individual laying hens behave and use resources when housed in large groups in non-cage housing systems. As more hens in commercial settings are housed in large groups, and their welfare assessed accordingly, understanding individual hen behavior and resource use is paramount. Therefore, a wireless body mounted sensor system was developed to track the location of individual laying hens in a non-cage environment. The development of this new technology stimulated ethical discussion surrounding development of technology with regards to animal welfare assessment through a Philosophy of Technology lens. Yet, technology in agriculture is a double-edged sword, especially as regards animal welfare. Therefore, technology should be utilized when appropriate and relinquished when necessary. By acknowledging what is gained or lost (from farm to fork) with regards to animal welfare by utilizing a technological tool.

Along this vein, the impact of using a hen-worn sensor system on hen resource use and agonistic behaviors was investigated. Harness presence had a minimal negative long-term effect on resource use and agonistic behavior, suggesting that hens were able to habituate to wearing the sensor. Following this work, two parsimonious sampling strategies were identified for monitoring the behavior of individually identifiable hens: continuous observation for 30 minutes every 1.5 hours and instantaneous scans every 15 minutes.

Using this newly identified sampling strategy, individual hen behavior and sensor data were collected at 19, 28, 48, and 66 wk of age along with physical assessments as described in the Welfare Quality[®] Assessment Protocol for Poultry. Mean differences in the amount of time hens spent standing, sitting, and perching were observed, and differences in the variability of behavior performance were observed for many of the assessed behaviors. This highlights that even though group averages may not change, hens individually may be variable in their physical condition and behavioral repertoire. The most robust physical parameter to measure with regards to current, past and future behavioral profiles was claw length, and the optimal age for performing welfare assessment was 48 wk.

Output from the hen-worn sensor system and video-based behavioral observations were collated in ArcMap 10.0, part of the Geographic Information System (GIS) software package. By combining the behavior and sensor data in GIS, a spatiotemporal representation of individual behavior was developed. For this study, data from 48 and 66 wk of age were used to characterize individual hen behavior through utilization distributions, hot spot mapping, and conspecific overlap calculations. The behaviors of feeding, foraging, and preening were specifically targeted to identify spatiotemporal patterns for a behavior that was constrained by the location of the resource (feeding), an appetitive behavior that was not constrained by a location for its performance (foraging), and a grooming and social behavior that could indicate a hen's affective state (preening). These results provide new insight into individual hen behavior in a non-cage system and present a platform for a new type of agricultural research, which integrates wildlife tracking techniques, to understand the individual experience in large group. This information may be able to provide insight into hen preference and can be utilized when developing best practices or designing new housing environments.

To my family: for teaching me to approach life with an open mind and an open heart.

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Chapter 2 corresponds to the peer-reviewed version of the following article: Daigle CL, Banerjee D, Biswas S, and Siegford JM. 2011. Noncaged laying hens remain unflappable while wearing body-mounted sensors: Levels of agonistic behaviors remain unchanged and resource use is not reduced after habituation. *Poultry Science* 91:2415-2423.

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KEYS TO SYMBOLS OR ABBREVIATIONS

10EV30 = ten minutes every thirty minutes

15EV1 = fifteen minutes every one hour

15EV2 = fifteen minutes every two hours

30EV1.5 = thirty minutes every one and a half hours

A = Aggression

AMS = Automatic Milking System

B = Behavior

C = Circadian

CA = California

cm = centimeter

d = day

D = dynamic

FW = area of room containing food and water

GIS = Geographic Information System

h = hour

ID = identification

IS = instantaneous scan

L = location

m = meter

MA = moral agent

min = minute

MP = moral patient

MSU-PTRC = Michigan State University Poultry Teaching and Research Center

NC = non-circadian

NON = hen not wearing a sensor

NONE = area of room with no resources

OBM = outcome based measure

OD = observation duration

PERCH = area of room containing perch structure

POT = Philosophy of Technology

R = resource use

RBM = resource based measure

RFID = radio frequency identification

RSSI = radio signal strength information

s = second

S = static

sec = second

SEN = hen wearing sensor

SI = sampling interval

SOAL = subject of a life

TDMA = time division multiple access

TS = time sampling

wk = week

WQ = Welfare Quality[®]

INTRODUCTION

Non-cage laying hens face a myriad of challenges when housed in large groups. Hens in non-cage environments have the space and opportunity to perform natural behaviors and move freely, but they may have difficulty accessing necessary resources, developing stable social relationships, or receiving specialized care. Assessment of animal welfare requires information be collected from an individual animal; which in a non-cage environment can be challenging, as the hens look similar. Therefore, a wireless body-mounted sensor system was developed to track the location of individual non-cage laying hens across time.

Welfare assessments of large groups can be challenging to perform and interpret, as the group average may not reflect the welfare of all individuals housed within. However, as welfare assessment programs are developed to provide producers information about husbandry and environmental conditions, and these assessments become more complex, technology may be able to provide information at the level of the individual animal that cannot be gained through visual observation. Many different forms of technology have been developed to track individual hens in the production environment. These identification systems are primarily used to track individuals from farm to table, but researchers can also use them to track behavior and movement of individual animals. The most common type of individual identification for poultry is the RFID sensor (Toth et al. 2010), and printing bar codes on animal beaks and legs is being examined for practicality (Fröschle et al. 2009; Mc Inerney et al. 2010, 2011). Yet, many of these technological developments provide information passively and will only provide information about hen location if the hen passes by a receiver or bar scanner. Therefore, there was a need to develop an actively responding sensor to provide information about individual hens.

Yet, technology in agriculture is a double-edged sword. Technology can facilitate animal care by making it easier for the farmer to care for animals; however, because these advancements in technology ease the workload of the farmer, they subsequently make it easier for farmers to care for more animals simultaneously, resulting in situations where farmers may spend less time with the animals thus furthering the distance between farmer and animal. Since a true understanding of animal welfare requires assessment at the individual level, technological advancements make it logistically more challenging to assess individual welfare because there are more individuals to assess and tailor management and environmental conditions for – thus creating a conflict between technology and animal welfare. The Philosophy of Technology (Heidegger 1977) provides a genre of thought in which to assess agricultural technologies and should be incorporated into animal welfare assessment so producers and assessors can clearly understand what is gained or lost with regards to animal welfare by utilizing a technological tool.

With this in mind, the impact of the sensor on hen behavior and social interactions was important to understand. Hens are sensitive to phenotypic differences among their flock mates, and individuals who look different may be subjected to increased levels of aggression and may be thwarted from accessing important resources. Furthermore, identifying a strategy for collecting behavioral information from non-cage hens that represents their continuous behavior would facilitate efficient data collection and statistical analysis. This would not only expedite data collection but would also reduce the required amount of data needed to assess individual hen location and behavior. Additionally, by identifying links between hen behavior and physical condition, hens may need to be handled less intensively and observed more, increasing our understanding of hen welfare through sensor output. Conversely, if assessing the physical

condition of the hen provides insights to behavior, behavioral observations may not need to be performed to understand how hens are behaving based upon physical performance.

Finally, as hens are housed in larger groups in non-cage environments, they have more choice as to where to spend their time and perform natural behaviors. Furthermore, these environments are more representative of a stochastic environment in which hens must adapt their behavior to a wide variety of pressures. Understanding the link between where a hen is located and what she is doing while there can provide insight into what the hen perceives as important or favorable and changes in behavioral patterns could be indicative of welfare challenges. Furthermore, the variability of hen space use could allow researchers to begin applying behavioral ecology theory to animals in agricultural environments, thus facilitating best practices that capture more than we know about their behavioral needs and promoting animal welfare.

The works presented in this dissertation are inter-disciplinary and present an examination of wireless sensor technology and its interplay with animal welfare assessment: from development to application. The first chapter explores the ethics behind technology development in agriculture and encourages scientists to consider the Philosophy of Technology approach when developing future agricultural animal welfare assessment tools. As hens are sensitive to phenotypic differences among their flock mates, the second chapter addresses whether the sensor system impacts hen behavior. Chapter three explores what impact sampling technique can have on data from different types of behaviors and utilizes several statistical methods to identify efficient samplings strategies, using the non-cage hens in this sensor system as a case study. The fourth chapter uses the sampling strategy identified in Chapter 3 to track individual hen behavior and resource use throughout the lay cycle to not only illustrate the average and diversity of hen behavior and physical condition, but to relate behaviors to physical

parameters collected as part of the Welfare Quality[®] Assessment Protocol for Poultry (2009) as well. Finally, data collected from the sensor system were examined using techniques from wildlife ecology data management in Geographic Information Systems to provide a proof of concept spatiotemporal representation of individual hen behavior across time.

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CHAPTER 1: INCORPORATING THE PHILOSOPHY OF TECHNOLOGY INTO ANIMAL WELFARE ASSESSMENT

ABSTRACT

Changes in attitudes towards how animals are housed in agriculture are currently under question in the public eye – particularly for laying hens. Many arguments from the rights and utilitarian viewpoints have been made for changing environmental conditions and managerial practices for animals in an effort to respect the interests of the animal and better their welfare. Yet, these arguments have been based upon belief systems that were developed from information that can be collected by human perception only. Technological advancements can facilitate animal welfare assessment by providing humans with new information about what the animal perceives. Yet, little has been discussed surrounding the thought process behind which technologies are conceived, how they are developed, and why they are implemented. Here, using the laying hen as a model, we turn to the philosophy of technology to address what role technological advancements may have in our capacity to understand animals, how technology can affect their welfare, and what role technology may play in in furthering animal welfare assessment.

INTRODUCTION

Technological developments have shaped the evolution of agriculture in America. The industrialization post World War II changed the landscape of American agriculture and transformed it from a network of agrarian family farms to a larger, commercialized system where many animals were managed by a few people – and this was made possible by advancements in technology. However, little consideration has been given to how these technological advancements affect the animals for which they are developed. Many of these developments have been created to facilitate animal care from the farmer's perspective and have been developed out of what we, as humans, perceive as necessary for the animal. Animal welfare is becoming an important component to producers and consumers with regards to animals in agriculture. Assessment programs have been developed to assess animal welfare, and ethical philosophies surrounding animal agriculture are becoming main stream and are beginning to infiltrate the dinner table conversation. Yet, animal welfare requires understanding the perspective of the animal itself – and here is where technology can begin to bridge the gap. Here, I use the laying hen as a model for understanding how technological developments can be used to better capture the animal's perspective, how they may affect animal welfare and propose we begin to incorporate a philosophy of technology perspective to animal welfare as an amendment to conventional animal ethics. Technological advancements may allow us an opportunity to understand better what the animal is experiencing which could allow us to better improve upon its welfare. Incorporating the philosophy of technology into the ethical conversation surrounding the use of technology with animals in agriculture may provide more insight into what the animal perceives – which can ultimately help us gain a clearer picture of its welfare.

A common misconception is that animals perceive their environment similarly to humans. However, animals have physiological and morphological differences that effect their perception. Chickens have a distinct sensory apparatus and a different set of genetically based behavioral drives. It is, in fact, helpful to stress the philosophical underpinnings of the issue. In 1972, Thomas Nagel published “What Is It Like to Be a Bat?”, in which he claims in the title: Although we humans cannot imagine what it is like to be a bat, we do not doubt that there is something that it is like to be a bat (1974). One goal of the paper was to argue that there is a gap between attempts to explain cognition in terms of neural activity, on the one hand, and the experience of conscious or perceptual awareness, on the other. Nagel believed that attempts to reduce mental phenomena to the causal interaction of neurons in the brain simply misunderstand the phenomenon that is of most fundamental interest in the philosophy of mind. At the same time, Nagel closed the article by speculating that a new science of animal cognition might in fact illuminate his title question by giving us a better sense of how animals whose perceptual abilities and genetic drives are very different from that of humans do in fact perceive the world. Nagel’s skepticism about the reduction of mind to brain was not also a skepticism about science’s ability to help us understand what it is like to be a bat. Or a laying hen.

Understanding what is like to be a laying hen is fundamental to understanding laying hen welfare. Yet, many different interpretations of animal welfare exist and all involve the assumption that animals have needs and urges that should be considered when housed in agriculture. What is more, animal urges are developed and shaped based upon the information they perceive through their sensory mechanisms. The information from the 1965 Brambell Report highlighting these beliefs stimulated the development of codes by Britain’s Farm Animal Welfare Council which modified them into the 5 freedoms in 1979 (Norwood and Lusk 2011).

These five freedoms have been used as the basis for multiple theories on how we assess animal welfare. They are:

1. Freedom from hunger or thirst
2. Freedom from discomfort
3. Freedom from pain, injury or disease
4. Freedom to express normal behavior
5. Freedom from fear and distress

The second and fifth freedoms advocate an ability to avoid negative perceptual states: they refer directly to the way that animals perceive a situation as uncomfortable, fearful or distressing.

Hunger and thirst also have a perceptual underpinning in that they articulate the way that biological drives are perceived by a given individual. While injury or disease may or may not have a perceptual basis, pain is normally defined as a perceptual state. Normal behaviors are usually performed because they are rewarding for the animal, so it can be argued that the animal perceives the behavior as pleasurable or necessary. However, this can only be inferred from our interpretation of the animals' reaction to its own behavior. That can suggest that normal behavior lacks a clear perceptual dimension, though as we will discuss below, there is often an implicit presumption that inability to express normal behaviors is indicative of frustration or distress. To the extent that this is the case, all five freedoms have a perceptual dimension.

The role of perception can be seen clearly in two of the predominant approaches to assessing animal welfare are illustrated by Miriam Stamp Dawkins and David Fraser. Miriam Dawkins uses two questions to determine animal welfare: 1) is the animal healthy? and 2) does it have what it wants? (Dawkins 2004). Health could be derived from veterinary measures of

which an individual animal could be unaware (e.g. parasitic infection). Wants, however, are based on the animal's perception of its environment and its internal states. David Fraser argues that animal welfare consists of 3 basic spheres of interest: biological functioning, expression of natural behaviors, and affective state (Fraser 2008). For Fraser, affective states are delimited entirely by animal perception of its internal cognitive situation (e.g. pain, frustration, satisfaction or satiation) as related to its environment. Hence, key approaches to animal welfare rely both implicitly and explicitly on Nagel's observation that although it may be difficult to know "what it is like" to be a given animal in a certain situation, we doubt neither that there is something that it is like or that "what it is like" is a crucial dimension of a given animal's well-being.

Regardless of which approach to welfare is taken, evaluation of farm animal welfare needs to ensure that new production methods 1) do not compromise health, 2) do not compromise productivity, 3) allow the animal to make a choice to have what it wants, 4) do not inhibit the animal's ability to perform natural behaviors, and 5) do not result in negative affective states. Because farms are becoming larger and housing more animals, and welfare assessments are becoming more important to animal agriculture, new technologies are being developed to ease the farmer workload and with the right approach, collect information to facilitate animal welfare assessment. However, developments in how we utilize technology to manage animals in agriculture stem firstly from the drive to increase productivity and efficiency of animal care, with animal welfare as a secondary consideration. So when animal welfare does become a consideration in the use or development of a new technology, these technological advancements may face many challenges when evaluated from an animal welfare perspective. Animal welfare includes multiple parameters including health, productivity, stress hormone levels, behavior, and

preference indicators (Norwood and Lusk 2011), and without evaluation we cannot fully understand the overall or long-term impacts of the technological development.

Understanding what it's like to be a bat (or a chicken) can prove difficult if we, as humans, do not possess the same sensory capacity as the animals we are caring for – yet consistent with Nagel's speculations from 1974; technology can help bridge the divide. Technological advancements provide humans with the opportunity to understand animal perception and present animals with environments that are perceived as comforting or positive by the animals themselves. For example, the use of dim biolux lighting in broiler houses to reduce aggression and increase foraging (Kristensen et al. 2007) and farrowing crates for housing sows right after parturition to reduce piglet mortality (Marchant et al. 2001) are examples of husbandry techniques that implemented technological developments to increase the overall level of welfare for these animals that may appear unpleasant to humans. Furthermore, animal sensory mechanisms are very different from humans thus affecting how they perceive the world. The large size of the middle ear ossicle and presence of sensory receptors in the trunk allows elephants to communicate with conspecifics up to 32 kilometers away using seismic vibrations (Rasmussen and Munger 1996). Chickens have tetrachromatic color vision (compared to the human trichromatic color vision), which allows them to see into the UV spectrum, perceive different reflective properties of the feathers on their conspecifics, and thus experience color intensities different from humans (Lewis and Morris 2000). Since animals have the ability to perceive their environments differently than humans would expect, they may be experiencing impacts to their welfare that we, with our set of sensory perception, cannot perceive. Therefore, technology that is focused on capturing the animals' viewpoint can help us understand what the

animal perceives and allow us to identify why they find environments suitable – thus improving their welfare.

However, technological development in agricultural animal welfare can be a double edged sword. Technology can facilitate animal care by making it easier for the farmer to care for animals through advancements such as electronic sow feeders that can use eating behavior to detect oestrus and infer lameness (Cornou et al. 2008), automatic lighting programs to facilitate safe growth rates in broiler chickens (Classen et al. 1991), hormone therapy to synchronize breeding in sheep (Titi et al. 2010) and cattle (Lamb et al. 2010), genetic selection for animals with more manageable temperaments so they can be handled safely (Barrozo et al. 2012), and automatic milking systems that give cows control over when they would like to milk (Jacobs and Siegford 2012). However, because these advancements in technology ease the workload of the farmer, they subsequently make it easier for farmers to care for more animals simultaneously. Since a true understanding of animal welfare requires assessing parameters that can only be measured at the individual level (e.g. affective states like ‘suffering’, ‘pain’, and ‘happiness’ or more broadly ‘emotions’ (Fraser, 2008) or being healthy and having needs met (Dawkins 2004)), technological advancements make it logistically more challenging to assess individual welfare because there are more individuals to assess and tailor management and environmental conditions for – thus creating a conflict between technology and animal welfare.

Much of the initial technological advancements in agriculture were a result of the post-World War II Industrial Revolution where governments placed considerable importance on mechanization, intensification, and specialized farming (Hardeman and Jochemsen 2011). Most of these technological advancements were designed to facilitate animal care, produce more food, and inadvertently resulted in a situation where fewer people were needed to care for animals.

This situation resulted in fewer people directly interacting with animals in agriculture. However, through the reduced number of people interacting with animals in an agricultural setting, and the distance between animals and humans becoming greater due to technological innovations, farm animals could have their essence as an animal lost in the eyes of humans and be seen as commodities designed for profit in a capitalistic society. Raymond Anthony describes some of these caveats of technological development in agriculture from an Environmental Virtue Ethic of Care approach where he describes that “the philosophy of technology that we have adopted regarding food conceals the nature of animals and transforms them into mere commodities” (Anthony 2010). Anthony continues to argue that this has happened through the processes of proprietorship (where the physical distance and conceptual detachment from the tradition of more wholesome food production eclipses any sense of responsibility towards local communities and its people), uniformity (the animality or subject of a life is overshadowed by its intended incarnation as an interchangeable, nondistinguishable morsel of food), and alienation (the separation of the animal from its subjectivity when they are characterized as food). Therefore, we should look to not only utilize technology as a tool for facilitating safe and effective food transfer and animal care, but to also look towards technological advancements as a way of highlighting the essence of the animal and understanding the individual.

Chickens as a case study

Deliberation among animal rights groups, animal scientists, producers, and legislators has transpired through the previous decades over the proper housing for commercial laying hens. Animal rights groups are openly criticizing traditional housing and management systems for laying hens in attempt to alter public opinion and change consumer habits (Shields and Duncan

2006). Limited space and opportunities to perform natural behaviors are some of the strongest arguments illustrating how cages compromise welfare. This criticism is received by a consumer base that on one hand is continually embracing new technological advancements in their daily life and on the other has been swayed to purchase ‘hormone free milk’ and ‘antibiotic free meat’. Consumers have been presented with arguments that could lead them to believe that laying hens are suffering at the hands of producers by being forced to live in a cage. Because this is a message that consumers are exposed to, legislation is being passed state by state across the United States to ban the housing of laying hens in cages. Producers, activists and legislators are reaching board room compromises in an attempt to avoid a polarizing and costly public battle.

In November 2008, 63% of California’s voting population passed a proposition to phase out the use of battery cages by 2015, and Michigan passed a law in October 2009 making it the second state in the nation to ban the use of battery cages to house laying hens (HSUS 2009). The general public has decided that they are ready for change in the egg industry and their voice is being heard. However, scientists, legislators, and animal rights advocates are still wrestling with how to satisfy their constituents and practically understand and assess the welfare in commercial situations while respecting the needs of the birds. With all sides of the argument trying to be heard, the most important voice, the chicken’s, may have been pushed to the side and muted – and technological advancements may provide a voice for the chicken’s actual needs.

Although the modern White Leghorn differs dramatically from their evolutionary ancestor, the Red Jungle Fowl, in many respects, the majority of these genetic differences reflect changes that have made it more profitable for humans to keep hens for egg production. Many behavioral characteristics of the Red Jungle Fowl are retained in the modern layer. Captive red jungle fowl – regardless of a regular feeding regime – choose to spend most of their daily

activities engaged in feeding activities (Dawkins 1989). Hens are extremely motivated to access nest areas for egg laying (Cooper and Appleby 1995, 2003; Smith et al. 1990) and perches during the day (Bubier 1996) and at night for roosting (Olsson and Keeling 2000). Fowl exhibit complex patterns of dominance and social hierarchy that are maintained through pecking behavior, as observed originally by Thorleif Schjelderup-Ebbe (Schjelderup-Ebbe 1922), which is still observed in groups of modern laying hens (D'Eath and Keeling 2003). In addition, nesting hens generally exhibit fear responses in the presence of predators (such as hawks) and retain vocalizations to differentiate between aerial and ground predators (Suarez and Gallup Jr. 1983).

Breeding companies are constantly developing new strains of laying hens, and strains currently in use are extremely efficient in converting feed and water into a marketable product. They lay larger eggs and do so more frequently than domesticated fowl. They have been bred to reduce “broodiness”, or the genetic drive to sit on a nest and incubate eggs, but breeding has not been successful in eliminating the drive to build and maintain a nest altogether. The disconnect between the genetic drive to perform natural behaviors and genetic selection towards a more profitable animal has forced producers to re-examine current housing practices to better accommodate behavioral and physiological needs of the hens. However, alternative housings that provide hens with more space and freedom of movement, also causes them to live in unnaturally large groups. Chickens have the capacity of remembering up to thirty distinct individuals and will congregate in groups of thirty or less in the wild (Wood-Gush 1971). Further, a smaller group size allows hens to develop peck orders that are determined by agonistic interactions which are maintained through time. So, from the hen’s perspective, housing hens in

groups of hundreds to thousands can make remembering and identifying individuals along with establishing a stable social environment challenging.

These larger group sizes also make it more difficult to assess the individual (as the hens look very similar) and therefore inherently more difficult to understand the welfare of the individual hen. One device currently under development to manage the non-cage laying hen welfare from the perspective of an individual hen is a wireless body-mounted sensor. This sensor is attached to a single hen using a nylon figure-eight harness and communicates with a network of sensors located in strategic points throughout the hen's environment. This sensor collects data on the hen's location (proximity to stationary sensor) as well as information about what behaviors the hens is performing. Valuable welfare-relevant information can be collected at the individual level with this sensor. Feeding, drinking, walking, preening, dust bathing, and proximity to important resources for each sensor-wearing hen can be identified using data produced from this sensor network – and all of these behaviors are important in understanding welfare from a health and emotional standpoint.

However, the information from this sensor must be interpreted in context for it to have any relevancy towards welfare. If the hen is recorded to be feeding, then that could be taken as a positive indicator towards welfare since we are able to assess that the hen is able to access food and feed. The information from this sensor can identify that the hen is performing the natural behavior of feeding which allows it to acquire something it wants thus reducing frustration, which could result in a positive affective state while gaining the necessary nutrients required for good health and biological functioning. However, the sensor is only capable of providing real-time data and information must be compiled over a larger portion of time to truly understand the individual hen's feeding behavior. A larger picture that includes feed duration, food conversion,

feather cover, and ability to access feed needs to be incorporated into an overall assessment when it comes to understanding the welfare of that hen with regards to feed acquisition. Further, there is wide variation among hens in temperament, behavioral patterns and resource use. Therefore, since the sensor can collect information from a single individual, the output from the single hen should be used to understand what that particular hen is currently experiencing and not extrapolated to represent an entire population.

This is just one example of how a developing technology can collect information that cannot be collected by human observation or perception. Yet the human element of understanding context and interpretation is required to truly maximize how the data from this technology can impact animal welfare assessment.

The way forward???

As new technologies are being developed to assess animal behavior, productivity, health and welfare, different interest groups (including those from the animal rights and animal welfare perspectives) may approach these technological advancements from different angles. In order to understand how each group might approach the development of technology for assessment of animal welfare it may be useful to highlight the way that perspectives on welfare are grounded in different philosophical beliefs. Ethical philosophies that are most commonly used with regards to animal welfare and animal agriculture are utilitarianism and rights views. Briefly stated with regards to technological developments, utilitarians would seek to understand if the technology brings more pleasure than would occur without the existence of the technology, while those with a rights view would seek to ensure that the presence of the technology respected the dignity or

inherent value of the animal thus allowing us to provide it with the opportunity to express its desires, initiate actions, and complete goals so they may satisfy their desires.

As exemplified in the work of Peter Singer, utilitarianism stresses the net value of the outcome that an action produces. Thus from the utilitarian perspective, the use of new technology for animal production is an action that will be justified only if the benefits outweigh the costs, when the outcomes of using the technology are compared to the status quo. If the technology would negatively affect production practices and product costs. Utilitarians would need to consider whether the benefit to animal welfare would outweigh the cost to the farmer or the consumer. Net utility could also be affected by how many animals (in terms of pleasure) are considered equal to the costs paid by the farmer. The classic utilitarian would regard the technology as good and right if it produced as much or more of an increase in the happiness or satisfaction of all affected by it when compared to an alternative action, and we would be wrong if we did not implement it (Singer 1993). Although there are many distinct ways to configure a utilitarian ethic, whether you were a hedonistic, egalitarian, or a preference utilitarian, the focus for animal welfare would be on developing technologies that bring pleasure (or relief from pain and distress) to the animal.

For example, the automatic milking system (AMS) is a technology that appears to meet the requirements of a utilitarian. The AMS provides the cow with the opportunity to choose when it would like to be milked. It is reasonable to surmise that the technology permits relief from distress that cows may feel when their udders are full. Furthermore, the cow receives a food reward for being milked thus creating a pleasurable memory for the cow surrounding milking. Of course, a complete utilitarian analysis would also need to consider the pleasure or utility of the farmer, so both party's interests and needs would be considered equally important.

The AMS allows the farmer increased flexibility in his/her schedule because s/he does not need to be at the farm at specific times to milk the cows allowing them to occasionally sleep in or attend a family gathering, and consumers are still able to purchase safe, healthy milk from cows that were raised in alignment with their values at a reasonable cost. The interests of the farmer and consumer could potentially outweigh any benefit the new technology may have provided to the animal's welfare. Although the AMS appears to pass the test of providing benefit both to cow and producer, if costs to humans outweigh benefits to animals, a utilitarian will insist that the new technology should not be adopted.

In contrast, the rights view begins by considering whether the parties involved are moral agents or moral patients, then moves on to a determination of the moral responsibility that agents have for their actions. Moral agents (MA) are capable of moral action, are rational, and capable of thinking morally. Moral patients (MP) must merely be sentient; their interests are affected but they do not think about the morality of their actions and are usually acted upon by moral agents. Tom Regan argues that animals are not MA because they do not have the ability to rationalize, but I do not completely agree with his argument. Many animals (including rats) have shown they have the capacity to learn from their previous experiences and the actions of others and have used that information to make future decisions about fitness and resource acquisition (Clayton and Dickinson 2006). This may be an argument for a different day, but if animals can rationalize their choices, understand how their choices affect other animals, and how their previous actions can affect their future experiences, and then they could potentially be considered MA which could have implications for how we view animals and their interactions with technology and other animals.

Further in Regan's view, individuals must be assessed as to whether they are a subject of a life (SOAL) before they are considered someone who is a bearer of rights. A SOAL must have beliefs and desires, have a memory capable of perception with a sense of the future, should be able to experience pleasure and pain, have preferences and welfare interests, will initiate actions in pursuit of its desires, develop a psychophysical identity over time, and understand how life experiences independently affect their interests (Regan 2004). The rights perspective would be interested in animal choices, animal preferences, animal past experiences, and whether the animal is experiencing pain. Many of these parameters have been measured and quantified for different agricultural animals and have shown to have implications for animal welfare, so when it comes to technology development, the rights view would seek to ensure that new technologies are designed in a way to provide animals with the freedom to choose what they prefer, can learn from their choices, and that animals do not experience pain due to the presence of the new technology. However, focuses just on animal interest may cause us to choose technologies that are impractical in an agricultural setting (Pastell et al. 2008) or may not measure a random proportion of the population, creating a bias (Turner et al. 1984). If animal management is too difficult due to changes in technology, farmers may be less inclined to implement or maintain the new technology that could increase welfare in favor of better, more practical, or easier management practices.

Utilitarian thinking will seek to utilize technological advancements based upon the net value gained while rights based thinking will choose to use technology only if it provides the animals with the freedom to honor individual preferences and respect individual interests. Yet, these two philosophies fall short when assessing technological advancements with regards to animal welfare. I propose we need to look beyond whether the technology can provide net gain

to the involved parties or inherent value of those associated with the technology, and look towards the value of the technology itself. Is technology necessary to enhance animal welfare and its assessment? If so, how much are we truly gaining from its implementation or advancement? Do we lose information from current tools when we implement new technologies? I propose we need to utilize a viewpoint that addresses the role of technology in animal welfare from a Philosophy of Technology perspective because technological innovation itself should be assessed before we make large investments in its development. We need to have an honest objective understanding of the true benefits of technological developments and whether they are prudent before assuming new products will universally enhance production practices or our understanding of animal welfare.

Incorporating the Philosophy of Technology into animal welfare

The utilitarian and rights viewpoints have been the most common philosophies utilized when discussing issues surrounding animals in agriculture. However, as technology becomes a more integral part of daily production practices, we should begin to incorporate a new way of thinking that is neither rights nor utilitarian based when deciding to use or using information from technological innovation. The Philosophy of Technology provides a framework for assessing the usefulness of technology, and when applied to animal welfare, may provide new insight as to the necessity or extent of information collected from these new technologies when assessing production practices.

In 1977, Martin Heidegger describes in his book “The Question Concerning Technology” a way of approaching technological development (Waddington 2005). Heidegger cautions

against the slippery slope humanity faces when it comes to modern technological development. Heidegger warns of becoming dependent on technology, exploiting resources (or animals) to gain the maximum yield at minimal expense, and emphasizes the need to separate ourselves from technology to objectively assess what and why we are creating. The philosophy of technology (POT) can provide guidance as to how we treat devices being created, how they are replacing previous technologies already in place, and reminds us to allow ourselves to utilize technology but allow ourselves to be removed from and not driven by technology. POT can help us to keep our Gestell (our drive to develop new technologies merely for the sake of understanding more or to see what can be done) in check by questioning what information is lost by moving beyond current technological developments and challenging forth (changing the nature of the materials or animals) the technology beyond its current state. We can have a free relationship with technology if we allow ourselves to use technology when necessary, and be willing to relinquish technology when appropriate (Dreyfus 1995). Improved or new technical devices do not necessarily serve the same function as the old ones but instead can simply alter the nature of the function (Zimmerman 1990). POT can force us to question whether we are relying on technology in agriculture, whether the new technology will continue to add to our understanding of animal welfare, or if the new advancements will overlook basic aspects of welfare because it was developed to provide information that omits knowledge gained from a previous device or assessment method.

Many of the impacts of modern technological advancement on animal agriculture has brought to life most of Heidegger's fears surrounding the development of technology. We have relied on technology in agriculture and taken living beings and reframed (changed their purpose) them into standing reserve (items that have become disposable, easily ordered and arranged or

endlessly replaceable and have little value) while humans challenge forth (or control the development) new technological devices to manage them without experiencing the essence of technology we have already brought forth (Heidegger 1977). Heidegger warned that technological reframing would compel entities (animals) to be revealed in inappropriate ways. POT assesses new technologies to identify what new information they are revealing. POT strives to understand whether the information gained from the new technology would do the same or more as the technology it is replacing or whether we are challenging forth past the essence of current technology by creating new devices. We should not allow technology to drive our decisions, but instead take an active role in how we view technology and be willing to separate ourselves from technology when necessary. POT, if taken from an animal perspective, can slow the process of implementation of technologies to identify whether they are necessary for animal welfare and truly allow the animal to retain its essence of being an animal.

A prime example of compromised animal welfare due to an inappropriate relationship with technology is reflected in the effects of genetic selection. Strong selection for increased production traits has resulted unintentionally in reduced reproductive performance in turkeys, negative immune performance in broiler chickens, leg weakness in pigs (Rauw et al. 1998), and blind chickens (Thompson 2008). Drawing from the blind chicken example, neither the utilitarian nor rights view provide a good framework for explaining why this is a problem. A utilitarian would argue that blind chickens are acceptable because the chicken is benefiting from being blind because they are at a reduced risk of aggression from conspecifics, are able to find food and have never missed sight because they never had the opportunity to experience it. Further, the producer would benefit from a reduced amount of aggression-related mortality while caring for chickens that produce at a rate comparable to chickens possessing sight and thus not

suffering any monetary consequences. A rights viewpoint emphasizes the importance of animal choices, preferences, past experiences and animal pain. A blind chicken is not experiencing pain, and cannot draw from previous experiences where they possessed sight that could influence their current or future choices or preferences. Therefore, even though breeding blind chickens feels morally wrong, the rights perspective does not provide a solid framework for explaining why this feels wrong. Yet, using our technological capabilities to breed blind chickens to relieve distress feels unnecessary and inherently wrong.

The Philosophy of Technology provides a different way of thinking that can help justify why breeding blind chickens feels morally wrong. Breeding blind chickens is a technological advancement that can be done, and POT will remind us to assess what we are compromising through genetic selection and whether these technological advancements should be done. Further POT can provide insight into other technological advancements in animal production, including understanding animal welfare, and help us develop a free relationship with technology that allows us to utilize technology when appropriate yet choose not to use it when unnecessary.

Even in cases where we cannot undo that such as regarding genetic selection, we can make changes to facilitate good care in sub-optimal situations. This is where animal welfare can step in and encourage the development of animal appropriate technologies that are designed to accommodate the challenges genetically selected individuals face and help caretakers understand the environment from the animals' perspective thus helping us to understand what is important to the animal and its welfare.

The interests of animal rights are nestled within the science of animal welfare. Animal welfare uses parameters important to animal rights such as good health, preference, perception, pain, and animal actions to understand if the animal is experiencing good welfare in its current condition. Both Dawkins' and Frasier's animal welfare schools of thought descend from the Five Freedoms and require us to understand what the animal is perceiving, how it is feeling in its current environment, and requires that good animal welfare include a positive affective state or the animal being satisfied by having what it wants – all of which are important to those with either a utilitarian or rights view.

Assessing new technologies with welfare in mind may be met with similar challenges as if we were assessing new technologies from the rights perspective. Animal welfare-based technology assessment take animal health into account regarding whether the technology will have the potential to increase animal welfare, but it may advocate for a technology that may be impractical to implement or may not provide as much benefit to the animal or information to the researcher as originally envisioned.

Technological development assessment from the animal welfare and animal rights perspective may cause us to develop new technology because we can, instead of learning how we can improve welfare or get more information from the technologies we currently have. By utilizing the principles from philosophy of technology in addition to the animal rights and animal welfare ideology in our evaluation of new agricultural technological developments, we may be able to enter into a free relationship with agricultural technology by improving upon current technologies, implementing practical animal-based technologies, and retain the essence of both the animal and the technology, while improving animal welfare.

One final point...

A cyclic relationship exists between the need to develop new technologies and working alongside the technologies already in place. Humans work alongside (bring forth) technology until the need for innovation forces us to challenge forth (or create) new technology. The same type of relationship is happening with animal welfare assessments. Humans have been working alongside (bringing forth) animals, and the need to understand and assess animal welfare has forced us to develop (challenge forth) animal welfare assessment protocols. These animal welfare assessment programs may be facilitated with input from technological advancements.

Technological advancements developed to ease farmer labor could be argued to have contributed to the commodification of animal products. These technologies have made it easier to provide food to a growing human population, but it has come at a cost. Technology can create a distance between humans and animals causing us to never learn (or to forget) where food comes from or appreciate the value of the animal that died to provide us with nutrition. Moreover, humans cannot perceive the animal's umwelt (the world from the animals' perspective) in the same manner as an animal (von Uexkull 1909). If we can challenge forth new technology that brings us closer to understanding the animal at the animal level (e.g. animal-appropriate and animal-focused technology) then humans may have the opportunity to return to bringing forth with technology and can help bridge the gap between human and animal perception, potentially increasing animal welfare.

However, as new technologies are being developed to understand the world from the animal's perspective and facilitate animal welfare assessment, we should be wary of the

technology treadmill (Thompson 1998). Technological advances have been a critical source of productivity and efficiency gains in farming, and farmers that are earlier adopters of the right technologies have typically been financially successful. The implementation of technology into animal welfare assessment should not be associated with economic gain or loss to the degree that it would change market prices. We will be assessing the interest of the animal, which farmers should not be financially punished to understand, but this is a capitalistic society and gathering additional information about the animals will require a financial commitment. Further, if farmers are constrained by consumer choice and economic feedback – or pushed by moral values, they may be motivated to adopt technologies that will enhance animal welfare and our understanding of animal welfare that will allow them to sell products in different markets.

Yet farmers should be cautious about technologies that claim to enhance animal welfare because they may not know out of what welfare philosophy the technology was born, whether the technology can give information on a single individual or multiple individuals, and the information gathered must be taken in context to truly be relevant to an animal's welfare state. Farmers may find technologies that are capable of collecting behavioral information on individual hens in non-cage housing systems seductive, but they need to understand that the welfare of one does not equal the welfare of many and any information collected must be taken in context to have any relevancy to animal welfare.

Technology can help us understand. Technology does not have to have bias, or previous experience to affect how it perceives the world around us. We may be able to develop technologies that will allow us to assess light level and intensity, sound frequency and volume, and vibration strength from the perspective of an animal, and that information will be useful in helping us assess how the animals are responding to the environment around them. Technology

(if built to be so) can be truly objective and operate with no pre-conceived notions. So, even if we cannot ever know what it's like to be a bat (or a chicken) we may be able to create unbiased, animal-based technology to collect objective information about the animal or the animal's environment, in a manner in which the animal perceives it. We may be able to then combine this information with the animal's behavior, to understand how the animal perceives the environment and what factors are important to its existence. This will allow humans to take technological and behavioral information in context and combine this with our values to determine a scientifically sound assessment of welfare.

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CHAPTER 2: NONCAGED LAYING HENS REMAIN UNFLAPPABLE WHILE WEARING BODY-MOUNTED SENSORS: LEVELS OF AGONISTIC BEHAVIORS REMAIN UNCHANGED AND RESOURCE USE IS NOT REDUCED AFTER HABITUATION

ABSTRACT

Unique markings or body-mounted sensors facilitate data collection from individuals in large groups of similar-looking conspecifics but may have unintended consequences on behaviour. A wireless sensor attached to the back of laying hens via a harness has been developed to monitor space use and activity. Prior to collecting experimental data, impacts of the sensor on resource use and social interactions were assessed. Four rooms of 135 hens each were weighed and 10 hens/room were randomly fitted with sensors at 11 wks of age (0 d). Instantaneous scan samples recorded the number of hens (SEN: sensor wearing hen and NON: hen without sensor) using resources (feeder, water, nest box, perch) every 5 min over 24 h on -5 d, -4 d, -2 d, -1 d, 1 d, 2 d, 4 d, 8 d, and 16 d. Logistic regression determined that SEN feeder use was less on 1 d and 2 d and more on 16 d than NON. SEN water use was reduced only on 1 d. SEN nest box use increased on 1 d, 2 d, and 16 d. SEN perched more on 1 d, 2 d, and 4 d, and less on 8 d. Initial resource use was affected by wearing a sensor, but by 16 d all resources were used similarly or more by SEN than NON. No difference in body weight was observed on 17 d suggesting that long-term resource use was not affected. No differences were observed among the number of agonistic observations -5 d, 8 d, and 16 d. With the exception of SEN hens acting as aggressors towards NON hens, agonistic interaction types occurred close to expected proportions. These factors indicate that hens habituate to wearing sensors within 2 wk.

INTRODUCTION

Body-mounted, wireless sensors are useful tools for remotely monitoring elements of animals' behavior including their location, levels and types of activity, and movement. Sensors can be attached through a myriad of methods such as pit tagging (fish and amphibians (Gibbons and Andrews, 2004)), gluing to the skin (sea lions (Willis and Horning, 2005), horses (Keegan et al., 2004)), or fitting the animal with a collar (arctic foxes: (Pamperin et al., 2008)), harness (elephants: (Rothwell et al., 2011), cattle: (Pastell et al., 2009; Robert et al., 2009)), and in some cases, a sweater (koala: (Takahashi et al., 2009)). Specifically in birds, sensors have been attached by gluing them to feathers (Yoda et al., 2001) and wrapping them around wing or body by using a harness (Fleskes, 2003).

Location, behavior, and resource use of wild birds have been monitored using sensors since the early 1970's, with harnesses used as the primary method of attaching the sensor to the birds (Gilmer et al., 1974; Greenwood and Sargeant, 1973). However, wearing a harness may alter birds' physicality by altering their balance or restricting wing movement, which may cause birds to move or behave differently. It is therefore important to validate that data collected from body-mounted sensors attached in this way are representative of data from birds not wearing sensors.

The impact of wearing body-mounted sensors has been studied in several types of wild birds, and the results suggest there are impacts of varying degree on behavior, resource use, or both. Wild female Mallards (*Anas platyrhynchos*) fitted with back-mounted radio transmitters spent less time feeding, more time resting and preening, and had smaller clutches of eggs when compared to female Mallards not wearing transmitters (Pietz et al., 1993). Female European Golden Plovers (*Pluvialis apricaria*) with back-mounted transmitters exhibited no differences in

feeding activity but did roost less than plovers not wearing transmitters (Whittingham, 1996). Further, captive male Dickcissels (*Spiza americana*) wearing leg-mounted transmitters showed a 24 h post-attachment spike in fecal glucocorticoids (Wells et al., 2003) but showed no long-term response to harness placement. Female captive Blue-Winged Teal (*Anas discors*) fitted with sensors attached using backpacks increased the amount of time they spent engaged in comfort behaviors and reduced the amount of time spent in the water throughout the three-month study (Garrettson et al., 2000).

In addition to a bird's own physical and behavioral response to wearing a sensor, having a sensor attached to the body could influence social interactions by altering the individual's appearance. For example, female Zebra Finches (*Taeniopygia guttata*) spent more time with males marked with a red leg band and less time with males marked with a green leg band when compared to time spent with unmarked males, indicating that social perception was altered by physical markings (Nancy, 1988). Further, male Blue Throats (*Luscinia s. svecica*) marked with a blue and orange leg band spent less time guarding their mate and sang at a higher rate compared to unmarked males and female Blue Throats showed a preference for males with symmetrical leg bands regardless of color (Fiske and Amundsen, 1997). Wild Turkey hens (*Meleagris gallopavo*) wearing a back-mounted transmitter experienced no long-term effects on reproduction, social status, or feather wear (Nenno and Healy, 1979). Thus, effects of sensor presence vary by species and can be different based upon the parameter of interest (e.g., reproduction vs. resource use). Further, the location of the sensor on the bird's body plays an important role on the degree of effect (e.g., leg-mounted vs. back-mounted) due to different areas of the body being more or less evolutionarily important in social interactions, resource use, and behavior.

A wireless sensor (Figure 1) has been developed to monitor the space use and activity levels of non-cage laying hens (Quwaider et al., 2010). The sensor is mounted on the back of the hen, to achieve maximum sensor stability and signal quality for both acceleration and radio signal information while avoiding tissue damage to the hen. The sensors are placed inside a casing attached to a figure eight nylon harness designed to fit around the hens' bodies while not restraining the wings. The casing is colored to match the hens' feather color and marked with a unique identifying number using white paint to allow for easy identification of individual hens on video recordings. This body-mounted sensor has been developed to observe individual hen movement and activity in large flocks in non-cage housing systems, therefore, it is important to first ensure that the presence of the sensor does not affect the behavior of the hens, unintentionally compromising accuracy and quality of future data.

Hens have complex social structures that are usually stabilized by 7 to 9 weeks of age (Wood-Gush, 1979). Body weight (Cloutier et al., 1995; Cloutier and Newberry, 2000) comb size (Cloutier et al., 1996), and previous interactions (Cloutier et al., 1995; Cloutier and Newberry, 2000) are important factors in determining social status. Hens can be sensitive to phenotypic differences and altering their physical appearance can alter social interactions with conspecifics. For example, hens who look different from their flock mates may become victims of feather pecking and exhibit different levels and types of behaviors than non-marked hens (Dennis et al., 2008), and roosters with dubbed wattles and combs were victims of increased aggression (Siegel and Hurst, 1962). Further, a strong positive association has been determined between a hen's comb size and her competitive ability which is retained over time (O'Connor et al., 2011).

The sensors in this study have been used in previous experiments to collect data regarding sensor performance (Quwaider et al., 2010). Anecdotal observations made during these studies suggested that wearing the sensor did not affect hen resource use and agonistic interactions. Additionally, no signs of hen skin or feather damage were observed as a result of wearing a sensor. The null hypothesis for this study was that no differences would be observed between harnessed and non-harnessed hens with regard to resource use and agonistic interactions at the individual and flock level. The objectives were to validate that patterns of resource use (feeder, drinker, nest box, and perch) were unchanged after sensor placement and to identify whether there were differences in levels of agonistic interactions between hens wearing and not wearing sensors.

MATERIALS AND METHODS

Animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Teaching and Research Center (MSU-PTRC). Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University Institutional Animal Care and Use Committee. Four identical rooms (6 m x 4.5 m) at MSU-PTRC were used. Each room was furnished in the same configuration with nest boxes, perches, tube feeders, and a water line with nipples. Sixteen nest boxes (each 0.4 m long x 0.3 m wide x 0.3 m high) in a 16 x 2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1 m x 6 m slatted area at a

height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8cm of wood shavings at time of data collection. Food and water were provided daily ad libitum. Daily care, including egg collection, feeding, and hen inspection, occurred at least once a day. Two incandescent light bulbs (60 lux at bulb level) on an automatic timer provided light 15 h per day in each room. Temperature was maintained between 16°C -22°C using a ventilation fan and forced air heating.

Hy-Line Brown laying hen pullets (135 hens/room; n = 552) were reared in each of the rooms as described above with accommodation made for their smaller size (i.e., smaller perches, which were removed at 6 wk) and immaturity (i.e., access to nest boxes was granted at 10 wk of age). Each room provided 0.21 m² floor space, 17.8 cm of perch space, 0.01 m² of nest box space, 4.83 cm feeder space per hen. Thirteen nipples provided enough drinking space for 10.3 hens per nipple. Hens (n = 10 per room) were fitted with sensors on 0 d when they were 11 wk of age (sensor-wearing hens = SEN; see (Quwaider, Daigle, Biswas, Siegford and Swanson, 2010) for image and description of sensor and sensor network). The remaining hens in each room did not receive sensors (non-sensor hens = NON). Hens in each room were videotaped for 48 h (-5 and -4 d) prior to any handling or sensor attachment. On -3 d, all hens were weighed and fitted with uniquely numbered leg bands. Leg bands were not visible from the ceiling mounted cameras, so individual tracking of hens without sensors was not possible. All hens were videotaped for an additional 48 h (-2 and -1 d). On 0 d, all hens were handled and 10 hens per room were selected and fitted with a sensor. The SEN hens were randomly selected from across the range of body weights (e.g. one random hen was selected from weights 700-800 g, one random hen was selected from weights 801 - 900 g etc.) obtained on -3 d in an attempt to select

for hens of varying social ranks (Cloutier and Newberry, 2000). Subsequent sensor data to monitor the hen's behavior prior to and immediately after the onset of lay was the motivation for performing this study before comb development. Therefore, we were unable to include comb size as a parameter to select hens for wearing sensors (as described in O'Connor et al., 2011). Hens were observed for 48 h on 1 d and 2 d and for 24 h subsequently on 4 d, 8 d, and 16 d. All hens were weighed again on 17 d.

Data recording and statistical analysis

For resource use data collection, instantaneous scan samples were taken every 5 min over 24 h on all observation days (-5 d, -4 d, -2 d, -1 d, 1 d, 2 d, 4 d, 8 d, and 16 d), and counts recorded the number of hens (SEN and NON) using each resource. For agonistic interactions, the number of agonistic interactions (Table 1) that occurred during six 20 min periods spread throughout the lights on portion of the day [0630 – 0650; 0820 – 0840; 1010 – 1030; 1540 – 1600; 1730 – 1750; 1920 – 1940] on -5 d, 8 d, and 16 d from all rooms was recorded from video using continuous observation. An increased level of agonistic interactions could have been expected immediately after placement of sensors on hens due to factors associated either with environmental disturbance, animal handling, or sensor placement. Therefore, agonistic interactions were recorded on 8 d and 16 d to identify whether sensor presence resulted in a sustained increase in agonistic interactions. All analysis for this paper was conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA). For all analyses, results were considered statistically significant at a probability of α less than 0.05.

Resource use

To determine whether overall flock patterns of resource use differed before and after sensor placement, use of the feeders, nipple drinkers, nest boxes, and perches was recorded (Table 2). The number of hens using each resource was divided by the total number of hens per room to create a proportion describing the percentage of a room's population using a resource. The proportions of all hens in a room using resources of interest before and after sensor placement were then transformed for normality and compared with a t-test. Satterthwaite t-tests were used to compare body weights of SEN and NON hens on -3 d and 17 d.

Data on the proportion of SEN and NON hens using each resource at each time point after sensor placement (1 d, 2 d, 4 d, 8 d, and 16 d) were analyzed using a logistic regression model (PROC LOGISTIC). The model included room, day, treatment (SEN vs. NON), and the interaction between day and treatment. Each resource was analyzed separately. Data on the proportion of hens using each resource were averaged over rooms. For all variables, the Firth's penalized likelihood was used to account for the large number of zeroes in the dataset. Odds ratio Wald Confidence Limit Contrast statements were used to identify specific differences between SEN and NON hens for each day.

Agonistic interactions

The location (Figure 2) of each agonistic interaction was noted [food-water area (FW, 10.33 m^2), perch (PERCH, 8.05 m^2), or not close to any resource (NONE, 7.27 m^2)], and whether hens involved in the interaction were two NON hens (NON-NON), a NON hen

aggressor and a SEN hen recipient (NON-SEN), a SEN hen aggressor and a NON hen recipient (SEN-NON), or two SEN hens (SEN-SEN).

A general mixed model procedure (PROC GLIMMIX) with a Poisson distribution was used to identify differences in the level of agonistic interactions across time and whether location in room affected the amount and type of interactions observed. The model included the total number of agonistic interactions recorded using day, area, their interaction, and included room as a random effect. Least-squared means were used to identify differences between area and day.

Levels of agonistic interactions that involved a SEN hen were analyzed for 8 d and 16 d only. The proportion of observations for each type of agonistic interaction (Table 1) were calculated per room per day and compared to the expected percentage using a Wilcoxon Mann-Whitney test (PROC NPAR1WAY). Further, the effect of the sensor on location of agonistic interaction was analyzed using a generalized linear model (PROC GENMOD) with a Poisson distribution where the fixed effects and interactions between sensor presence, day, and area were analyzed. Least-squared means were used to identify differences between area and day.

RESULTS

Resource Use

On average, no differences were found in percentage of hens using the feeder before and after sensor placement (before = 51.6%, after = 51.5%, $t = 0.19$, $P > 0.05$) or drinker (before = 30.8%, after = 31.0%, $t = -0.68$, $P < 0.05$). Differences were observed in the percentage of hens that used the nest box before and after sensor placement (before = 20.0%, after = 23.0%, $t = -9.12$, $P < 0.001$) and perch (before = 56.0%, after = 57.4%, $t = -4.75$, $P < 0.001$). SEN weighed

(g) less (mean \pm SEM; 921 ± 21) than NON (963 ± 5) on -3 d ($t = -2.32$, $P < 0.05$). However, SEN gained more weight (369 ± 11) than NON (339 ± 2) during the 20 d observation period ($t = 2.83$, $P < 0.01$), and by 17 d no difference ($t = -0.25$, $P > 0.05$) in body weight (g) was observed between SEN (1297 ± 22) and NON (1302 ± 5).

Significant differences were found between proportions of SEN and NON hens using the various resources at some of the time points (Figure 3). Feeder use by SEN was less on 1 d ($X^2 = 12.13$, $P < 0.01$) and 2 d ($X^2 = 6.88$, $P < 0.01$), and more on 16 d compared to use by NON hens ($X^2 = 48.17$, $P < 0.001$). Feeder use was the same for both treatments on 4 d ($X^2 = 0.014$, $P > 0.05$) and 8 d ($X^2 = 0.002$, $P > 0.05$). Drinker use was reduced on 1 d ($X^2 = 4.80$, $P < 0.05$) for SEN hens compared to NON hens. No differences were observed between SEN and NON hen drinker use on 2 d ($X^2 = 0.22$, $P < 0.05$), 4 d ($X^2 = 0.003$, $P > 0.05$), 8 d ($X^2 = 0.002$, $P > 0.05$), and 16 d ($X^2 = 1.54$, $P > 0.05$). Nest box use by SEN hens was increased on 1 d ($X^2 = 181.64$, $P < 0.001$), 2 d ($X^2 = 0.65$, $P < 0.001$) and 16 d ($X^2 = 75.64$, $P < 0.001$) relative to use by NON hens. No differences were observed between SEN and NON nest box use on 4 d ($X^2 = 0.89$, $P > 0.05$) and 8 d ($X^2 = 2.37$, $P > 0.05$). SEN used the perch more on 1 d ($X^2 = 10.62$, $P = 0.001$), 2 d ($X^2 = 11.01$, $P = 0.001$) and 4 d ($X^2 = 8.97$, $P < 0.01$), and less on 8 d ($X^2 = 20.34$, $P < 0.001$) compared to NON hens. No difference ($X^2 = 0.15$, $P > 0.05$) in perch use between the treatments was observed on 16 d.

Agonistic interactions

When examining overall levels of agonistic behaviors, there was no effect of day on the number of observed agonistic interactions (mean \pm SEM; -5 d: 2.32 ± 0.37 , 8 d: 2.91 ± 0.25 , and 16 d: 2.74 ± 0.30 ; Figure 4a; $F_{2,24} = 0.91$, $P > 0.05$). Each of the three areas of the room (FW: 3.00 ± 0.20 ; NONE: 3.61 ± 0.15 ; and PERCH: 1.36 ± 0.47) had different levels of observed agonistic interactions (Figure 4b; $F_{2,24} = 11.85$, $P < 0.001$) with the fewest agonistic interactions occurring in the PERCH area. No interaction effect was observed between area and day ($F_{2,24} = 0.11$, $P > 0.05$).

When considering the impact of sensor presence on the type of agonistic interaction, Figure 5 illustrates that no differences were observed between the observed and expected percentage of interactions for NON-NON ($Z = -1.64$, $P < 0.10$), NON-SEN ($Z = -1.64$, $P < 0.10$), or SEN-SEN ($Z = 0.81$, $P > 0.05$). No SEN-NON interactions were observed throughout the study, so therefore, fewer SEN-NON interactions occurred relative to the expected percentage ($Z = 3.57$, $P < 0.001$). Effects were observed for the level of agonistic interactions for area of room (Figure 6; $X^2 = 71.55$, $P < 0.001$). After hens were fitted with sensors (i.e., 8 d and 16 d), fewer agonistic interactions were observed in the PERCH area (0.58 ± 0.22) than in either FW (2.01 ± 0.11) or NONE (2.22 ± 0.10). No differences in levels of agonistic interactions were observed between SEN and NON hens for 8 d (1.57 ± 0.144) or 16 d (1.65 ± 0.11 , $X^2 = 0.18$, $P > 0.05$), and no interaction effects between day and area ($X^2 = 4.79$, $P > 0.05$), day and sensor ($X^2 = 1.41$, $P > 0.05$), area and sensor ($X^2 = 1.55$, $P > 0.05$), or day, area and sensor ($X^2 = 0.70$, $P > 0.05$) were observed across the model.

DISCUSSION

Harness presence had a minimal negative long term effect on non-cage laying hen resource use and agonistic behavior, suggesting that hens were able to habituate to wearing the device. This was the first study to assess the effect of marking non-cage laying hens on resource use and agonistic behavior. Resource use appears to have been affected initially by sensor placement as indicated by a reduction in feeder and drinker usage by SEN hens and an increase in nest box and perch use. Hens fitted with sensors may have initially spent more time using resources, such as perch and nest boxes, that allowed them to isolate themselves from the flock in order to acclimate to their new sensor. Aviary-housed hens have shown a linear increase in nest box use even after egg production peaked indicating the nest boxes may be used for behaviors other than egg laying (Carmichael et al., 1999). SEN may have also spent more time on the perches because they may have been spending more time maneuvering throughout the perches due to the change in body weight distribution. Also, this breed of hen rarely used the perch (they would sleep on the floor) so small changes in the number of hens using the perch could have greatly influenced the results. Specific behaviors of individual SEN hens were not recorded, but it is likely that SEN hens were spending more time preening and adjusting to the presence of a sensor on their backs during the first few days after sensor placement since preening is considered a comfort behavior (Black and Hughes, 1974; Nicol, 1989) and might also be required to rearrange feathers on the hens' body to accommodate the sensor.

Hens could see the sensor on either herself or her flock mates; however, the sensor itself did not appear to attract other hens' attention. SEN were observed flying, jumping, and accessing all resources seemingly without difficulty, so it is not assumed that the harness presence

impacted movement, agility, or their ability to perform behaviors. However, although the sensor weighed very little (<10 g), SEN hens may have required time to adjust to the slight change in body weight distribution following SEN placement. Further research investigating comfort behaviors may highlight whether comfort behaviors only occur as hens habituate to and accommodate the sensor or if they are sustained over a period of time.

Resource use for SEN and NON hens fluctuated throughout the duration of the study. Prior to hen handling, feeder and perch use were not constant between -5 d and -4d, after hen handling and before sensor placement (on -2 d and -1 d), feeder, water, and perch use greatly fluctuated within the group of all NON hens. Therefore, almost none of the resources were consistently used throughout the duration of the study among the NON hens or in the period before handling and sensor placement. This may be a reflection of their stage of development, or since there were fewer hens in the SEN group, the differences in resource use due to individual variability may be more apparent. Initial changes in resource use by SEN hens that were observed were larger than the fluctuations observed in the pre-sensor period or by NON hens, and some of those changes lasted for a week or longer. Regardless, the changes that were observed occurred in directions that do not suggest a poor welfare state for either the SEN or NON hens.

Habituation can be defined as a “decreased response to repeated stimulation” and can be used as a proxy measure for behavioral plasticity (Groves and Thompson, 1970). The increased level of feeder use by SEN hens compared to NON hens on 16 d could be due to a rebound effect as hens may have been spending more time eating to recoup the caloric intake from the feeding time they lost during 1 d and 2 d (Duncan and Wood-Gush, 1972). Another possible reason for increased feeder time could be associated with the extra effort required to carry the extra weight

associated with the sensor. Even though the sensor weighed very little (10g – which was 1.07% of the hen's body weight on -3 d and 0.77% of the hen's body weight on 17 d), it may have been enough weight to alter how hens were moving through their environment, required more energy than normal, and resulted in the hen feeding more to gain the necessary calories for movement. SEN were observed flying, jumping, and accessing all resources without difficulty, so it is assumed that the harness presence did not impact movement, agility, or their ability to perform behaviors. Further, at 11 – 14 wk, the hens were still growing, so it is possible that the hens were growing and feeding at different rates between -3 d and 17 d. The increased nest box use by SEN hens compared to NON hens on 16 d could be attributed to factors associated with the onset of lay or changes in social dynamics. Therefore, after an initial 1-2 day habituation period, SEN hens appeared to habituate to wearing the sensor and harness and returned to using resources at a rate similar to or greater than that of NON hens.

Levels of agonistic interactions remained unchanged across all observed days, and NON-NON, NON-SEN, and SEN-SEN interactions occurred at proportions that were not significantly different from expected. This suggests that wearing sensors had no effect on overall levels of agonistic interactions within the flocks. The presence of the sensors did not visually change the appearance of SEN hens making them targets of other birds (due to markings and physical appearance of sensor casing), nor did the sensors stimulate increased aggression by SEN as a result of redirected aggression by SEN hens uncomfortable in their harnesses (McFarland, 1966). Moreover, frustrated hens have been found to exhibit higher levels of aggression than hens who were not placed in a frustrating situation (Duncan and Wood-Gush, 1971). Since SEN hens were never involved in a SEN-NON interaction, they may not have been frustrated by the sensor presence and thus not motivated to perform more aggressive behaviors. Alternatively, they may

have been trying to isolate themselves to avoid attracting the attention of other hens while they made adjustments to the sensor.

The lack of SEN-NON interactions is consistent with other studies investigating amount and direction of aggressive interactions for group-housed chickens. Dennis and colleagues (2008) investigated whether the proportion of marked individuals in small and large groups of broiler chickens affected the delivery and receipt of aggressive and non-aggressive pecks. Consistent with the present study, regardless of group size or proportion of marked hens, marked hens gave the least number of aggressive pecks compared to unmarked flock mates. Conversely, Dennis and colleagues observed marked hens receiving more aggressive pecks compared to their unmarked counterparts. The results from this and our study emphasize that marked hens in groups of marked and unmarked hens may be less likely to deliver aggressive pecks or initiate aggressive interactions, yet marked hens may not always be the recipient.

More agonistic interactions were observed in the FW and NONE areas compared to the PERCH. There could be many reasons for this. For example, hens perform higher levels of frustration behaviors when they cannot access food (Duncan and Wood-Gush, 1972; Zimmerman et al., 2000). The FW area contained two highly-valued and inelastic resources (i.e., food and water) in a small area; thus hens could have been performing agonistic behaviors to gain access to these resources. The NONE area had no physical barriers, allowing hens to see from one end of the room to the other, and was mostly used by hens moving between FW and PERCH areas. Thus, alternatively, more agonistic interactions could have occurred here because NONE provided hens with an open area with sufficient physical space to perform agonistic interactions such as standoffs or fights, or because hens may have been more likely to make visual or physical contact with unfamiliar individuals in NONE when compared to other areas. Higher

levels of aggressive behavior in broiler chickens were observed in pens without physical perch barriers compared to those with perch barriers (Ventura et al., 2012) and aggressive interactions in broilers usually occurred in the open areas of the pen (Cornetto et al., 2002; Pettit-Riley et al., 2002). This suggests hens housed in rooms with large amounts of uninterrupted space may perform more aggressive behaviors, and providing physical barriers that break up the visual and physical space may be useful for mediating aggression levels.

Levels of agonistic interactions in large flocks are usually lower than in small flocks because hens no longer rely on a stable peck order as a social strategy (D'Eath and Keeling, 2003; Estevez et al., 2002). Instead they use a more competitive ability strategy, incorporating individual physical characteristics and status signaling to determine dyadic dominance on an as-needed frequency (D'Eath and Keeling, 2003; Estevez, Newberry and Keeling, 2002; Hughes et al., 1997; O'Connor, Saunders, Grist, McLeman, Wathes and Abeyesinghe, 2011). Increased aggression in the NONE area compared to FW and PERCH supports the competitive ability theory where agonistic interactions occur on an as-needed basis rather than used to establish a dominance hierarchy.

Hens rarely used the perch during the observation period. Therefore, few agonistic interactions were observed in the PERCH area simply because there were few hens in that area. Again, since few hens used the perch, a small number of hens could greatly influence the results and may account for the wide variation of perch use recorded in SEN and NON perch use throughout the duration of the study. Hens usually use the perch for resting, and perches are normally a destination for a hen, not a transitional area (e.g., the NONE area). These factors combined with the low competition for perches, resulting from the wide physical distribution of perches across the length of the rooms and sufficient space per hen to perch (17 cm perch

space/hen) could account for the reduced number of agonistic interactions observed in the PERCH area.

The impact of social factors or changes in social structure cannot be overlooked when assessing the impact of the sensor on non-cage laying hen welfare. However, identifying the social hierarchy before or after sensor placement for this large group of hens was not possible. Understanding the sensor's impact on social structure or agonistic interactions may benefit from future investigation. SEN hens never initiated an agonistic interaction with a NON hen, but they did initiate interactions with other SEN hens. This may indicate that SEN hens were of similar rank or they may have been intimidated by NON hens. Another possibility is that SEN may have been able to identify those few unique looking individuals with sensors, remember what happened in their past encounter, and find the agonistic interaction worth the investment when they may not be able to remember past encounters with NON. SEN were initially lighter than NON which could have contributed to their lack of agonistic interaction initiation, but the difference between the groups' body weights were so small, they could be due to differences in crop fill (Savory, 1985).

In summary, the body-mounted sensors were mounted on the back of non-cage laying hens without having large or long-lasting impacts on resource use and agonistic behavior. The hens may have been able to habituate to the harnesses in part because each harness was custom fitted for individual comfort. Additionally, the back of the hen is a part of the birds' anatomy that is not evolutionarily important for signaling status or improving competitive ability for resource acquisition in groups of all-female non-cage laying hens. Even though impacts on resource use and agonistic behavior were observed in this study, future studies utilizing body-mounted sensors or markings for individual identification should take into account the possible physical

impacts of sensor attachment on bird movement and the social context in which the phenotypic modification could be perceived by conspecifics before determining whether the sensor will provide unbiased information.

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APPENDIX

Table 2.1: Definitions of behaviors developed to identify agonistic interactions (Adapted from Newberry and Cloutier, 2002)

Behavior	Description
Peck	A hard, fast stab with the beak at another hen, usually at the head or comb
Claw	A strike at another hen with the claws of one foot while keeping the other foot on the substrate
Leap	A jump into the air with both feet off the ground followed by a strike at another hen with one or both feet
Stand-off	When 2 hens stand staring at each other for >2 seconds
Fight	Two hens perform a series of agonistic acts towards each other in rapid succession including leaps, claws, and/or pecks, until one hen retreats

Table 2.2: Definitions of behaviors developed to identify resource use

Resource	Description
Feeder	Hen has head in feeder and is pecking at grain with beak
Drinker	Hen has head turned upwards towards water line and is pecking at nipples of the drinker with beak
Perch	Hen is standing, walking, or resting on perch, the rail in front of nest boxes, or black slats underneath raised perches
Nest box	Hen is standing or resting inside of a nest box

Figure 2.1: (a) Photograph of a laying hen wearing a wireless sensor. (b) Sensors are packaged in a plastic case to prevent entry of dust and moisture without damaging hen skin or feathers. The sensor is mounted on the back of the hen using a figure-eight nylon harness. Both the sensor case and harness are colored to blend in with the hen's feathers to avoid attracting the attention of other hens in the room. The picture was taken several days after the hen was fitted with the sensor.

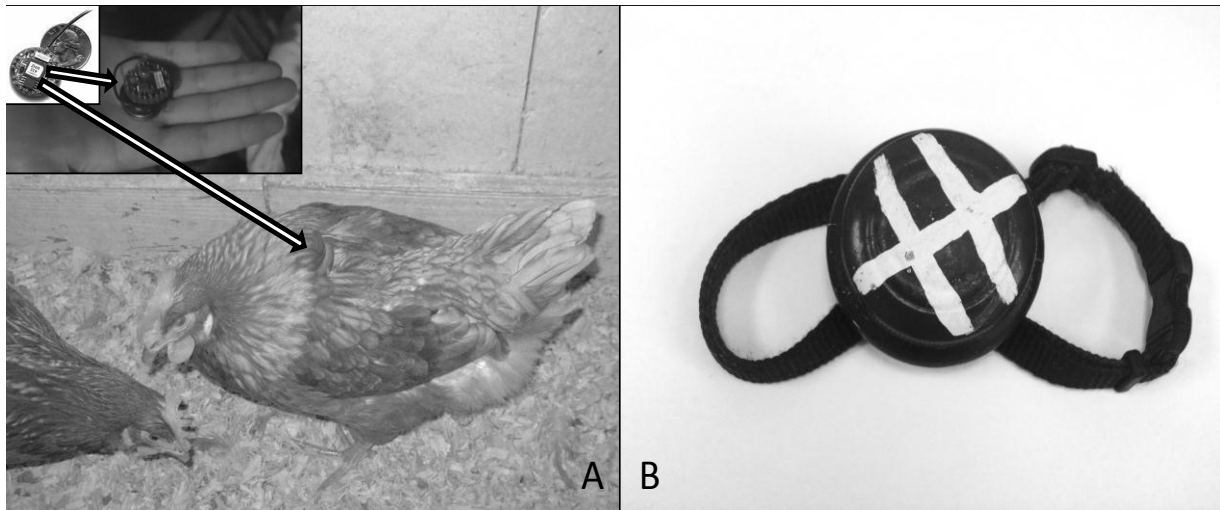


Figure 2.2: Dimensions and orientation of resources in room. The location of hens in the room were recorded and designated as one of the 3 shaded areas (PERCH, FW, or NONE).

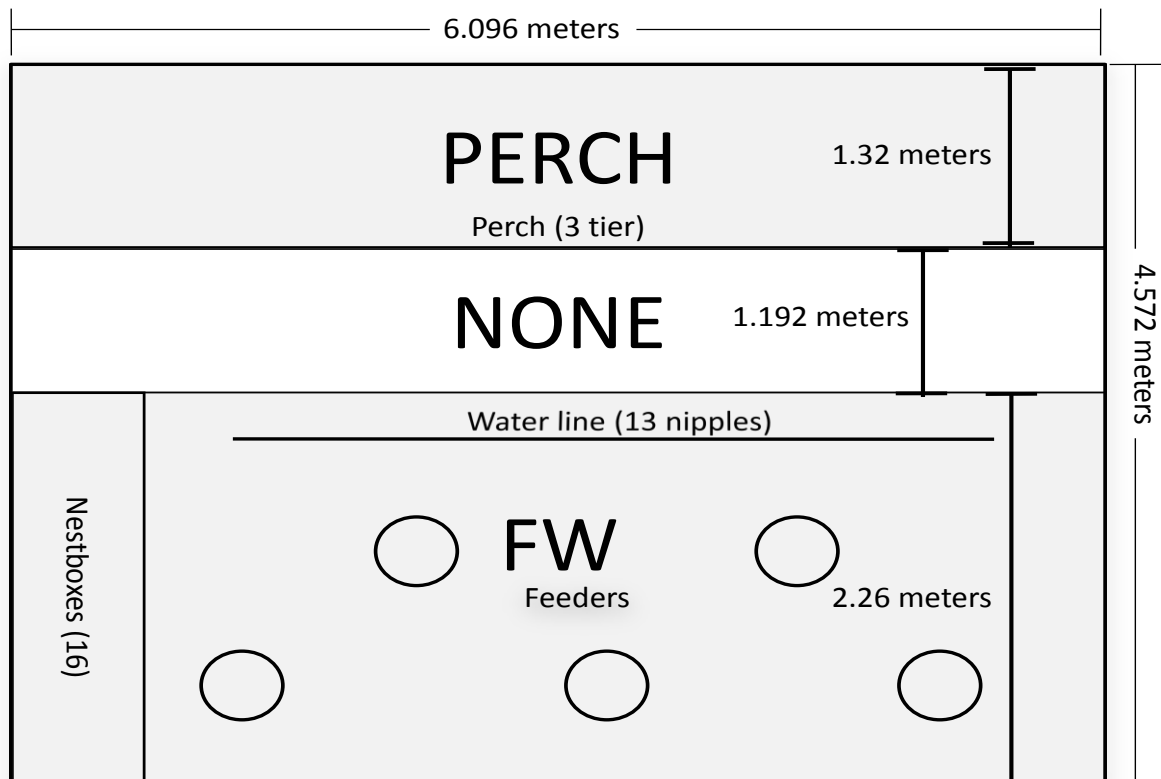


Figure 2.3: Percentage of hens using the (a) feeder, (b) drinker, (c) nest box, and (d) perch across days. Differences between treatments ($P < 0.05$) are indicated with an asterisk. In each graph, non-sensor hens (NON) are represented by the solid line and sensor hens (SEN) are represented by the dashed line.

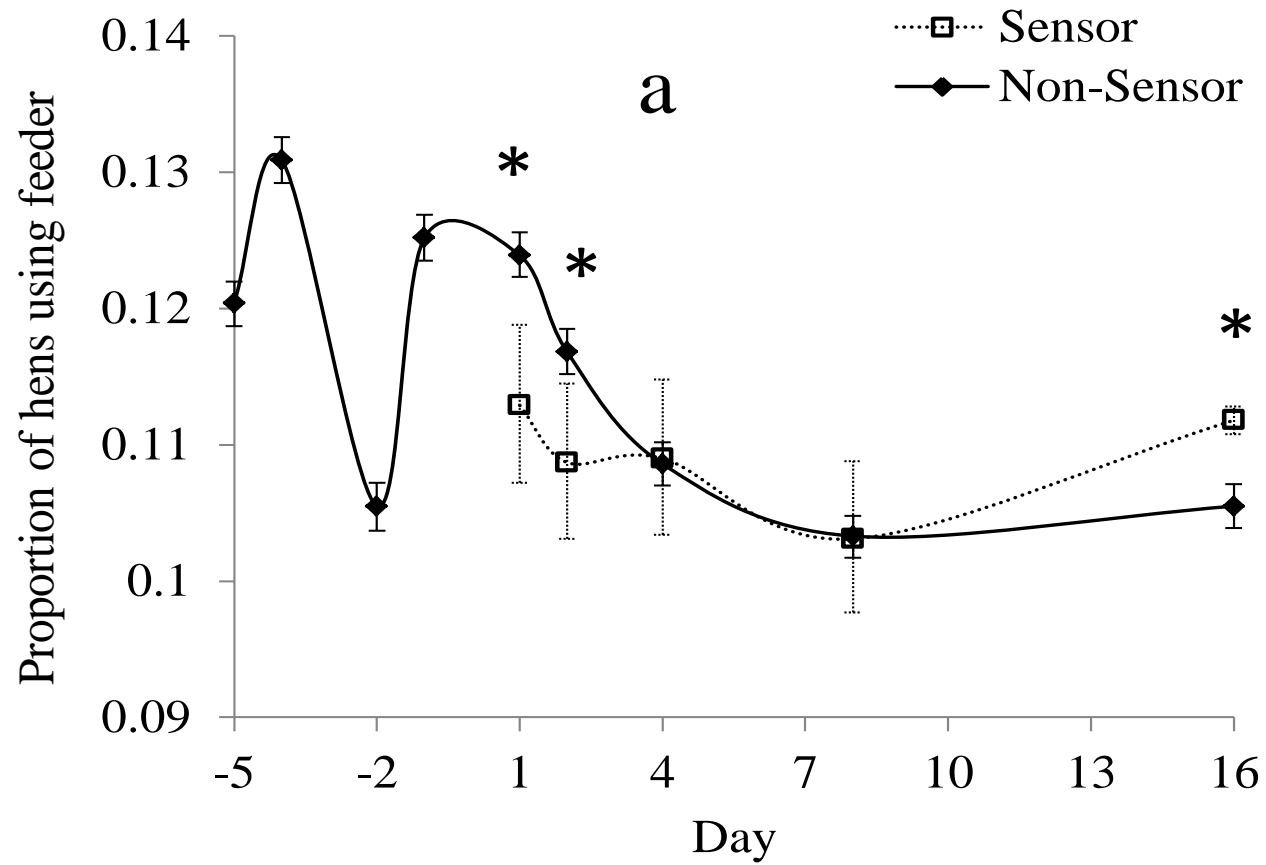


Figure 2.3 (cont'd)

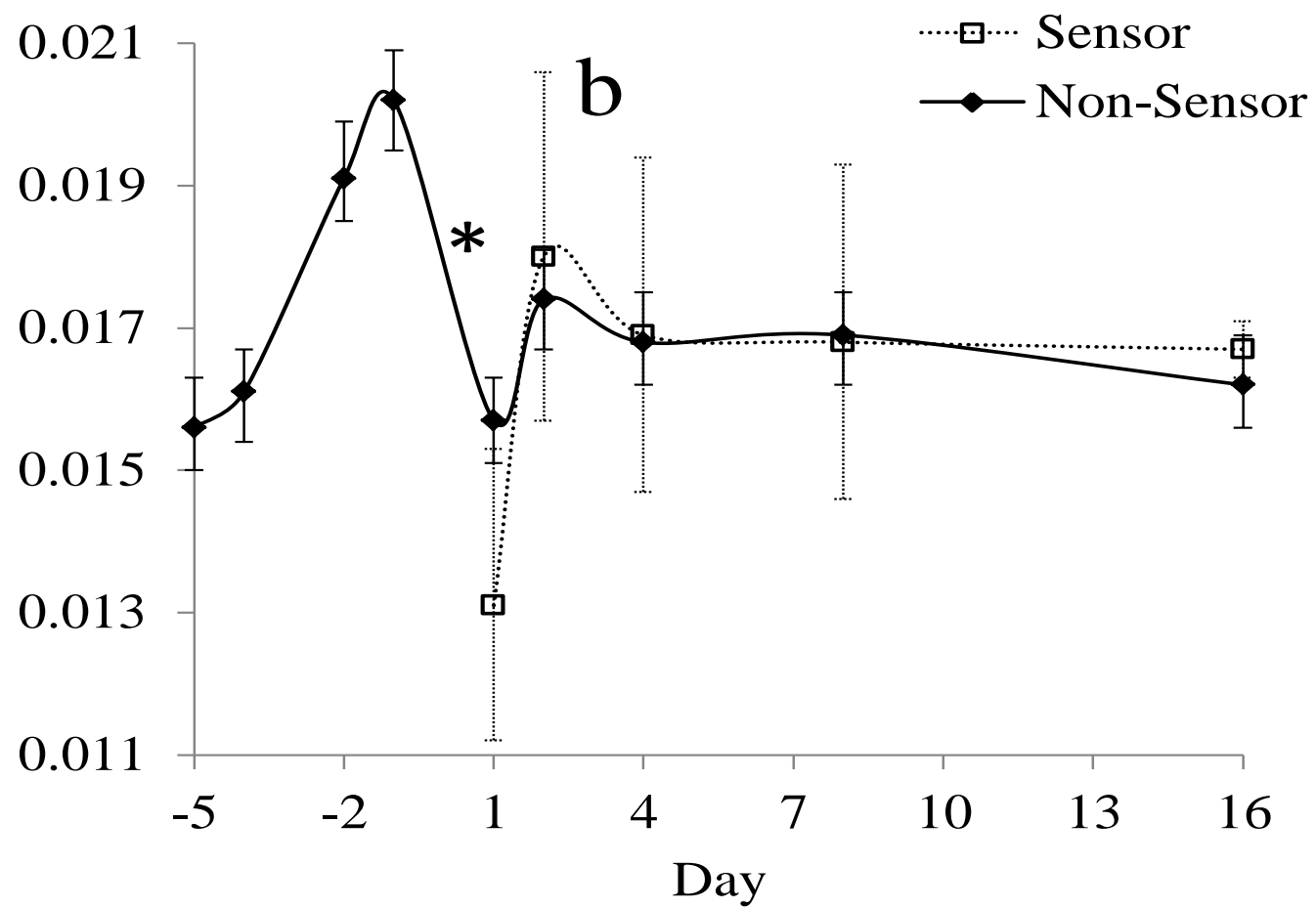


Figure 2.3 (cont'd)

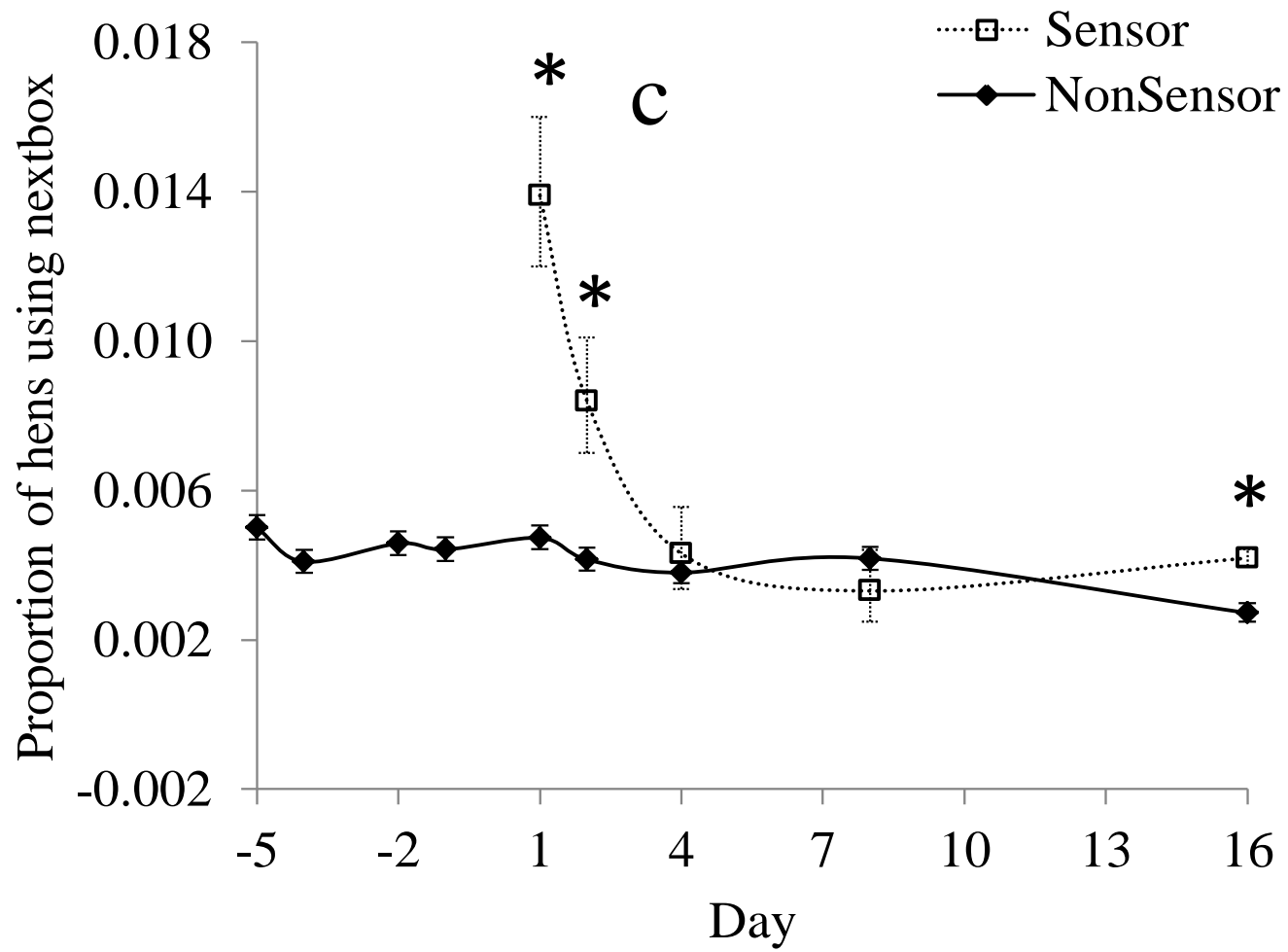


Figure 2.3 (cont'd)

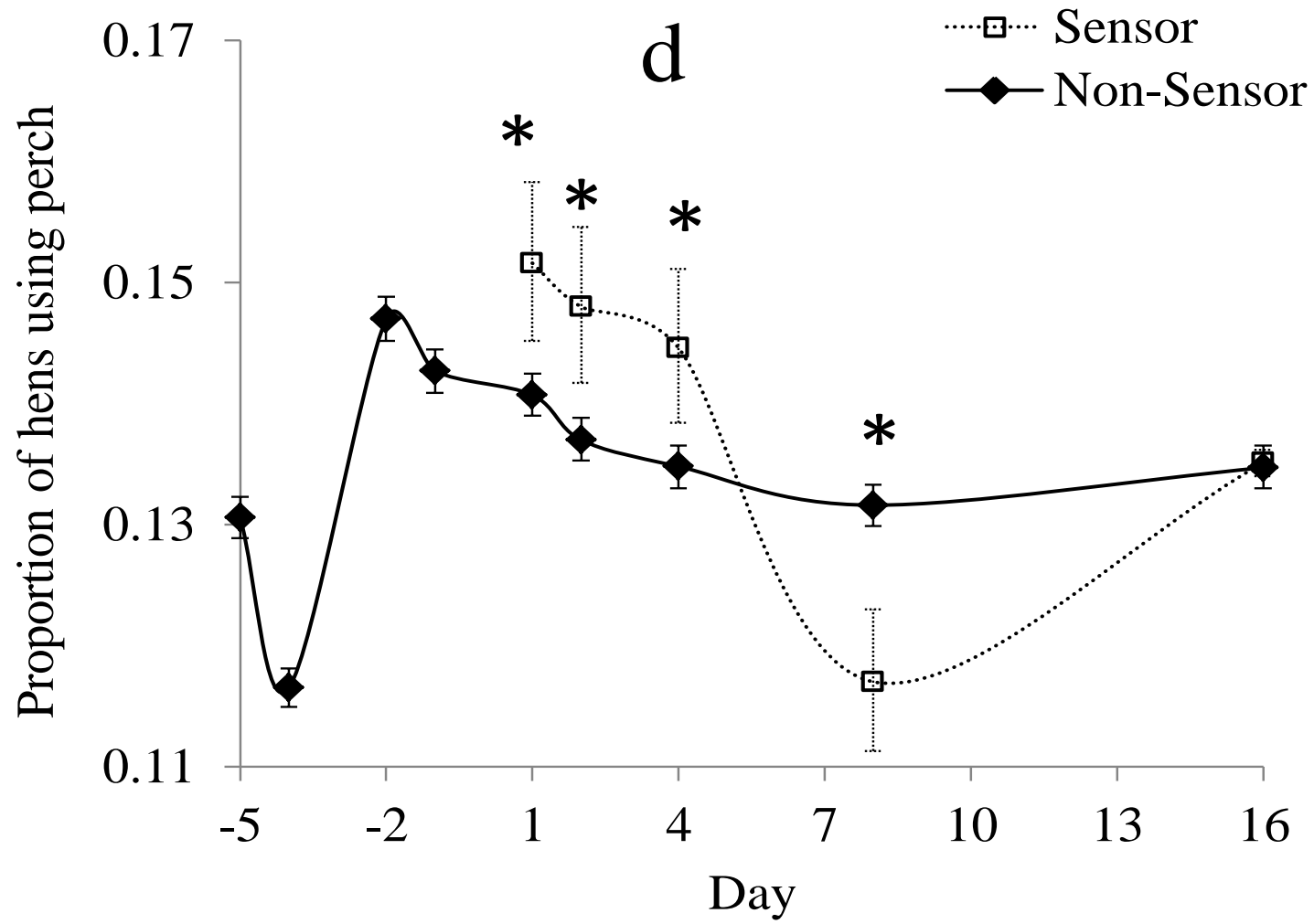


Figure 2.4: (a) Average number of agonistic observations per 20-minute time period across days, (b) Number of agonistic observation by area in room. Observations are summed across all days and differences ($P > 0.05$) are indicated by different letters.

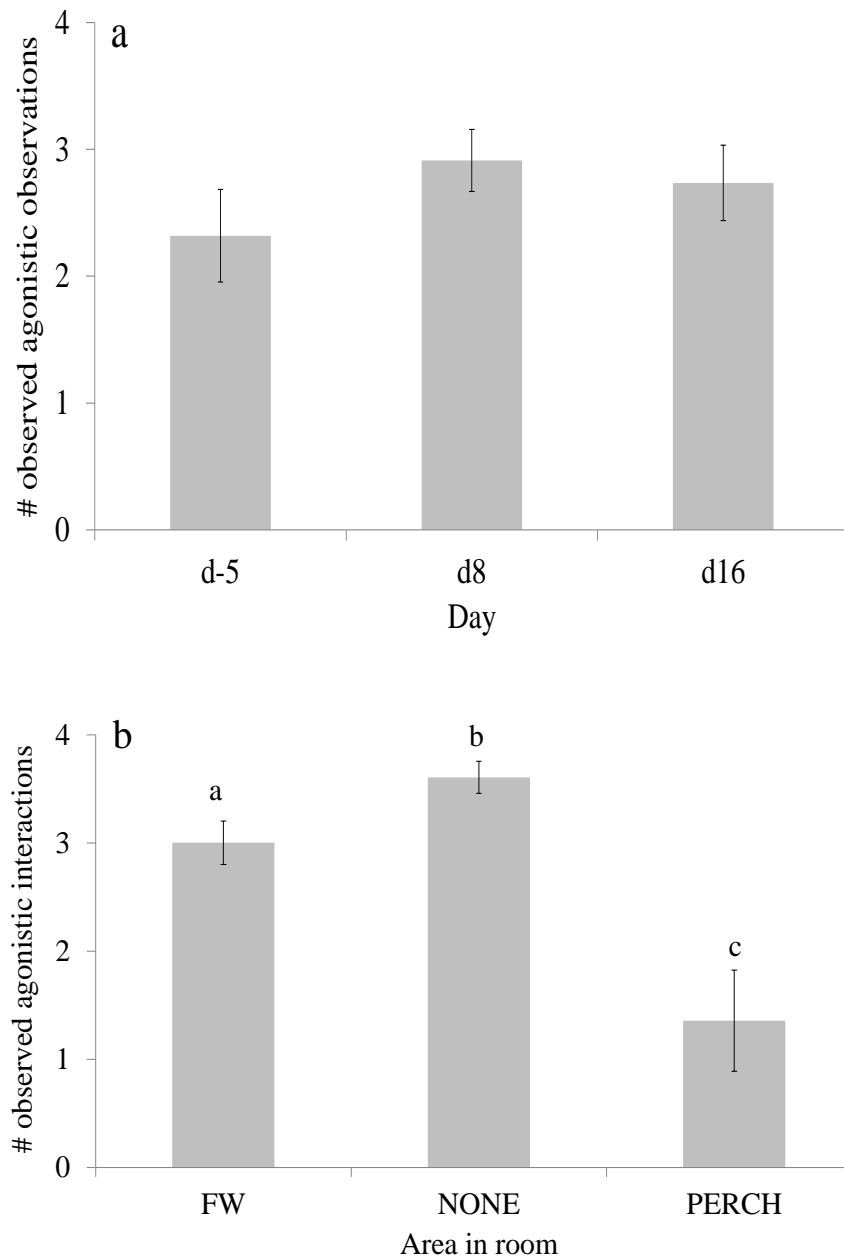


Figure 2.5: (a) Average number of agonistic observations per 20-minute time period across days, (b) Number of agonistic observation by area in room. Observations are summed across all days and differences ($P > 0.05$) are indicated by different letters.

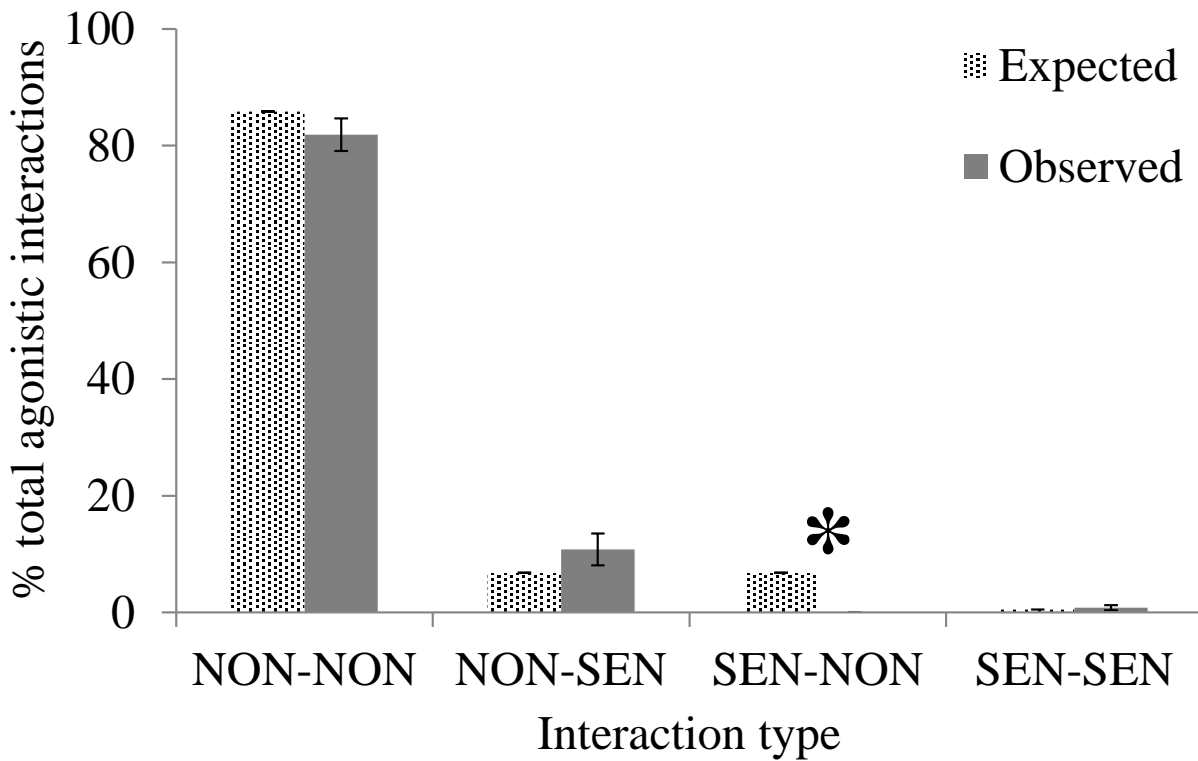
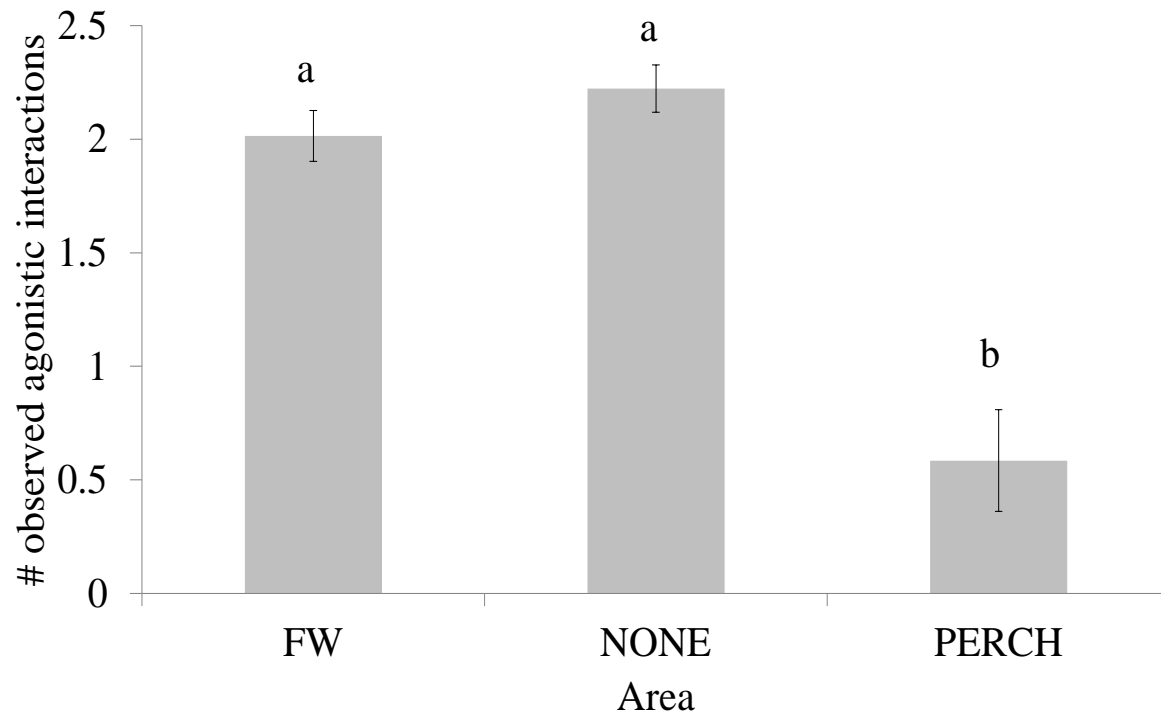


Figure 2.6: (a) Average number of agonistic observations per 20-minute time period across days, (b) Number of agonistic observation by area in room. Observations are summed across all days and differences ($P > 0.05$) are indicated by different letters.



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CHAPTER 3: WHEN CONTINUOUS OBSERVATION JUST WON'T DO: DEVELOPING ACCURATE AND EFFICIENT SAMPLING STRATEGIES FOR THE LAYING HEN

ABSTRACT

Continuous observation is the most accurate way to determine animals' actual time budget and can provide a 'gold standard' representation of resource use, behavior frequency, and duration. Continuous observation is useful for capturing behaviors that are of short duration or occur infrequently. However, collecting continuous data is labor intensive and time consuming, making multiple individual or long term data collection difficult. Posture, behavior, and resource use were recorded from 15 h of continuous video for each of six non-cage laying hens. Data from a continuous observation was compared with scan sampling intervals of 5, 10, 15, 30, and 60 min and subsamples of continuous observations performed for 10 minutes every 30 minutes, 15 minutes every 1 h, 30 minutes every 1.5 hour, and 15 minutes every 2 h. Three approaches were utilized to determine an effective sampling interval and assess its caveats. General linear models identified how the time budget from the sampling techniques differed from continuous observation. Correlation analysis identified how strongly the sampling techniques were associated with the continuous observation. Regression analysis identified how well the sampling technique was associated with the continuous observation, its change in magnitude, and whether the sampling technique had bias. Methods for identifying an effective sampling technique are outlined and results for non-caged laying hens are presented.

KEYWORDS: laying hen, sampling interval, time budget, validation, resource use, behaviour, posture

INTRODUCTION

Behavioral data collection is a type of assay. All assays require validation and refinement to meet specific objectives before results can be trusted as accurate; therefore, data collection protocols using behavioral observations should be subjected to similar rigorous validation (Mitlohner et al. 2001). Determining the proper sampling interval (the length of time between consecutive observation sessions) and sampling duration (the length of time across which behavioral observations are recorded) for collecting behavioral data is highly dependent on the behavior of interest, and interval appropriateness is also dependent on the total duration of the observation period (Altmann 1974). Different behaviors may not need to be collected at the same frequency since they may be performed for different durations of time. On one hand, if the sampling interval is shorter than the typical duration of the behavior, the same occurrence of a behavior may be recorded multiple times. Conversely, a sampling interval may be too large to capture the presence of the behavior, thus omitting it from the behavioral record. Continuous observation (recording all events and states as they occur during a fixed period of time) is the most accurate way of determining animals' time budgets, and can provide a nearly perfect representation of resource use and behavior frequency and duration (Altmann 1974). In particular, continuous observation can capture behaviors that are of very short duration, occur infrequently or are circadian, or are dynamic and difficult to identify from a still image (e.g., walking vs. standing). However, continuous observation is labor intensive and time consuming, making it difficult to collect data on multiple individuals or over long time spans. Further, statistically analyzing a continuous dataset can be challenging and mathematically difficult to support. Therefore, identifying behavioral sampling techniques for dynamic, static, or infrequent

behaviors that will provide an accurate representation of data that would be collected by continuous observation is important for expedient and accurate behavioral data collection.

A clear picture of the behavior of any one individual animal living within a group can be difficult to obtain. However, an increasing amount of research in applied ethology is examining the response of individual animals in such situations to assess their welfare, or quality of life, which must be done at an individual level.

For example, egg-laying hens (*Gallus gallus domesticus*) are increasingly housed in larger groups in complex environments rather than in groups of 4-9 in small, simple cages, the standard hen housing environment for the last 60 years. This change is occurring in response to social pressure to provide agricultural animals, such as chickens, a good quality of life. The European Union transitioned to non-cage housing systems in 2012 (CEC 1999), and egg producers in the United States are adopting non-cage housing for laying hens to meet voter and customer demands, respectively. In response, research has increasingly focused on the impacts of non-cage housing systems on hen production, behavior, and welfare (Coalition 2012). As applied ethologists study large groups of hens in non-cage environments, sampling techniques that can provide a reliable representation of data collected by continuous observation will facilitate the research process. Thus laying hens provide an appropriate model for examining the impact of different sampling techniques on the representation of a period of continuous observation.

Yet, previous research investigating the behavior and resource use of non-cage laying hens varied widely in methodological approach (Table 2). Therefore, there is a need to identify a

consistent sampling regime to facilitate easy data collection and comparison across different research studies.

Individual circadian rhythms (e.g. dust bathing and egg laying), social interactions (e.g. aggression, reproduction, grooming), and animal physiology (e.g. defecation, hunger, illness) may determine when a specific behavior occurs, guiding the type of data collection technique utilized (Abe et al. 1979; Vestergaard, Skadhauge & Lawson 1997). Furthermore, many static and dynamic behaviors (e.g., standing and walking) may look similar if an instantaneous scan is utilized to collect data. Thus, many factors, including type of behavior (infrequent, static, dynamic, etc.), internal physiology, and social factors may impact the presence of the behavior. These factors should be considered when deciding upon a behavioral sampling interval and the sampling technique should be customized to address the specific research question.

Sampling intervals for behavioral data collection have been investigated for farmed foxes (Jauhiainen & Korhonen 2005), young pigs (Arnold-Meeks & McGlone 1986), feedlot cattle (Mitlohner et al. 2001), and female broiler chickens (Kristensen et al. 2007) however, identifying an appropriate sampling technique is not always a straightforward process. D'Eath (2012a) highlights the complexity of identifying the impact of observer bias using multiple approaches to assess data from sow lameness scoring. Systematic differences in lameness scores were assessed with signed rank tests to identify how well the observers matched exactly with their lameness scores by calculating their proportion of agreement, the prevalence of bias adjusted Kappa, Spearman's and Kendall's rank correlations, and lameness scoring consistency over time were with a Wilcoxon signed rank tests. Further, different kinds of agreement should be assessed between two measures to clarify how they differ from and relate to one another. More specifically, statistical investigations should identify how much different measures do differ (e.g.

is there a bias?), how strongly the two measures are associated, and how well they match or completely agree (D'Eath 2012b). Therefore, multiple approaches should be used when identifying an efficient sampling strategy to ensure that the strategy chosen is representative of the continuous sampling gold standard and the caveats of the selected strategy are understood.

Behavioral research on individual animals living in large groups is becoming a more integral part of animal welfare assessment. Therefore, the need for researchers to understand individual productivity and health of animals in groups along with the need to efficiently sample individual animal behavior is growing. As mentioned previously, with the recent transition towards housing laying hens in large groups in alternative systems, there has been a large increase in non-cage laying hen behavior research, providing a relevant and easy animal model to address these overarching behavioral questions. By identifying the strengths and weaknesses of different subsampling techniques for different types of behaviors, researchers may be able to expedite data collection, understand what effects their sampling technique may have on interpretation of results, collect data that can be statistically analyzed, and have information that provides a good representation of continuous observation. The results of this study provide a framework for how to approach these unique problems along with specific data concerning laying hens. Therefore, our objectives were to compare and validate instantaneous scan sampling and time sampling methods with continuous observation of behavior, using non-cage laying hens as a model, to identify the optimal sampling techniques for static, dynamic, and infrequent behaviors.

MATERIALS AND METHODS

Animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Teaching and Research Center. Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University Institutional Animal Care and Use Committee. Three identical rooms (6 m x 4.5 m) at Michigan State University Poultry Teaching and Research Center were used. Each room was furnished in the same configuration with nest boxes, perches, tube feeders, and a water line with nipples. Sixteen nest boxes (each 0.4 m long x 0.3 m wide x 0.3 m high) in an 8 x 2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1 m x 6 m slatted area at a height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8 cm of wood shavings at time of data collection. Food and water were provided daily ad libitum. Daily care, including egg collection, feeding, and hen inspection, occurred at least once a day. Two incandescent light bulbs (60 lux at bulb level) on an automatic timer provided light 15 h per day (06:00 – 21:00) in each room. Temperature was maintained between 16°C -22°C using a ventilation fan and forced air heating.

Hy-Line Brown laying hen pullets were reared in each of the rooms as described above with accommodation made for their smaller size (i.e., smaller perches, which were removed at 6 wk) and immaturity (i.e., access to nest boxes was granted at 10 wk of age). Each room provided

0.21 m² floor space, 17.8 cm of perch space, 0.01 m² of nest box space, 4.83 cm feeder space per hen. Thirteen nipples provided enough drinking space for 10.3 hens per nipple.

Each room housed approximately 135 hens (total hens housed n = 405), and behavior, posture, and resource data for this experiment were collected from two focal hens per room. As part of a separate experiment, these hens had been fitted with an individually numbered backpack attached via a figure eight harness, visible on the hen's back. The presence of this numbered backpack made individual identification possible as the hen moved through the environment, and previous research (Daigle et al. 2012) showed that wearing the backpack had a minimal impact on long-term resource use or agonistic interactions.

Data collection

Hens were videotaped at approximately 20 weeks of age using ceiling-mounted cameras from 6:00 - 21:00 and their posture (stand, sit, walk), behavior (feed, drink, preen, dust bathe, forage, rest), and resource use (feeder, water, nest box, perch, other) were recorded from the video every 2 sec (in accordance with the objectives for the previously mentioned experiment). Behaviors were also categorized as static (sit, stand, rest, nest box use, perch use), dynamic (walk, preen, forage, feed, drink, dust bathe, feeder use, water use), and infrequent (drink, dust bathe, nest box). For all of the behaviors observed (Table 1), drinking behavior was observed to be the behavior that occurred with the shortest duration (approx. 30 sec, unpublished data). Therefore, even though this 2 sec sampling interval could be argued as not continuous, the observations from this dataset were treated as such because the possibility of missing a behavioral event or change was very low. Furthermore, the video was watched continuously,

though the changes in behavior were only recorded at the 2 sec interval. Behavior, posture, and resource use were recorded for each hen during the 15 h lights-on period. Selected data points within the 2 sec data set were extracted and used to create the sub-sampling datasets. To create an instantaneous scan sub-sampling dataset, behaviors, postures, and resource use observations were extracted from the 2 sec dataset and analyzed at scan intervals of 5, 10, 15, 30 and 60 min. Subsequently, behaviors, postures, and resource use observations were extracted from the 2 sec observations and analyzed for four different time sub-sampling techniques (where the behavior was periodically observed continuously for a fixed period of time): 10 min every 30 min (10EV30), 15 min every 1 h (15EV1), 30 min every 1.5 h (30EV1.5) and 15 min every 2 h (15EV2). These intervals and sampling bout durations were selected because they were straightforward to collect and represented a high degree of diversity with regard to behavioral duration, time between behavioral bouts and how many samples would be available for statistical analysis. Since previous behavioral research into laying hen behavior and resource use has used a wide variety of techniques (Table 2), these were selected to encompass most of the sampling rates previously recorded in the literature while investigating whether sampling techniques that require less effort would yield accurate results.

Statistical analysis

The number of observations for each behavior, posture, and resource were converted to a percentage of the total observations to calculate time budgets. These percentages were then square root-arcsine transformed to achieve normal distribution. Transformed data were used for all analyses. Findings are reported in the results using untransformed data.

To identify how time budgets from different sampling techniques differed from each other, an analysis of variance was performed on the transformed data using a General Mixed Model (PROC MIXED) in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The model included animal ID, treatment, room, animal ID nested within room, and the treatment x room interaction. Animal ID nested within room was a random effect. Treatments were considered the different sub-sampling techniques. Least square means identified differences among the different treatments.

To identify how strongly individual hens time budgets that were calculated from the sub-sample datasets were associated with the 2 sec dataset, Pearson product correlations were used to correlate average time budget percentages from the scan and time sub-sampling techniques with the 2 sec observation time budget percentages.

Pearson's correlations provide information regarding how the sub-sampling techniques co-vary with the continuous observation. However, to identify and illustrate the line of best fit, which can provide information about the sub-sampling techniques when the 2 sec observation is provided, regression analysis (PROC REG) was utilized. Stronger support can be made for identifying an appropriate sampling interval by using information from both the intercept and the slope provided from a regression analysis (Ledgerwood, Winckler & Tucker 2010). For example, when the results of a sampling technique perfectly match the results of the continuous observation, the regression equation would be $y = x$ (Figure 1a). If the intercept of the regression line is different than zero, then the results from the sampling technique may over- or underestimate compared to the results from continuous observation (Figure 1b). If the slope of the regression line differs from one, the degree to which the behavior is observed may differ between the sampling methods, thus the results from a sampling technique may not provide an accurate

representation of change in magnitude (Figure 1c). Therefore, using the time budgets from the 2 sec observation as the independent variable and the time budgets from the sub-sampling techniques as the dependent variable, regression analysis was utilized to identify the proportional and constant difference the sub-sampling technique represented when compared against the 2 sec approach. Specifically, regression identified whether the sampling technique had a positive or negative bias (had an intercept different from zero, i.e., so did the sampling technique over or under-estimate the percent of time spent performing the behavior), how strongly the sampling technique was associated with the 2 sec observation (represented by R^2), and how well the sampling technique matched the results from a 2 sec observation (measured by whether the slope of the regression line was different from 1).

Sampling interval and observation duration factorial analysis

The impact of sampling interval and observation duration on sampling technique results was assessed with a factor analysis. Four behaviors were chosen for this analysis based upon whether they were static (S), dynamic (D), circadian (C) or non-circadian (NC). These behaviors included preening (D-NC), feeding (D-C), standing (S-NC), and perch use (S-C). Factorial analysis (PROC MIXED) assessed whether the observation duration of different lengths (10min, 15min, 30min) and the sampling interval separating observation duration (30min, 60min, 90min, and 120min) impacted the time budget recorded. The model included duration of the continuous observation, the interval between continuous observations, and their interaction. Hen nested within room was the random factor and LS-means with a Tukey-Kramer adjustment identified differences between effects.

RESULTS

Differences were identified among the different sampling techniques as illustrated in Table 3, however, the mean percentage of time spent in a posture, performing a behavior, or using a resource did not differ from continuous sampling for all parameters examined ($P > 0.05$), except for preening at the 60 min scan interval, rest at the 30 min scan interval, and nest box usage at the 15EV2 time sampling interval.

Analysis using a Pearson's correlation (Table 4) revealed correlations (Pearson's Correlation: $r_6 > 0.86$, $P < 0.05$) for all postures between continuous and 5, 10, and 15 min scans and 15EV1 and 30EV1.5 intervals. All sampling strategies (excluding 60 min) were correlated with data from continuous observations (Pearson's Correlation: $r_6 > 0.81$, $P < 0.05$) for all resources except drinker. The same pattern was observed for feeding, foraging, and resting behaviors when compared with continuous observations (Pearson's Correlation: $r_6 > 0.93$, $P < 0.02$). Preening was correlated (Pearson's Correlation: $r_6 > 0.84$, $P < 0.05$) with continuous observations at the 5 min scan and 30EV1.5 time sampling intervals only; while drinking was correlated with continuous observations at the 15 min scan interval only (Pearson's Correlation: $r_6 = 0.88$, $P = 0.02$).

A regression analysis was performed on four of the nine sub-sampling techniques to illustrate how the results differ among sampling techniques that have strong and weak associations as determined by Pearson's correlations with the continuous observation.

Table 5 illustrates how strongly the four sampling techniques (5 min, 15 min, 30EV1.5, and 15EV2) are associated with the continuous observation (R^2), how well the sampling

technique and the continuous observation match (β) or change in magnitude, and whether the sampling technique had bias (intercept) for all postures, behaviors, and resource use. The 5 min scan sampling technique showed strong ($R^2 > 0.85$) associations for all assessed parameters except for drinking, preening and drinker use. However, a negative bias and change in magnitude was observed for the 5 min scan sampling technique relative to continuous observations for sitting and standing. Further, feeding behavior and feeder resource use tended ($0.05 < P < 0.10$) towards bias when using the 5 min scan sampling technique compared to the continuous sampling technique.

The 15 min scan sampling technique showed strong associations for feeding and resting behavior along with feeder, nest box, perch, and other resource use (Table 5). Behaviors, such as sitting, standing, walking, foraging, dust bathing, and drinker use, exhibited fairly strong associations ($R^2 > 0.70$) with continuous observation. No bias or change in magnitude was observed for any assessed parameters with the 15 min scan sampling technique, except for perch use, which tended towards a positive bias.

Strong and fairly strong associations were observed for all parameters assessed except preening and dust bathing with the 30EV1.5 sampling technique (Table 5). Bias and a change in magnitude were observed only for resting, and tended towards a bias for foraging. Fairly strong associations were observed for walking and other resource use.

No effect of sampling interval (SI), observational duration (OD), or their interaction was identified for the circadian behaviors: feeding and perch use. Effects of SI (Figure 2a), OD (Figure 2b) and their interaction were observed for the non-circadian behaviors: preening and standing. OD lasting 15min produced time budgets with a higher proportion of time performing

preening than OD lasting 10min ($t_{55} = -8.49$, $P < 0.001$) and 30min ($t_{55} = 8.28$, $P < 0.0001$) while SI of 120min yielded time budgets with more time preening than SI of 30min ($t_{55} = 11.11$, $P < 0.0001$), 60min ($t_{55} = 10.88$, $P < 0.0001$), and 90min ($t_{55} = 11.44$, $P < 0.0001$). A significant interaction between sampling interval and observational duration was observed where the percentage of time preening was more with the 15min OD/120min SI compared to all other SI/OD combinations. An identical pattern was observed for the fixed and interaction effects for standing.

DISCUSSION

The results of this study indicate that there are maximum and minimum thresholds at which a sampling interval can no longer provide an accurate representation of continuous observation or does not provide any additional accuracy. If sampling intervals are too large, behaviors may not be represented in the proportion in which they are performed. However, these results highlight that smaller sampling intervals may not necessarily yield better results though data collection will take longer. This suggests that an intermediate sampling regime might provide researchers with accurate results while maintaining efficiency. Dynamic behaviors appeared to be better represented using time sampling techniques compared with instantaneous scan sampling, while static behaviors appeared to be well represented by both time sampling and instantaneous sampling techniques, as long as the interval between scans was not greater than 30 minutes. And results from this study may facilitate sampling technique decisions for future behavioral researchers (Figure 4). Furthermore, sampling interval and observation duration do

not have an impact on outcome for circadian behaviors, but may impact the representation of non-circadian behaviors, regardless of whether they are static or dynamic.

Results from this study then suggest that when recording data from focal hens in a larger group, using the 30 min every 1.5h time sampling technique may be the most parsimonious approach for collecting behavioral data on performance of postures, behaviors, and resource use by groups of non-cage laying hens. Performing scan samples every 15 minutes may also be an alternative efficient, accurate, and practical technique for collecting behavioral data from either an individual or a group of laying hens. One consideration when deciding between these two sampling techniques would be the amount of time required to perform the observations. If the behavioral observations are being taken from video recordings, then the amount of time required to decode the video may be less for the 15 min scan because the observer would not have to continuously watch video for 30 minutes and could quickly advance the video between the target time points.

Behaviors that required a longer amount of time to perform, behaviors that were performed consistently throughout the observation period, static postures, and their corresponding resources remained correlated with high accuracy to data from continuous observations even as sampling intervals increased. Dynamic behaviors and postures may be difficult to capture with scan sampling because still images of dynamic behaviors and postures can look similar to still images of static postures and behaviors. Therefore, time sampling techniques, such as 30 min every 1.5h, can help ensure that the behaviors that are recorded are accurately represented as either dynamic (e.g., walking) or static (e.g., standing) since the subject will be observed continuously for a short period of time. However, as dynamic behaviors are

more accurately represented by time sampling techniques, they may make up a larger part of the overall time budget and minimize the presence of static behaviors.

Infrequent behaviors that are strongly circadian, such as dust bathing, may be better captured using a scan sampling strategy that encompasses the entire day compared to a time sampling strategy that may miss entire portions of the day. Figure 3 illustrates how sampling technique can affect the representation of a circadian behavior. Dust bathing occurs approximately every 48 hours (Olsson & Keeling 2005), may not take up a large portion of the animals' time budget, and could be accurately represented with large or small scan intervals. Time sampling may be useful for capturing circadian behaviors, but only if the observation period includes the time of day in which the behavior is performed. Alternatively, if the infrequent behavior is of primary interest, focusing the observation period around when this behavior is most likely to be performed may be the best strategy for collecting this type of data.

The results from this study regarding nest box use should be interpreted tenuously. Hens in alternative housing systems may lay their eggs on the floor rather than in designated nesting areas due to multiple factors such as social pressure, preference or experience, causing some hens to never use the nest box as a resource (Brinch 2010; Sherwin & Nicol 1993). Oviposition of individual hens was not recorded in this study, but it is likely that half of the hens observed may not have used the nest box. Approximately 50% of the eggs laid were collected from the floor (Daigle unpublished data) and there were a large number of zeros for nest box use in the data set. Therefore, this behavior may have been difficult to capture with any sampling technique, simply because some of the hens studied may never have used the nest box.

Drinking behavior and water resource use were the two parameters that were captured with the least accuracy when sampling strategies were used rather than continuous observations across the light period. On average, a single drinking event lasts approximately 30 s (Daigle, unpublished data), and hens have been reported to spend 1.36 ± 0.24 percent of their daily time budget drinking, with most drinking occurring around 09:00 and 15:00 hours (Mishra et al. 2005). Therefore, even if data were to be collected at 5 min intervals, the likelihood that a drinking event would be missed would be high because the sampling interval is ten-fold longer than the behavioral event. Further, hens may not drink consistently throughout the day as there is a strong positive correlation between drinking and feeding behavior (Savory 2010). Thus, if a 30 min every 1.5 h time sampling technique was used with an observation period that omitted a peak drinking time for that hen, then actual drinking behavior and subsequent water resource use may not be accurately represented. Counter intuitively, the highest association for drinking behavior was observed for the 10min scan technique, and this could be due to a statistical artifact as drinking was difficult to capture, or this could indicate that a 10min scan sampling technique is a better approach for assessing drinking behavior. Therefore, if collecting drinking behavior or drinker use data is important to capture, a behavioral observation technique focused on those specific elements would provide better results.

The average amount of time spent preening was found to be different from continuous observations at the 60 min interval and a strong relationship was only observed between the continuous observation and the 5 min scan and 30 minutes every 1.5 h time sampling techniques. Preening is a dynamic grooming behavior that occurs in bouts (Mishra et al. 2005) lasting on average 107 s (Daigle, unpublished data). Therefore, scan sampling even at 5 min intervals may have captured the hen between bouts, resulting in inaccurate recording of her behavior since she

would have appeared to be resting, or performing another behavior. Even though preening occurs throughout the day, most preening behavior is performed in the morning and late evening (Mishra et al. 2005), so if the time periods used for time sampling omit parts of the day when preening mainly occurs or are too short to capture preening in an adequate capacity, then an accurate representation of preening behavior may not be possible.

Multiple statistical approaches to identifying a parsimonious sampling technique were presented here, yet most researchers will not be able to devote similar resources to identifying the ideal sampling technique before they conduct a study. If a single approach could be chosen to identify a sampling technique for analysis, using the results from the regression analysis provides the most robust information regarding sampling accuracy and bias. Mean comparisons do not yield much information, as most means were observed to be similar across sampling techniques even though differences were detected by different statistical approaches. Correlational analysis is simple to perform, but cannot account for influences associated with room replication or individual variation and the results from this factor analysis highlighted that observation and sampling interval may not influence the overall outcome.

CONCLUSION

The results from this study illustrate how to statistically identify a parsimonious sampling technique for behavioral observation. Further, this study highlights the strengths and weaknesses of different behavioral sampling techniques in comparison to data collected using continuous observation of different postures, behaviors, and resource use for non-cage laying hens. The methods outlined may provide a framework for future researchers in identifying a parsimonious

sampling technique for their study. Information from this work can be used to identify sampling techniques for future non-cage laying hen studies to help identify what sub-sampling strategy will provide the strongest representation of a continuous observation while maintaining efficiency. Future behavioral research should assess the validity and accuracy of the sampling interval before utilizing a sampling interval for large-scale data collection.

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APPENDIX

Table 3.1: Ethogram of behaviors developed to identify posture, behavior and resource use. A hen was counted as performing a new posture, behavior, or resource use when she stopped performing the previous behavior for >5 s or began performing a new behavior.

<i>Posture</i>	Description
walk	Walking more than 3 steps in succession with head up or when walking hen has not been standing, drinking, feeding, or foraging in litter for the previous 5 s
stand	Hen is upright and supported off of the ground or perch by legs
sit	Hen is upright with body touching the ground or perch
<i>Behavior</i>	
feed	Hen pecks at feed in the feeder. Recording starts at first peck
drink	Head is turned upwards towards water source, and hen uses beak to consume water from nipple drinker
preen	Hen may be sitting or standing and uses beak to manipulate, rearrange, pull, or clean body feathers on self
dust bathe	While squatting or lying, hen performs dust bathing activities including vertical wing shaking, bill raking, scratching, ground pecking, movements of the feet and wings to raise dust into the ruffled plumage, rubbing of head and sides in the dust, feather-ruffling and shaking dust out of the feathers. Starts with first wing shake
forage	Hen pecks at substrate while standing or stepping forward with head below rump level. Starts when hen makes >3 successive pecks at substrate, or when foraging hen has not been standing or walking with head up, or feeding, for the previous 5 seconds
rest	Starts when hen lies down (sternum resting on substrate) from an upright position or when lying bird has made no dust bathing or preening movements for the previous 8 seconds
<i>Resource</i>	
feeder	Hen has head in feeder and pecks at feed in the feeder
drinker	Head is turned upwards towards water source, and hen uses beak to consume water from nipple drinker
perch	Hen is standing, walking, or resting on perch, the rail in front of the nest boxes, or black base of slats underneath raised perches
nest box	Hen is standing or resting inside a nest box

Table 3.2: Overview of different types of instantaneous scans (IS) and time sampling (TS) techniques utilized in previous research to assess behavior (B), location (L), aggression (A), and resource use (R) of commercially housed chickens

Author	Sampling technique	Species	Parameters assessed
Cordiner and Savory, 2001	30min TS twice daily	laying hens	A
Estevez et al., 2002	15min TS	laying hens	A
Oconner et al., 2011	15min TS	laying hens	A
Oden et al., 2000	18, 20 min TS/day	laying hens	A
Hughes et al., 1997	60min IS and 30min TS	laying hens	A & L
Webster and Hurnik, 1994	8min IS	laying hens	B
Webster, 2000	8min IS	laying hens	B
Shimmura et al., 2007	10min scan for 4 hours twice daily	laying hens	B & R
Shimmura et al., 2008	10min IS across 2 hours 3x/day	laying hens	B & R
Olsson and Keeling, 2000	2min, 4min, and 15min IS	laying hens	B & R
Tanaka and Hurnik, 1992	5min IS	laying hens	B & R
Albentosa and Cooper, 2006	60min IS	laying hens	cage height preference
Keeling and Duncan, 1991	15min IS	laying hens	flock activity
Mueller et al., 2009	5min TS for 1 hour	broilers	L
Widowski et al., 1992	5min IS	broilers	L
Leone and Estevez, 2008	1min IS and 15min TS	broilers	L & A
Channing et al., 2001	2 ISs/day	laying hens	L & B
Kristensen et al., 2007	15min IS	broilers	L & B
Donaldson and O'Connell, 2012	120min IS	laying hens	L & R
Cordiner and Savory, 2001	15min and 30second IS	laying hens	R

Table 3.3: Least square means, standard errors, and ANOVA P-values for percentages of postures, behaviors, and resource usage of six Hy-Line brown laying hens housed in a non-cage system as measured by different sampling techniques (values are percentage of the total duration for 15 h). Superscripts of different lowercase letters indicate statistical differences ($P > 0.05$) between values in that row.

	Sampling Method									
	Scan Samples						Time Samples			
	Con	5	10	15	30	60	10EV30	15EV1	30EV1.5	15EV2
<i>Posture</i>										
sit	15.5	15.5	15.5	16	18.2	16.2	16.1	15.1	14.4	13.7
stand	77.1	76.5	76.3	77	76.6	77.6	77.6	78.4	78	79.1
walk	7.4 ^{ab}	8.1 ^{ab}	8.2 ^a	7.0 ^{ab}	5.2 ^{ab}	6.2 ^b	6.4 ^{ab}	6.6 ^{ab}	7.6 ^{ab}	7.2 ^{ab}
<i>Behavior</i>										
feed	13.7	13	13.9	12.6	12.6	12.3	14.2	14.2	14.2	10.6
drink	2.6	3.2	3.1	3	2.6	3.2	2.3	2.6	2.7	3.5
preen	15.6 ^a	16.7 ^{ab}	16.8 ^a	16.7 ^a	16.8 ^a	20.8 ^b	15.4 ^a	15.4 ^a	15.5 ^{ab}	18.0 ^{ab}
dust bathe	0.8 ^{ab}	0.8 ^{ab}	0.9 ^{ab}	0.8 ^{ab}	1.1 ^{ab}	1.1 ^{ab}	1.2 ^a	1.0 ^a	1.5 ^a	1.0 ^b
forage	23.3 ^{ab}	22.5 ^{ab}	22.4 ^{ab}	25.6 ^{ab}	27.7 ^a	22.4 ^b	24.6 ^{ab}	24.7 ^{ab}	22.6 ^{ab}	23.5 ^{ab}
rest	44.0 ^a	43.8 ^{ab}	42.8 ^{ab}	40.9 ^{ab}	38.8 ^b	39.3 ^{ab}	42.4 ^{ab}	42.2 ^{ab}	43.5 ^{ab}	44.4 ^{ab}
<i>Resource</i>										
feeder	13.5	12.9	13.8	13.1	12.5	12.3	13.7	13.7	14	10.5
drinker	2.6	3.2	3.1	3	2.6	3.1	2.2	2.5	2.4	3.5
nest box	1.0 ^a	1.0 ^a	1.1 ^a	1.1 ^a	1.0 ^a	1.0 ^a	1.0 ^a	1.0 ^a	1.1 ^a	0.0 ^b
perch	22.1 ^{ab}	21.8 ^{ab}	21.4 ^{ab}	20.1 ^{ab}	20.8 ^{ab}	17.5 ^b	20.8 ^{ab}	21.0 ^{ab}	18.7 ^b	24.2 ^a
none	60.8	61	61.4	62.5	62.5	61.2	62.2	61.6	63.6	61.9

Table 3.4: Pearson product correlation coefficients between data collected using various behavioral sampling techniques compared to data collected using a continuous sampling methodology. ^aThese r-values were not estimable (NE) because all observations of these behaviors were zero, thus there was no variation. An observational technique using an infrequent sampling strategy would not be expected to capture behaviors that also occur infrequently. * $P < 0.05$ ** $P < 0.001$ ‡ $P < 0.0001$

	Sampling Method								
	Scan Samples					Time Samples			
	5	10	15	30	60	10EV30	15EV1	30EV1.5	15EV2
<i>Posture</i>									
sit	0.997 [‡]	0.977**	0.937*	0.944*	0.39	0.773	0.872*	0.924*	0.839*
stand	0.986**	0.950*	0.882*	0.666	0.218	0.711	0.863*	0.881*	0.422
walk	0.972*	0.904*	0.932*	0.768	0.682	0.985**	0.982**	0.993 [‡]	0.976**
<i>Behavior</i>									
feed	0.995 [‡]	0.986**	0.946*	0.989**	0.716	0.993 [‡]	0.992 [‡]	0.998 [‡]	0.961*
drink	0.543	0.26	0.879*	0.206	0.287	0.54	0.362	0.603	0.645
dust									
bathe	0.998 [‡]	0.999 [‡]	0.974**	0.974**	0.974**	0.561	0.559	0.971*	NE ^a
forage	0.997 [‡]	0.985**	0.939**	0.910*	0.7	0.989**	0.985**	0.993 [‡]	0.937**
preen	0.901*	0.581	0.623	0.733	0.188	0.609	0.547	0.844*	0.731
rest	0.979**	0.961**	0.944**	0.978**	0.742	0.974**	0.975**	0.992 [‡]	0.955*
<i>Resource</i>									
feeder	0.994 [‡]	0.985**	0.940**	0.989**	0.726	0.994 [‡]	0.993 [‡]	0.998 [‡]	0.959*
drinker	0.565	0.29	0.886*	0.226	0.293	0.559	0.631	0.624	0.656
nest box	1.000 [‡]	1.000 [‡]	1.000 [‡]	1.000 [‡]	1.000 [‡]	1.000 [‡]	1.000 [‡]	1.000 [‡]	NE ^a
perch	0.998 [‡]	0.988**	0.977**	0.978**	0.901*	0.983**	0.986**	0.981**	0.815*
none	0.990**	0.981**	0.955*	0.953*	0.779	0.978**	0.980**	0.980**	0.920**

Table 3.5: Regression coefficients (β) and their respective intercepts for data collected using various behavioral sampling techniques (30EV1.5, 15 min, 5 min and 15EV2). ^a indicates the strength of the relationship. ^b indicates the difference from 1. ^c indicates a difference from zero. NE indicates that this parameter was not estimable because all observations were zero. INF indicates that this parameter was infinity.

5 min									
	R^2	$F_{1,4}$ ^a	P value	β	$F_{1,4}$ ^b	P value	intercept	t_6 ^c	P value
<i>Posture</i>									
Sit	0.989	458.78	<0.0001	1.15	7.93	0.048	-0.006	-2.9	0.044
Stand	0.964	136.34	0	1.39	10.68	0.031	-0.035	-3.31	0.03
Walk	0.881	37.9	0.004	1.18	0.92	0.392	-0.004	-0.79	0.473
<i>Behavior</i>									
Feed	0.992	581.41	<0.0001	0.9	6.83	0.059	0.003	2.1	0.104
Drink	0.306	3.2	0.148	1.81	0.64	0.467	-0.013	-0.77	0.487
Preen	0.744	15.56	0.017	0.88	0.3	0.614	0.006	0.69	0.528
Forage	0.988	399.76	<0.0001	1.01	0.05	0.832	-0.001	-0.59	0.589
Rest	0.958	114.57	0	1.01	0.02	0.906	-0.001	-0.15	0.887
Dust bathe	0.932	70.02	0.001	1.09	0.5	0.518	-0.002	-1.27	0.274
<i>Resource</i>									
Feeder	0.99	474.79	<0.0001	0.9	5.55	0.078	0.003	2.01	0.115
Drinker	0.347	3.66	0.129	1.84	0.76	0.433	-0.013	-0.82	0.456
Nest box	1	INF	<0.0001	0.99	INF	<0.0001	0	INF	<0.0001
Perch	0.996	1292.22	<0.0001	1.05	3.09	0.154	-0.003	-2.12	0.101
Other	0.978	218.89	<0.0001	1.02	0.06	0.826	-0.001	-0.21	0.846

Table 3.5 (cont'd)

15 min									
	R^2	$F_{1,4}^a$	P value	β	$F_{1,4}^b$	P value	intercept	t_6^c	P value
<i>Posture</i>									
Sit	0.796	20.47	0.011	1.26	0.89	0.4	-0.01	-0.92	0.408
Stand	0.707	13.06	0.023	1.54	1.59	0.276	-0.047	-1.26	0.275
Walk	0.779	18.67	0.012	0.94	0.07	0.798	0.001	0.13	0.904
<i>Behavior</i>									
Feed	0.905	48.4	0.002	1.02	0.01	0.911	-0.003	-0.52	0.631
Drink	0.686	11.94	0.026	2.38	4.02	0.116	-0.022	-2.01	0.115
Preen	0.231	2.5	0.189	0.75	0.28	0.624	0.011	0.59	0.584
Forage	0.812	22.56	0.009	0.73	3.08	0.154	0.016	2.1	0.104
Rest	0.885	39.57	0.003	1.04	0.05	0.832	-0.005	-0.46	0.672
Dust bathe	0.728	14.41	0.019	1.08	0.08	0.797	-0.003	-1.07	0.344
<i>Resource</i>									
Feeder	0.877	36.7	0.004	1.01	0.01	0.945	-0.002	-0.26	0.81
Drinker	0.714	13.46	0.021	2.36	4.48	0.102	-0.022	-2.11	0.103
Nest box	1	INF	<0.0001	1.03	INF	<0.0001	0	INF	<0.0001
Perch	0.933	70.67	0.001	1.25	2.9	0.164	-0.015	-2.19	0.093
Other	0.901	46.54	0.002	0.83	2.07	0.224	0.015	1.56	0.194

Table 3.5 (cont'd)

30EV1.5									
	R^2	$F_{1,4}^a$	P value	β	$F_{1,4}^b$	P value	intercept	t_6^c	P value
<i>Posture</i>									
Sit	0.875	36.06	0.004	1.03	0.04	0.86	0.002	0.3	0.78
Stand	0.743	15.42	0.017	0.94	0.06	0.821	0.004	0.17	0.872
Walk	0.995	957.51	<0.0001	1.02	0.42	0.554	-0.001	-1.12	0.325
<i>Behavior</i>									
Feed	0.989	450.14	<0.0001	1	0.01	0.934	0	-0.07	0.95
Drink	0.744	15.54	0.017	1.01	0	0.961	0	-0.05	0.96
Preen	0.363	3.84	0.122	0.64	1.26	0.325	0.017	1.36	0.247
Forage	0.993	726.25	<0.0001	1.04	1.1	0.354	-0.004	-2.19	0.094
Rest	0.988	408.66	<0.0001	1.16	8.07	0.047	-0.011	-2.94	0.042
Dust bathe	-0.215	0.12	0.751	0.15	3.78	0.124	0.003	0.69	0.528
<i>Resource</i>									
Feeder	0.989	443.95	<0.0001	1.5	0.01	0.922	0	-0.07	0.95
Drinker	0.745	15.57	0.017	1	0	0.991	0	0.01	0.99
Nest box	1	INF	<0.0001	0.51	INF	<0.0001	0	INF	<0.0001
Perch	0.952	99.87	0.001	1.24	3.87	0.121	-0.009	-1.55	0.196
Other	0.841	27.5	0.006	0.85	0.91	0.394	0.011	0.84	0.45

Table 3.5 (cont'd)

15EV2									
	R^2	$F_{1,4}^a$	P value	β	$F_{1,4}^b$	P value	intercept	t_6^c	P value
<i>Posture</i>									
Sit	0.163	1.97	0.233	0.91	0.02	0.901	0.004	0.14	0.896
Stand	-0.241	0.03	0.874	-0.1	3.37	0.14	0.097	1.83	0.141
Walk	0.745	15.6	0.017	0.84	0.56	0.496	0.003	0.46	0.667
<i>Behavior</i>									
Feed	0.957	112.14	0.001	0.98	0.03	0.871	-0.005	-1.39	0.238
Drink	0.319	3.34	0.142	1.2	0.09	0.776	-0.001	-0.11	0.915
Preen	0.339	3.57	0.132	1.29	0.19	0.689	-0.009	-0.34	0.75
Forage	0.934	71.44	0.001	1.1	0.57	0.493	-0.004	-0.59	0.588
Rest	0.951	98.73	0.001	1.49	10.6	0.031	-0.033	-3.32	0.03
Dust bathe	NE	NE	NE	NE	NE	NE	NE	NE	NE
<i>Resource</i>									
Feeder	0.953	102.61	0.001	0.98	0.04	0.86	-0.005	-1.31	0.261
Drinker	0.329	3.45	0.137	1.19	0.09	0.783	-0.001	-0.09	0.934
Nest box	NE	NE	NE	NE	NE	NE	NE	NE	NE
Perch	0.632	9.57	0.037	0.8	0.58	0.489	0.014	1.11	0.331
Other	0.84	27.25	0.006	1.12	0.91	0.394	-0.01	-0.6	0.583

Figure 3.1: Scatterplots of the relationship between the results of a continuous observation and a sampling technique illustrating a perfect relationship (a), a scenario where the sampling technique over estimates the performance of the behavior (b), and a scenario where the sampling technique changes in magnitude with the amount of behavior performed (c).

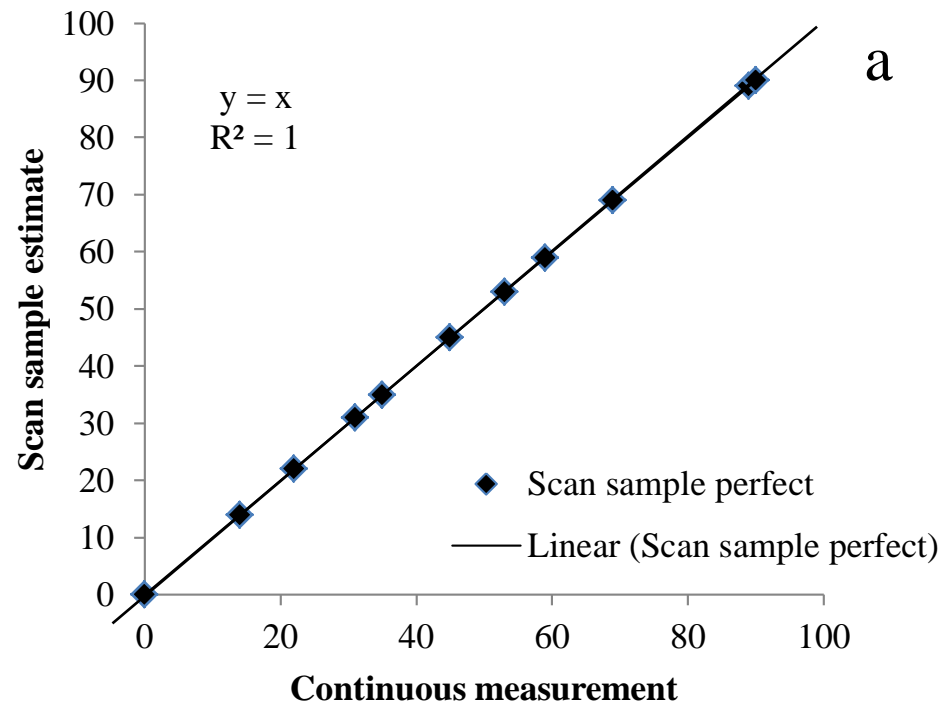


Figure 3.1 (cont'd)

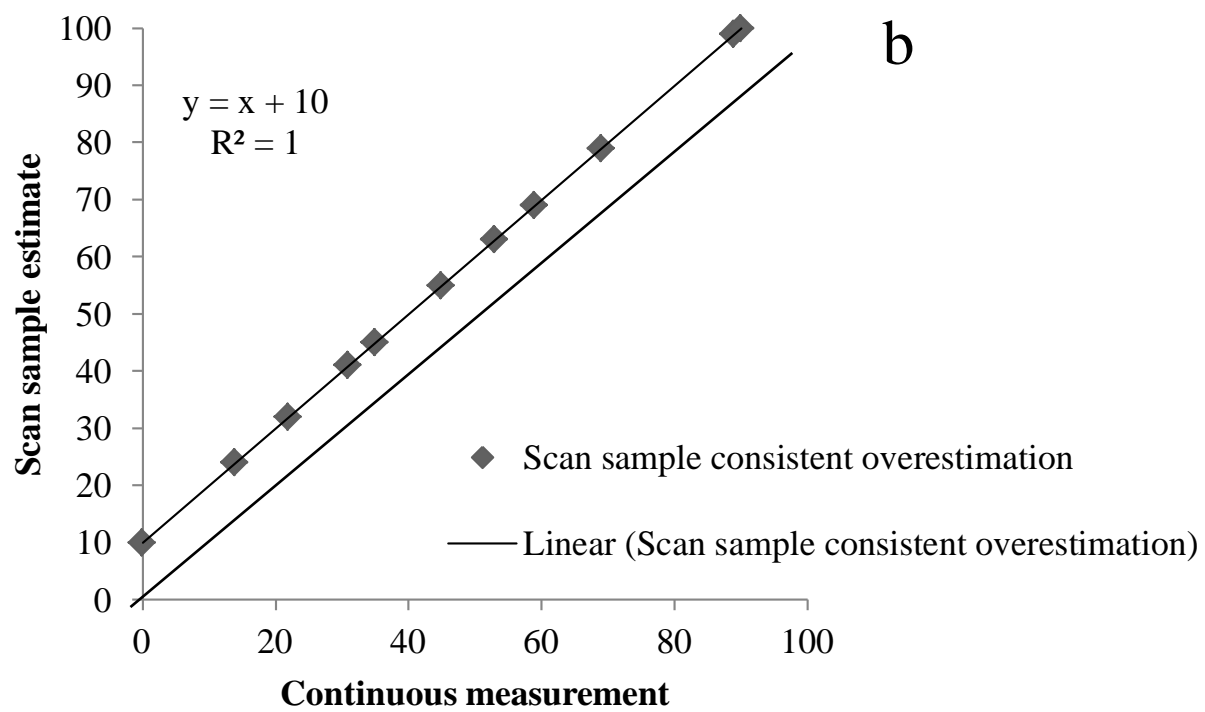


Figure 3.1 (cont'd)

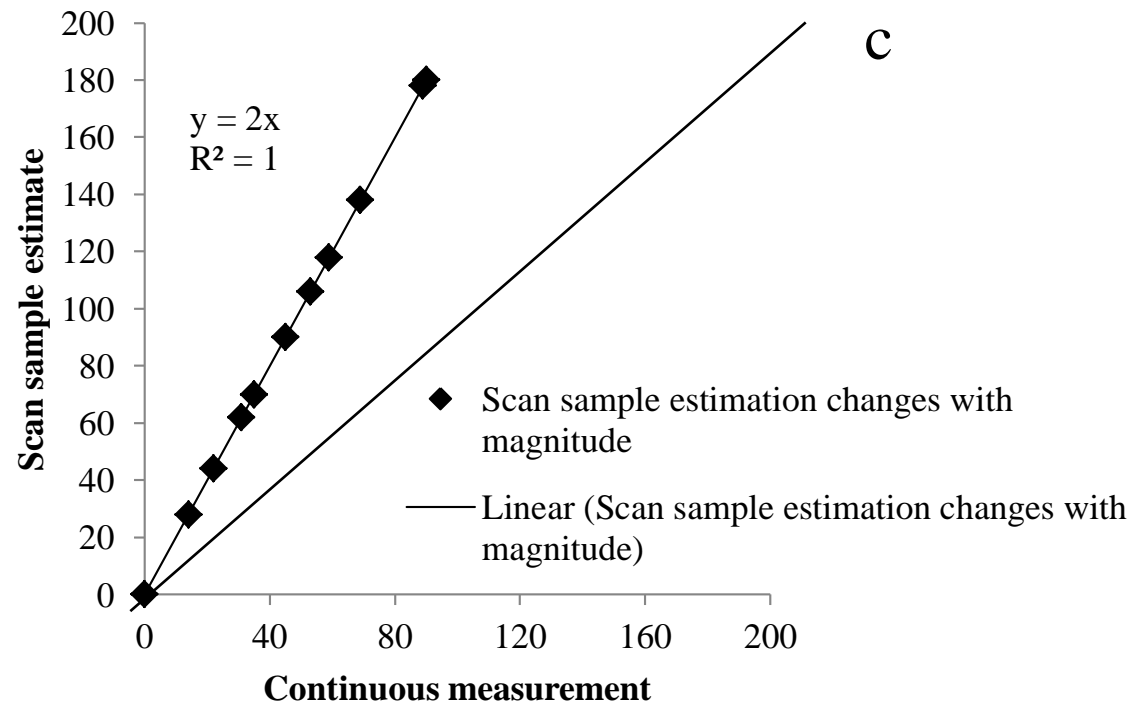


Figure 3.2: Percentage of time budget for static, dynamic, circadian and non-circadian behaviors represented by different durations of observation duration (a) and the amount of time between the beginnings of behavioral observations (b).

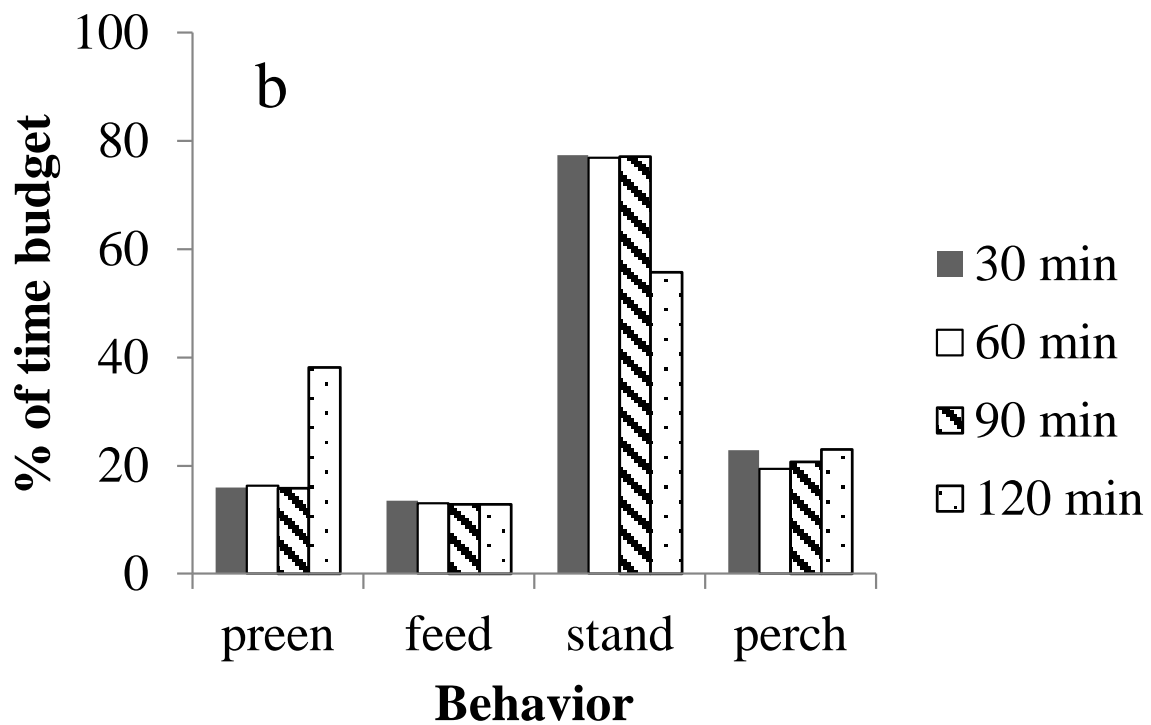
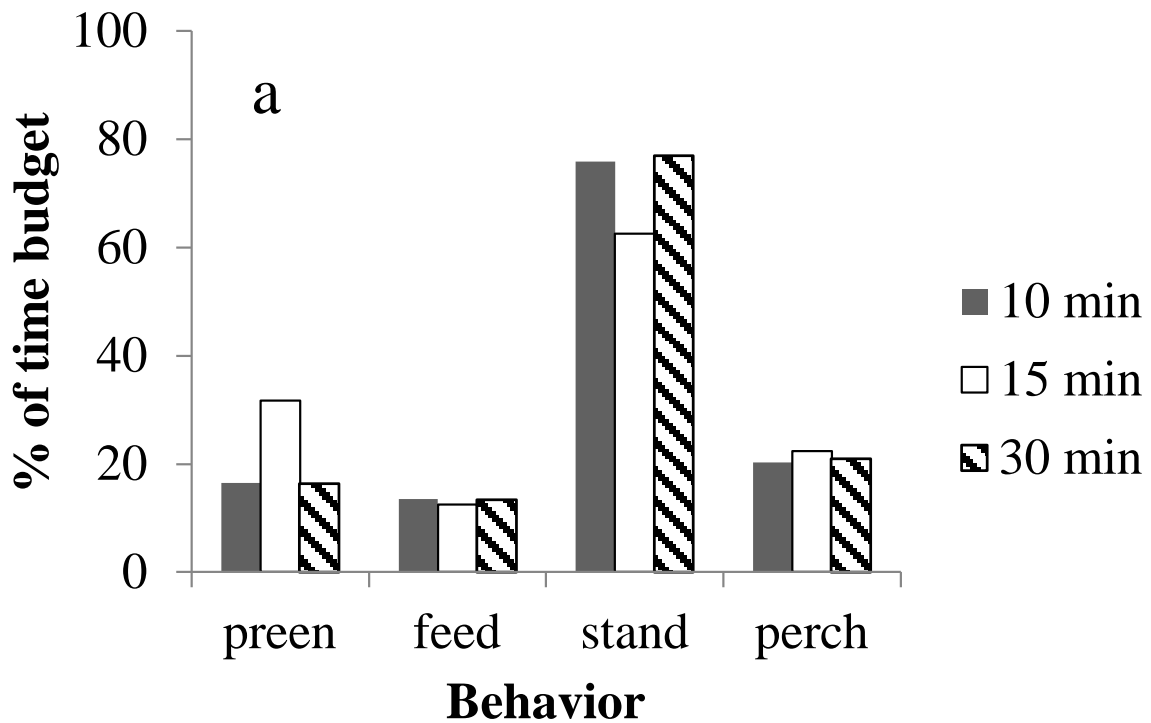


Figure 3.3: Percent of time per hour hen spends dust bathing across light period.

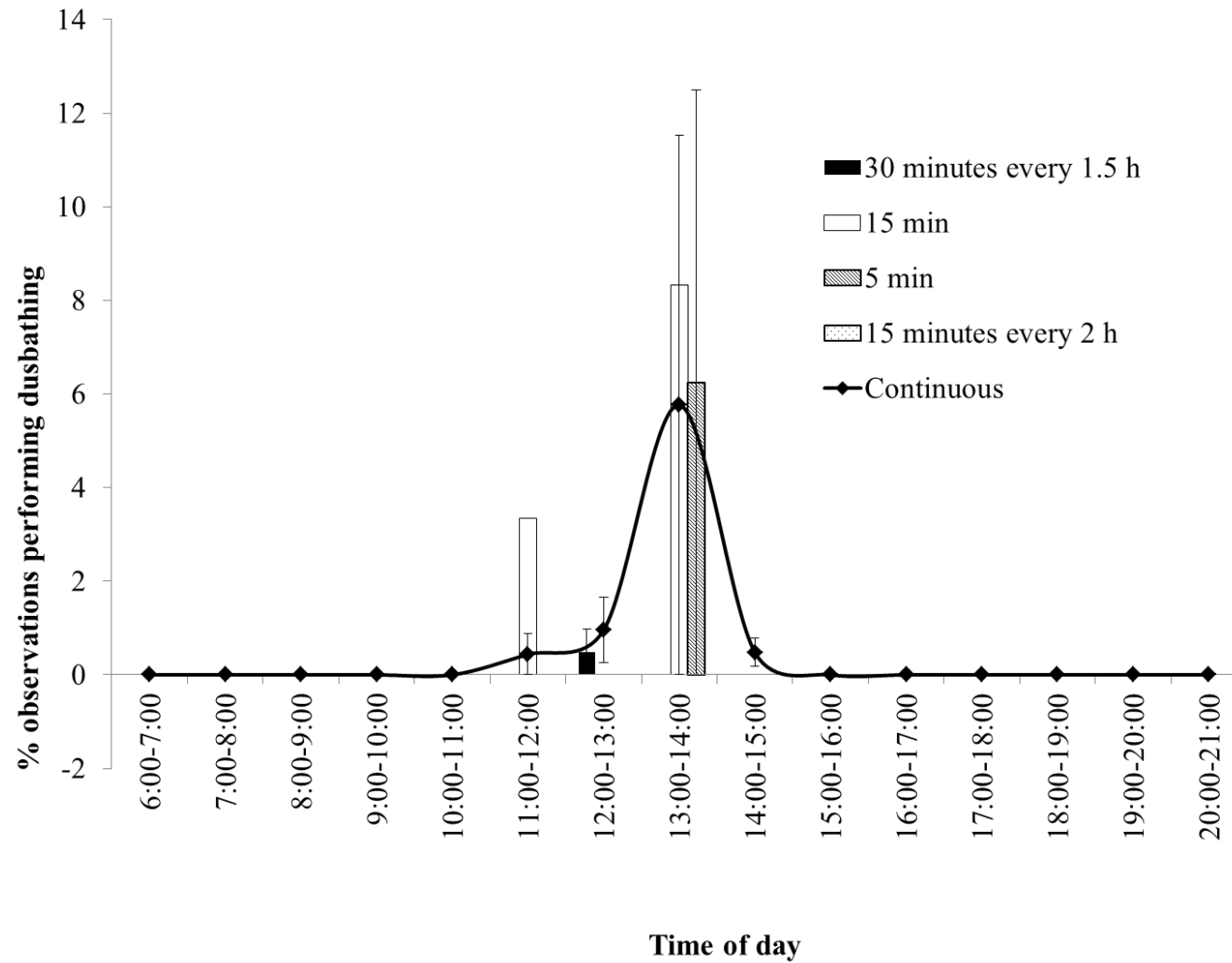
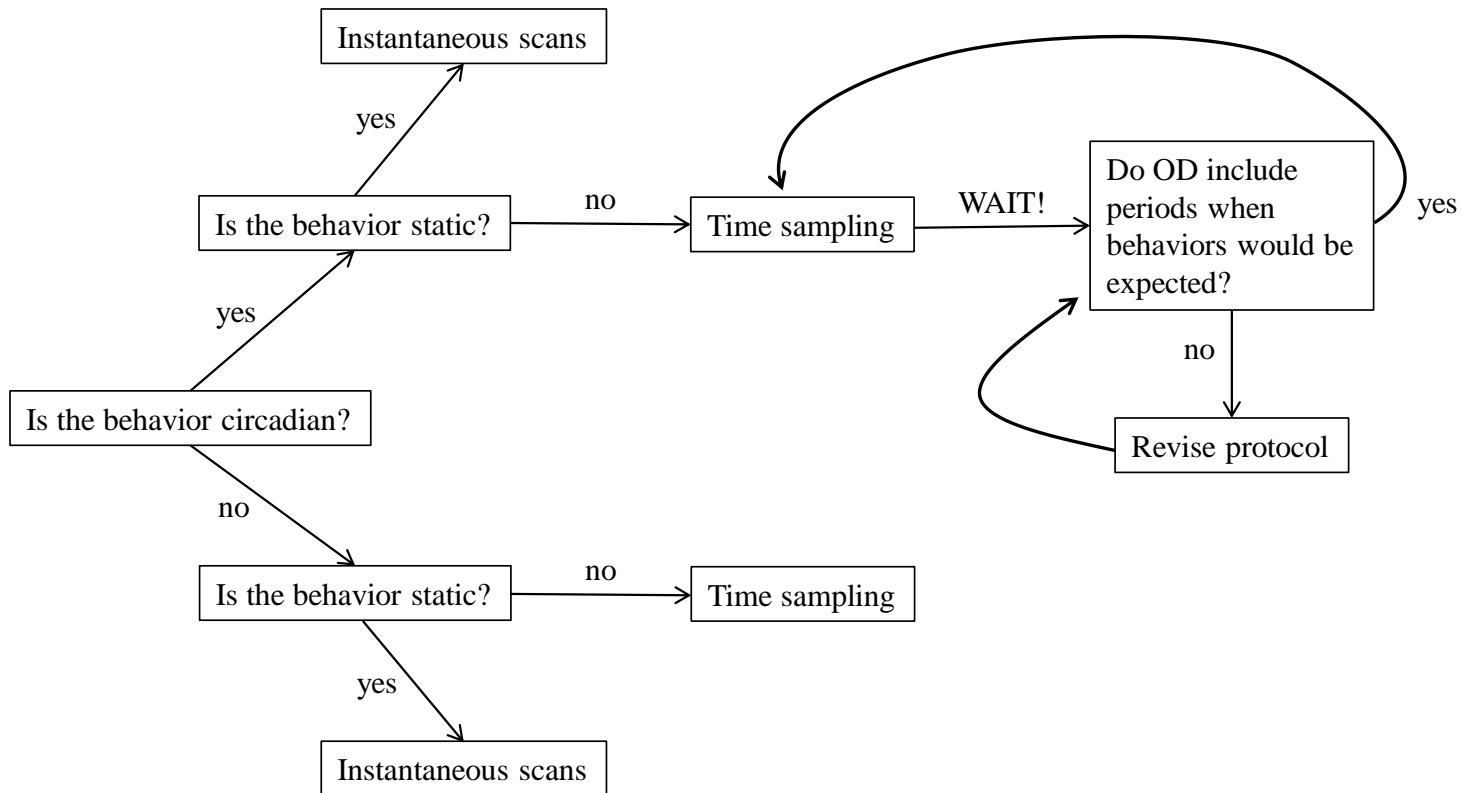


Figure 3.4: Decision tree to facilitate selection of appropriate sampling technique based upon characteristics of behavior to be assessed. OD represents observational duration.



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CHAPTER 4: UNDERSTANDING THE INDIVIDUAL NON-CAGE LAYING HEN THROUGH THE BODY-BEHAVIOR CONNECTION: ASSOCIATIONS BETWEEN WELFARE QUALITY PHYSICAL PARAMETERS AND BEHAVIORAL OBSERVATIONS THROUGHOUT A LAY CYCLE

ABSTRACT

The integration of outcome-based measurements to animal welfare assessment programs can provide a new perspective into the individual animal's experience. Identifying the variability of the individual experience can facilitate understanding of animals at the periphery of the welfare spectrum, compared to those at the average. Welfare Quality[®] physical measurements and behavioral observations were taken from the same fifteen non-cage laying hens throughout their production cycle. The average amount of time performing the behavior and the amount of variation in its performance was compared at four different ages: 19, 28, 48 and 66wk. The same analysis was performed for all Welfare Quality[®] physical measurements. To identify associations between hen behavior and her physical condition, correlations were performed between the physical measurements and hen behavior across all ages. No differences were observed among the four ages for most behaviors, but the amount of variability differed for most behaviors observed. Physical measurements taken at 19wk differed from those taken at later ages. The most robust physical measurement was claw length, as it provided the most correlations for the widest variety of behaviors at all ages. And the optimal age for gathering lifelong behavioral information from physical measurements was 48 wk. These results highlight the importance of assessing welfare at the individual level. While the average response of the flock may appear consistent across time or treatment, differences among the hens within the same flock may vary drastically.

KEYWORDS: Welfare Quality, laying hen, behavior, group welfare, outcome based measures

INTRODUCTION

Traditionally, animal welfare audits and certification programs have used resource-based measures (RBM), such as the availability of adequate food, water, veterinary care, and space, to assess how well a housing system meets animals' needs. However, many are beginning to emphasize the importance of outcome-based measures (OBM), such as behavior or individual physical condition, as part of animal welfare assessment (Butterworth, et al. 2011). As the laying hen industry transitions from small conventional cages to group housing, the hens housed in these alternative systems may face different challenges than those historically faced by their conventionally caged counterparts.

Outcome Based Measures are typically taken at the group level and individual measurements are collated to provide an average response for the group. However, many of the fundamental concepts of animal welfare are rooted in an individual's response to a situation, thus the concept of animal welfare inherently implies the importance of the individual experience (Dawkins 2003, Duncan & Mench 1993, Fraser 2008). Most theories of animal welfare apply to individuals because only individuals possess the characteristics (e.g., affective state, perception, needs, motivation) that make lives better or worse. Individual variation with regards to genetics, experiences, and temperament, can also impact how the individual perceives its current situation and ultimately its welfare. Yet, issues of practicality limit the ability of on-farm assessors to examine each individual, and welfare must be assessed at the group level for there to be any chance of improving the welfare of an individual in a production system. Therefore, RBMs are extrapolated and weighted to infer how an animal is performing based upon its environmental conditions (Veissier, et al. 2011). However, the average condition of the group may not accurately reflect the condition of a specific individual and the condition of individuals within

the same group may vary widely. The challenge for non-cage laying hen welfare assessments is two-fold: reconciling that group welfare is a complicated oxymoron, and identifying whether OBM provide a good proxy for understanding individual hen behavior.

The Welfare Quality[®] (WQ) assessment protocol for poultry (WelfareQuality[®] 2009) is a European developed living document designed to use OBM measures to show the outcome of the “interaction between the animal and its environment (housing design and management)”. This protocol was selected for this study because the information generated from the WQ provides practical and accessible feedback to producers while examining welfare at the individual level. RBM and OBM measurements emphasize four primary areas: good feeding, good housing, good health, and appropriate behavior to address the Five Freedoms (Brambell Committee 1965). The WQ incorporates measurements taken from the environment and the hens themselves to attempt to understand the hen’s perspective and the hen-environment interaction along with assessing the human-animal relationship. The WQ for laying hens utilizes OBMs including keel bone deformation, skin lesions, foot pad dermatitis, toe damage, on-farm mortality, culls on farm, enlarged crops, eye pathologies, respiratory infections, enteritis, parasites, comb abnormalities, and beak trimming abnormalities to understand the welfare of hens with regards to pain, injury and disease. These measurements have been chosen as they could indicate how hens are behaving, their potential pain experience, and feeling as well as their overall physical health. Yet the direct links between behavior and the physical conditions scored have not been fully investigated.

One OBM scores the number of pecking wounds found on the hen’s comb. The comb is an ornamental organ important to social recognition, status signalling, and can be an indicator of

productivity and health status (Mukhtar & Khan 2012). Hens with larger comb areas and which were more yellowish-red compared to pure-red have been reported to have a better competitive ability (O'Connor, et al. 2011). Hens from a high feather pecking line perform more comb pecking than hens selected for low feather pecking (van Hierden, et al. 2002), and hens have also been observed to sustain comb injuries as a result of agonistic interactions. Yet, the relationship between comb injury and individual hen behavior has not been explored to identify whether the relationships observed in the laboratory setting accurately reflect expectations of individual hen behavior in large groups in either commercial or laboratory settings.

The WQ also assessed hen foot health and perch availability. Foot pad dermatitis is a type of contact dermatitis that can be caused by prolonged contact with wet litter possessing a high ammonia content (Wang, et al. 1998). Perches also affect foot health, but the presence of perches on foot health has yielded mixed results [for review see: (Pickel, et al. 2011)]. Hens in alternative systems may be forced to spend longer periods of time standing on litter to access necessary resources (e.g., food and water) which could affect the development of foot related health issues. Conversely, hens with access to perches may have feet that benefit from the presence of the perch as an alternative location for standing off of manure and wet litter – as long as the perches are constructed of a material and shape that facilitates good foot health.

Although perches may potentially aid foot health; increased perch use has been associated with a higher prevalence of keel bone deformations (Abrahamsson, et al. 1996). Up to 73% of hens housed in an aviary system have been reported to have keel bone deformities (Freire, et al. 2003). Peak force on the keel bone of sitting hens is five times higher than the peak force exerted on the feet. This is true regardless of perch design which could help explain the high prevalence of keel bone deformations observed in hens housed with perches (Pickel, et

al. 2011). Hens with known keel bone fractures took longer to land from a perch than hens without keel bone fractures, and hens with fractures that were injected with butorphanol decreased their latency to land compared to hens injected with saline (Nasr, et al. 2012), which suggests that keel bone fractures are painful. However, even though welfare concerns are raised by the presence of keel bone fractures and deviations, little is understood with regard to how this impacts individual hen behavior.

Hens may experience feather damage for a variety of reasons, and the WQ scores feather damage in three areas of the body to attempt to differentiate the motivation or behavioral encounters behind the patterns of feather damage and loss. The three areas of feather damage (head/neck, back, and vent/rump) assessed in the WQ protocol may be indicative of different behavioral occurrences including feather pecking, feather picking (analogous to trichotillomania) (Bordnick, et al. 1994), agonistic encounters, and interactions with the environment (e.g., feeder troughs, nest box, wire mesh). Regardless of why feather damage and loss has occurred, overarching welfare concerns are associated with feather damage and loss. Hens with damaged or lost feathers have more difficulty with thermoregulation (Yahav, et al. 1998), exhibit reduced feed efficiency (Su, et al. 2006), and have lost a layer of physical protection from conspecific and environmental interactions. Hens subjected to feather pecking may spend more time in a heightened emotional state as they may be more vigilant against individuals performing feather pecking. This may detract from the time and energy they can devote to maintenance and comfort behaviors. Hens housed with individuals performing feather pecking exhibited higher levels of fear as reflected in longer time spent in tonic immobility (Vestergaard, et al. 1993). Negative affective states have been associated with the development of feather picking (Bordnick, et al.

1994), and individuals subjected to feather pecking at the rump are at an increased risk of injury from vent pecking and death due to cannibalism (Pöttsch, et al. 2001).

WQ measurements are implicitly assumed to serve as indicators of good physical health and functioning (e.g., keel bone deformation, foot pad dermatitis, plumage damage, comb pecking wounds) and to provide insight into hens' behavior or the animal-environment interaction. However, in many cases the link is tenuous or has not been demonstrated in commercial production conditions. By explicitly linking behavioral tendencies with a hen's physical condition, the information gained by collecting physical measurements of hens as part of the WQ may create a more robust assessment of animal welfare for the individuals assessed. The OBM selected for assessment in the WQ were selected because they met specific criteria including validity, reliability, and feasibility (WelfareQuality® 2009). These factors were also selected due to their known relationship with overall welfare concerns, but no research has verified how well the WQ score relates to individual behavior.

Historically, other welfare assessment protocols have summarized individual scores to provide an overall farm-level score (Johnsen, et al. 2001). The WQ utilizes resource-based and individual outcome-based measurements to develop an overall picture of welfare, and assessments have been reported to be taken at the individual, pen, and farm level (Temple, et al. 2011, Temple, et al. 2013). However, different hens housed within the same environment may exhibit different responses to the same set of conditions, and thus experience different levels of welfare. These differences can be attributed to a myriad of factors including individual growth patterns, physical conformation differences, social pressures, past experiences, individual perception, and coping style. Therefore, identifying an average level of welfare for a group may

mask welfare concerns of specific individuals, as animals that experience poor welfare would be at the tail of a distribution curve.

Manifestations of different physical changes that impact welfare may occur at drastically different rates. A keel break can happen instantly, while a keel deviation may take several weeks of repeated perch use and keel remodeling to develop. Foot pad dermatitis can take up to a week to manifest but may not be immediately apparent during early stages of development. Claws take time to grow and require repeated wear and use to stay short. An aggressive encounter can change a hen's comb pecking score within minutes, and likewise a hen's feather condition. Thus, each WQ score may be able to provide information on a unique time scale about a hen's behavior, and each may have different utility in assessing immediate, past, or potentially future welfare concerns. Measurements taken today could be reflective of either an immediate welfare concern or may be indicative of behavior and environmental conditions that persisted across the previous weeks or months. Therefore, identifying an age at which the most robust picture of hen welfare can be obtained would enhance the usefulness of the WQ measurements.

As animals are housed in more complex and stochastic environments, they have increased opportunity to make choices about when and how they access resources and perform natural behaviors. These choices can impact their physical condition, and conversely their physical condition can impact the behaviors they choose to perform with the cause and effect relationship difficult to extrapolate. Understanding the choices hens make is difficult. Humans are equipped with a different set of sensory mechanisms than animals, may process information in our brains differently, and animals may make choices based upon information we cannot or do not perceive (Sherwin 2007). However, understanding the connection between animal behavior

and physical health and function may illuminate some of the mystery behind why animals make choices (e.g., environmental preference, resource use) observed by their behavior.

Choices—and resulting behaviors—made at an early age can also influence future body condition and future choices. An animal's coping style (or personality, temperament, axes, constructs, or behavioral syndrome) has been linked to behavioral and physiological characteristics such as exploratory behavior and boldness (Stowe, et al. 2010, van Oers, et al. 2011) and can be consistent over time and across contexts (Re'ale, et al. 2007, Sih, et al. 2004). Because behavior can be shaped by choice and experience, and experiences shape future choices, understanding the cyclic relationship between the body and behavior is becoming increasingly important - especially as regards animal welfare. However, observing the behavior of individuals in large groups can be challenging, and identifying links between the physical body and behavior can enhance the information gained from OBM measurements, thus providing insight into individual behavioral choices with regard to welfare. Therefore, the objectives of this study were to assess how posture, behavior and resource use of individual laying hens change as they age, assess whether the variability of posture, behavior, and resource use changes among individual hens changes as they age, and identify relationships between hen WQ scores and behavior.

MATERIALS AND METHODS

Animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Teaching and Research Center (MSU-PTRC). Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University

Institutional Animal Care and Use Committee. Three identical rooms (6 m x 4.5 m) at MSU-PTRC were used. Each room was furnished in the same configuration with nest boxes, perches, tube feeders, and a water line with nipples. Sixteen nest boxes (each 0.4 m long x 0.3 m wide x 0.3 m high) in an 8 x 2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1 m x 6 m slatted area at a height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8 cm of wood shavings at time of data collection. Food and water were provided daily ad libitum. Daily care, including egg collection, feeding, and hen inspection, occurred at least once a day. Two incandescent light bulbs (60 lux at bulb level) on an automatic timer provided light 15 h per day in each room. Temperature was maintained between 16°C -22°C using a ventilation fan and forced air heating.

Hy-Line Brown laying hen pullets (n = 405) were reared in each of the rooms as described above with accommodation made for their size (i.e., smaller perches, which were removed at 6 wk) and immaturity (i.e., access to nest boxes was granted at 10 wk of age). Each room provided 0.21 m² floor space, 17.8 cm of perch space, 0.01 m² of nest box space, and 4.83 cm feeder space per hen. Thirteen nipples provided drinking space for 10.3 hens per nipple. At 10 wk, all hens were weighed and fitted with uniquely numbered leg bands. All room parameters met or exceeded United Egg Producers and the Federation of Animal Science Societies housing requirements for non –cage laying hens.

At 11 wk, all hens were handled and 10 hens per room were selected and fitted with a sensor. The remaining hens in each room did not receive sensors. Previous research indicated that wearing the sensor did not have any long lasting effects on hen resource use or agonistic interactions (Daigle, et al. 2012).

The sensor-wearing hens were selected from across the range of body weights obtained at 10 wk in an attempt to select for hens of varying social ranks (Cloutier & Newberry 2000). Collection of data from the sensor to monitor the hen's behavior prior to and immediately after the onset of lay was the motivation for fitting the hens with sensors prior to comb development. Therefore, it was not possible to include comb size as a parameter indicative of social status to select hens to wear sensors as described in O'Connor, et al. (2011).

Behavioral data collection

Hens in each room were videotaped for 48 h at 19, 28, 48 and 66 wk of age with ceiling-mounted cameras. These weeks were chosen for observation because they correspond to time periods within the hen's production cycle right before she began laying eggs (19 wk), during her peak production (28 wk), mid-way through her production life cycle (48 wk) and when she was at the end of her production cycle (66 wk). Though ten hens per room were fitted with sensors, video data were only decoded for five individual hens per room. Data collection as part of a separate experiment was limited to five individuals based upon capabilities of the sensor network. Therefore, the individual behavioral data collection for this research was restricted to the same five individuals. Furthermore, the individuals assessed for this study were selected retrospectively based upon whether they survived the entire length of the lay cycle. Several of

the hens that had been used to collect sensor data early in the lay cycle died; therefore, an alternate sensor hen was used to collect sensor data. The goal was to track hens through the entire production cycle. Therefore, we identified five sensor-wearing hens per room that had survived the entire lay cycle and subsequently decoded video data from these individuals for the duration of the lay cycle.

Continuous observation of individual hen posture, behavior, and resource use (Table 1) was made over a 30 min period every hour and a half (06:00-06:30, 07:30-08:00, 09:00-09:30, 10:30-11:00, 12:00-12:30, 13:30-14:00, 15:00-15:30, 16:30-17:00, 18:00-18:30, and 19:30-20:00) during the lights on period (Daigle & Siegford in press). Data were collected across a 48 h time period with the dark period (21:00-06:00) omitted. Infrared cameras were used, which enabled night-time viewing, but movement between perches or areas of the room was not observed (though hens were observed transitioning between standing and sitting as they readjusted during the night). Posture, behavior, and resource use were recorded using mutually exclusive categories and are reported in duration of time spent (sec) in that state across the 48 h period.

Welfare Quality Assessment

All sensor wearing hens were evaluated using the WQ throughout the lay cycle. For this analysis, five hens per room ($n = 15$) were analysed, and these hens were the same hens from which behavioral observations were recorded. The same individuals were assessed throughout the entire study. Parameters measured included body weight (kg), claw length (cm), comb pecking wounds, plumage damage, foot pad dermatitis, and keel bone scores (Table 2). WQ

parameters were measured once every other wk from 16-66 wk of age. WQ parameters that were measured in the 2 wk before, during, and 2 wk after video recording were averaged to create a single WQ score for each hen at each data collection time point.

Statistical analysis

All analyses for this paper were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA). Results were considered statistically significant at a probability of α less than 0.05. The residuals for each variable were tested for normality. Variables with residuals that caused both the skew and kurtosis to be greater than one were considered outliers and removed from the data set.

Mean WQ parameters, posture, behavior and resource use of individual laying hens as they age

Boxplots were created for each WQ parameter and hen activity (posture, behavior, and resource use) at the four time points of interest (19, 28, 48, and 66 wk of age). To identify whether there was a difference in either the mean WQ parameter or the mean amount of time spent performing each activity, mixed model analysis (PROC MIXED) was used to identify mean differences across the four age points for each WQ parameter and activity. The model included a repeated measure of age, a random effect of hen nested within room, and hen was the subject. Least squared means with a Bonferonni correction were used to identify specific differences between the age groups.

Variability of WQ parameters, posture, behavior, and resource use among individual hens as they age

To examine whether the variability of either the WQ parameters or time spent performing each activity was homogenous across the different age time points, the COVTEST option was utilized as part of a General Linear Model (PROC GLIMMIX). The model used age as a fixed effect; hen nested within room as the random effect, hen as the subject and age as the group. Contrast statements were employed to identify specific differences among the standard errors of the different age groups.

Relationship between WQ parameters and hen activity

To identify whether the physical condition at one age had any relationship with activity at another age, correlations (PROC CORR) were performed. WQ parameters taken at 19 wk were correlated with the duration of time spent in each activity at 19, 28, 48, and 66 wk. This analysis was repeated for WQ parameters taken at 28, 48, and 66 wk.

RESULTS

Mean change in WQ parameters, posture, behavior and resource use of individual laying hens as they age

Hens spent more time standing (Figure 1a) at 48 wk than at 19 wk ($t = 3.36$, $P = 0.0046$) and 28 wk ($t = -3.67$, $P = 0.0041$). Hens spent more time sitting (Figure 1b) at 19 wk than at 28 wk ($t = 3.23$, $P = 0.0144$). No mean differences were observed among the four time points for amount of time spent walking (Figure 1c), feeding (Figure 1d), drinking (Figure 1e), preening (Figure 1f), foraging (Figure 1h), dust bathing (Figure 1g), resting (Figure 1i), or nest box use

(Figure 1j). More time was spent using the perch (Figure 1k) at 19 wk than was observed at 28 ($t = 3.19, P = 0.0162$).

Hens were lighter (Figure 2a) at 19 wk than at 28 ($t = -5.06, P < 0.0001$), 48 ($t = -6.55, P < 0.0001$), and 66 wk ($t = -5.46, P < 0.0001$). Claws (Figure 2b) were shorter at 28 wk compared to 19wk ($t = 3.41, P = 0.0088$) and 66 wk ($t = -8.81, P < 0.0001$). Subsequently, claws were longer at 66 wk than at 48 ($t = -6.21, P < 0.0001$) and 19 wk ($t = -5.41, P < 0.0001$). Plumage damage scores (Figure 2c) were lower at 19 wk compared to 28 ($t = -5.35, P < 0.0001$), 48 ($t = -7.35, P < 0.0001$), and 66 wk ($t = -7.89, P < 0.0001$). A similar pattern was observed for comb pecking scores (Figure 2d) which were lower at 19 wk than at 28 ($t = -7.89, P < 0.0001$), 48 ($t = -6.43, P < 0.0001$), and 66 wk ($t = -4.38, P = 0.0005$). Yet, comb pecking scores were higher at 28 wk than at 66 wk ($t = 3.51, P = 0.0066$). Keel bone scores (Figure 2e) were higher at 48 ($t = -3.30, P = 0.0120$) and 66 ($t = -3.71, P = 0.0036$) wk compared to 19 wk. Foot pad dermatitis scores (Figure 2f) were lower at 19 wk compared to 28 ($t = -5.97, P < 0.0001$), 48 ($t = -9.51, P < 0.0001$), and 66 wk ($t = -9.33, P < 0.0001$). Furthermore, foot pad dermatitis scores at 28 wk were lower than those measured at 48 ($t = -3.54, P = 0.0059$) and 66 ($t = -3.36, P = 0.0101$) wk.

Variability of WQ parameters, posture, behavior, and resource use among individual hens as they age

No difference in the variability of time spent drinking (Figure 1e), walking (Figure 1c), or foraging (Figure 1h) was observed among the four time points. More variability in feeding behavior (Figure 1d) was observed at 48 wk when compared to 28 wk ($X^2 = 4.44, P = 0.0351$).

Hens tended to be more variable in their sitting (Figure 1b) behavior at 19 wk than at 28 wk ($X^2 = 3.60, P = 0.0576$), yet an opposite pattern was observed for standing behavior (Figure 1a) with

hens standing more at 28 wk compared to 19 wk ($X^2 = 5.16$, $P = 0.0232$). Hens were more variable in their preening behavior (Figure 1f) at 66 wk compared to preening behavior at 19 wk ($X^2 = 4.69$, $P = 0.0303$) and 28 wk ($X^2 = 4.43$, $P = 0.0354$). Dust bathing behavior (Figure 1g) tended to be less variable at 28 wk than at 66 wk ($X^2 = 3.54$, $P = 0.0598$), yet resting behavior (Figure 1i) was more variable at 19 wk compared to resting behavior at 28 wk ($X^2 = 6.64$, $P = 0.0100$). Nest box use (Figure 1j) was more variable at 66 wk than at 19 wk ($X^2 = 4.15$, $P = 0.0417$), and perch (Figure 1k) use was more variable at 66 wk than at 28 wk ($X^2 = 4.33$, $P = 0.0374$).

Body weight (Figure 2a) was more variable at 66 wk than at 19 ($X^2 = 5.48$, $P = 0.0192$) and 28 ($X^2 = 4.23$, $P = 0.0396$) wk. Claw length (Figure 2b) was more variable at 66 wk than 28 wk ($X^2 = 4.69$, $P = 0.0303$). Plumage damage scores (Figure 2c) were less variable at 19 wk compared to 28 ($X^2 = 14.5$, $P > 0.0001$), 48 ($X^2 = 6.73$, $P = 0.0095$), and 66 ($X^2 = 14.41$, $P = 0.0001$) wk. Comb pecking scores (Figure 2d) followed a similar pattern with more variability observed at 28 ($X^2 = 6.46$, $P = 0.0110$), 48 ($X^2 = 10.27$, $P = 0.0014$), and 66 ($X^2 = 15.92$, $P < 0.0001$) compared to 19 wk. Keel bone scores (Figure 2e) were less variable at 19 wk compared to 28 ($X^2 = 11.14$, $P = 0.0008$), 48 ($X^2 = 11.71$, $P = 0.0006$), and 66 ($X^2 = 14.23$, $P = 0.0002$) wk. Foot pad dermatitis scores (Figure 2f) were less variable at 19 wk compared to 28 ($X^2 = 298.07$, $P < 0.0001$), 48 ($X^2 = 308.98$, $P < 0.0001$), and 66 ($X^2 = 303.93$, $P < 0.0001$) wk.

Relationship between WQ parameters and hen behavior

Relationships between WQ measurements and behavioral observations taken at 19, 28, 48, and 66 wk are presented in Tables 3, 4, 5, and 6 respectively with only significant relationships ($R > \pm 0.5$, $P < 0.05$) reported for this portion of the analysis. Irrespective of age, the WQ parameter claw length had the most associations with behavior, while body weight had the fewest (only being related to sitting, standing, preening, and walking). Furthermore, claw length and keel bone had associations across the widest variety of behaviors. Keel bone was the only WQ parameter that had significant associations with nest box use, while foot health and keel bone scores were associated with perch use. Welfare Quality parameters measured at 48 wk provided the most robust associations of all of the time points, with most of these associations occurring in relation to 28 wk behaviors. Furthermore, WQ parameters measured at 66 wk had the most associations with behaviors at 48 and 66wk, while WQ parameters measured at 28 wk had the least associations.

Claw length at 19 wk was negatively associated with feeding behavior at 28, 48, and 66 wk (Figure 3). Plumage damage score at 19 wk was positively associated with standing and sitting behavior at 66 wk. Foot pad dermatitis score at 28 wk was positively associated with feeding behavior at 48 and 66 wk, with the association becoming stronger as the hens age (Figure 4). Hens with higher foot pad dermatitis scores at 48 wk were observed to be using the perch less at 19 wk (Figure 5). The fewest associations with behavior were observed for WQ parameters measured at 28 wk.

DISCUSSION

Little is known about how individual laying hens behave and perform in non-cage systems, especially as they age. As far as we know, this is the first time individual behavioral observations and the WQ assessment protocol has been used to examine laying hen behavior and body condition across a lay cycle in a non-cage system. Furthermore, this is the first study to track the variability in response of individual hens as they age and to observe associations between a hen's body condition and her future and past behavioral profiles. Each physical measurement may provide different degrees of insight into either current or past welfare states of a hen. Each measure, therefore, requires different interpretation with regard to the hen's welfare based upon the time it takes for the physical condition to manifest or resolve. Of the time points observed in this study, assessing WQ parameters at 48 wk provides the most robust information about hen behavior throughout the lay cycle.

Behavior

The average amount of time performing many of the activities remained unchanged throughout the lay cycle. Previous studies on the behavior of brown laying hens in a perchery house noted differences in feeding and drinking behavior as the hens aged, but with regard to the ages that are comparable to those assessed in this study, similar patterns were observed (Channing, et al. 2001). Further, in another study, no differences were observed in feeding behavior of ISA brown hens in modified and conventional cages at 32 and 59 wk (Freire, et al. 1999). Many of the assessed activities (e.g., feeding, drinking, resting) could be considered ultimate needs (Alcock 2009), and therefore, would be required to be performed with little room for flexibility. For example, as food deprivation increases, laying hens perform more gakeel calls

(Zimmerman, et al. 2000) and are more willing to withstand aversive stimuli to access food (Faure & Lagadic 1994). Inelastic demands must be met to ensure an animal's survival, regardless of environmental condition or social status and could be expected to be performed at a similar rate no matter how different the experiences, preferences, or perceptions were for the individual hen (Dawkins 1983).

Other activities, including preening, dust bathing, and foraging, could be considered proximate (here and now) needs, which are still important for hens to perform (again, regardless of social status or environmental condition) though less vital to survival. For example, dust bathing is important to hen welfare as hens are motivated to dust bathe even in the absence of substrate (Black & Hughes 1974). Hens that once had opportunity to dust bathe and then were subsequently placed in a situation where they could no longer dust bathe showed signs of stress, including increased corticosterone levels (Vestergaard, et al. 1997). Hens will also work to access substrate for foraging (Bubier 1996) and will continue to forage in the presence of an aversive stimulus (Browne, et al. 2011). Further, preening is considered a comfort and grooming behavior and is performed more often in the presence of familiar conspecifics (Nicol 1989).

Because the performance of these behaviors (preening, dust bathing, foraging, etc.) is driven by internal motivation (Jensen & Toates 1993), and this motivation may be driven by the behavior's contribution to the individual's overall fitness (Akçay, et al. 2009), they are important to the hen to perform. Variations in their performance may provide insight into individual hen frustration or satisfaction. Yet, since these behaviors are not required for survival, hens may be willing to compromise their ability to perform them or alter how often or when they are performed in exchange for access to necessary resources, to avoid social stress, or to fulfil ultimate needs.

Perch use can vary among different strains and housing types. One specific strain (HyLine Brown) and one specific housing type (non-cage slats and litter) were used in this study. Therefore these results should not be generalized to all strains and housing types. From the results of this study, differences across the lay cycle were observed for standing, sitting, and perch use. Sitting and perching decreased at peak lay and increased as the hens aged while standing followed the opposite pattern. These results follow an inverse pattern of perch use compared to findings of Carmichael and colleagues (1999) where perch use increased after peak production and decreased with age. In general, perch use by the study hens was low. Similar patterns of perch use by Hy-Line Brown hens have been reported: compared to W-36 hens they used the perch less and were never observed perching during the morning hours of observation (Barbosa Filho, et al. 2008). However, in a study investigating perch orientation, Hy-Line brown hens were reported to perch more than Hy-Line White hens (Wall & Tauson 2007). Multiple factors are known to influence perch use, including strain, previous experience, accessibility and perch orientation. Therefore, identifying motivation behind perch use can be difficult to support. Further, the patterns of standing and sitting follow a counter-intuitive pattern, as hens would be expected to sit more and stand less as they age. However, if the 19 wk data point is omitted from the analysis, an increasing trend consistent with expectations is observed for sitting. Hens may have sat less at 19 wk because they were smaller in size and may have been less prone to displacement by other hens. Further, hens may have increased the amount of time sitting as they aged because individual behavioral patterns and territories may have been established and the motivation to rest increased. As sitting and standing are mutually exclusive, the observation of these behaviors following inverse patterns is not surprising.

Many of the behaviors changed in amount of variability that was observed over time among the hens, and this is where welfare concerns might exist. On average, no significant differences were found for the amount of time spent performing many of the observed behaviors. Yet, all of the hens were recorded to have spent at least some portion of their time engaged in all of the behaviors of interest (except dust bathing and nest box use) at 19 wk. However, by 66 wk some of the hens were never recorded feeding, drinking, preening, foraging, perching, or resting during the two consecutive lights on periods when these observations were made.

This would seem to suggest that these hens were either not performing certain vital maintenance behaviors at all, that they were performing them so infrequently that they were not captured with the sampling technique, or that they had shifted performance of such activities, including feeding and drinking to the dark period. However observation of hen behavior during the dark period showed that hens were not performing any behaviors, only transitioning between sitting and standing, and did not use any of the resources (except the perch) during the dark period (Daigle & Siegford, unpublished data). Understanding the precise factors contributing to these observations is difficult, but they could be related to physiological changes within the hen due to age or injury, or they could be attributed to social or environmental influences. These results highlight the importance of assessing welfare at the individual level because while the average response of the flock may appear consistent across time or treatment, differences among the hens within the same flock may vary drastically.

WQ scores

For all physical parameters assessed, measurements taken at 19 wk differed from those taken at a later age. This highlights the impact of age on physical condition and is consistent with previous reports of changes in plumage score, claw length, bumble foot prevalence, comb wounds, and keel bone deformations as hens age (Wahlstrom, et al. 2001). Furthermore, these differences emphasize that transitioning from pre-lay to lay can have drastic impacts on the hen's body.

Keel bone scores increased with age, in accordance with previous studies investigating keel bone condition in laying hens (Fleming, et al. 2004). Yet, the interpretation of keel bone scores can be challenging due to several factors. Keel bones can be subjected to either instantaneous changes (e.g., a break) or a slow change (e.g., due to repeated perch use or to healing and remodeling). The WQ scoring system gives hens with keel bone deviations and hens with broken keels the same score, even though the degree of pain—and therefore impact on welfare—experienced by a hen with a keel bone deviation compared to a keel bone break is unknown. Thus, the WQ keel bone scoring may lump individuals with different degrees of keel bone deformity (those with deviations and those with breaks) into the same category even though their welfare may be drastically different. However, some of the alternative four-point keel scoring systems require a histological examination of the keel bone, and identifying differences between moderate and slight deformities can be subjective (Scholz, et al. 2008, Vits, et al. 2005). The WQ palpation scoring can be done in an on-farm setting without the need for euthanasia and eliminates observer bias.

The keel bone is cartilaginous and capable of remodeling. Therefore, hens that may have developed a keel bone deviation due to perch use may not receive the same score later in life if

their perch use changes. Figure 6 illustrates changes in individual keel bone scores over time, illustrating the fact that keel bone scores may even improve as a possible consequence of this remodeling.

Differences in plumage condition could be attributed to a molt surrounding the onset of lay (Leeson & Walsh 2004), increased levels of feather pecking as the hens age, or aggressive interactions. The WQ includes assessing the plumage condition for the head/neck, back, and belly/rump region to calculate an overall plumage condition score. Therefore, each body region contributes to the hen's overall plumage score. The head/neck regions could provide information about either the amount of abrasion the hen is experiencing or the level of agonistic interactions in the flock. However, since these hens did not have to pass their head through cage bars to reach food, aggression was the most likely the source of any plumage damage in this region of the body. The back and belly/rump regions could provide insight into the amount of feather and vent pecking respectively. Increased levels of feather pecking and vent pecking have been observed as hens age (Pötzsch, et al. 2001), and the back and belly/rump scores contributed to a higher overall plumage score 70% and 27% of the time respectively, thus contributing to the higher overall plumage scores observed in this study. Levels of aggression are usually low in large flocks of (> 100) hens (Hughes, et al. 1997, Rodenburg & Koene 2007), yet the head/neck region of the body contributed to the overall plumage score 47% of the time in this study.

The hens in this study were housed in a slat and litter system. Therefore, their feet were in contact with manure and litter when they were not on the perch or nest box. Hens exposed to wet litter and manure can develop foot pad dermatitis in under a week and have been reported to recover within two weeks (Wang, et al. 1998). Hens in the present study also showed signs of

dermatitis development and recovery following pen cleaning within similar time frames (Figure 7).

Claws were longer at 66 wk compared to any of the other time points. Claw length could be a reflection of hen mobility. Claw length also followed a similar trend as sitting behavior, so as hens aged, and sat more, they may have not spent as much time engaged in claw shortening behaviors (e.g., foraging, walking).

As was found for individual behavior, variability in physical condition, as assessed by WQ parameters, increased with age. This illustrates that many hens begin the lay cycle with similar physical condition, but as they are subjected to environmental and social pressures, their individual experiences may cause them to make choices that lead to increased variability between hens with regard to behavior performance and subsequent physical condition. Further, as hens may not grow and develop at similar rates (e.g. lack of comb development) and this inherent physical difference may drive the hen's behavior. The hen's physical condition could impact a hen's social status thus forcing the hen to perform behaviors differently, in different rates, or develop new behavioral patterns to adapt to her behavioral repertoire to maximize survival.

Associations between behavior and WQ scores

Even though a predictive statistical model was not used, links were found to exist between certain behaviors and body conditions across time. Identifying whether the body condition caused the behavior, or the behavior resulted in the body condition is akin to identifying whether the chicken or the egg came first. However, relationship between the body

condition and behavior were identified and an attempt made to gauge the impact of age on these relationships, hen behavior, and physical condition. Some health issues take time to manifest (e.g., foot pad dermatitis); therefore, behaviors that are performed and become part of an established behavioral pattern may have physiological consequences that are not immediately identifiable. Conversely, health issues that arise and resolve (e.g., keel bone fracture, foot pad dermatitis) could impact future behavioral choices (e.g., perching and walking behavior) thus altering how the hen chooses to spend its time due to physical limitations.

Claw length, surprisingly, appears to be one of the more robust parameters to measure when identifying behavior as it was associated with the widest variety of postures and behaviors from all time points. Furthermore, measuring WQ parameters at 48 wk of age provided the most insight into individual hen behavior throughout her lay cycle. This may be because sufficient time has passed from entry into the laying facility for the hen to develop stable social and environmental patterns and allow the hen's body condition to reflect her behavioral choices. WQ measurements at 28 wk provided the least amount of information. This is a period where the hen is undergoing significant physiological changes as she both experiences peak production and reaches physical maturity. Therefore, a clear relationship between the body and behavior may be blurred due to overriding effects of such substantial concurrent physiological changes, or it could be due to factors associated with the latency required for physiological problems to manifest.

A consistent negative relationship was observed between feeding behavior and keel bone score (Figure 8), which could lend support to the claim that chickens can feel pain (Gentle 2001), keel bone fractures are painful (Nasr, et al. 2012) and the presence of pain, or physical impairment, may result in reduced feed intake or altered behavior. Lamé dairy cows spent less

time feeding, fed less often, and ate at a slower rate than non-lame cows (González, et al. 2008). Brown leghorn hens injected with naloxone hydrochloride, an opioid inverse agonist that inhibits pain-lowering endorphins, performed less feeding behavior than hens injected with saline (Wylie & Gentle 1998). Broken keel bones can cause an inflammatory response, catalyzing the release of inflammatory cytokines, which have been associated with a dose dependent decrease in feeding behavior (Larson & Dunn 2001). However, Morgan's Canon of Interpretation could argue that hens spent less time feeding because they were using the perch more and could not physically feed and perch simultaneously, yet no significant associations between feeding and perch use were observed at any of the ages investigated.

The positive association between foot health and feeding behavior is a good example of the hen's choice impacting its body condition. This trend provides insight into the degree of discomfort induced by the development of foot pad dermatitis compared to the discomfort of a broken keel bone. Hens in this system were required to stand on the litter to feed; therefore, hens that spent more time feeding consequently spent more time standing in the litter. The consumption of food has also been argued to act as a natural analgesic as the hen is focused on feeding and not on the pain she is experiencing. As mentioned previously, food pad dermatitis develops from contact with wet litter and manure, so the more time the hens spent feeding, the higher the likelihood they would develop foot pad dermatitis. Foot pad dermatitis can be associated with swelling and also stimulates the release of inflammatory cytokines; however, the pain associated with this response was not associated with reduced feeding behavior. This is an example of behavior influencing the body condition. However, the development of foot pad dermatitis appears to not have induced a situation painful enough to impact feeding behavior or override the motivation to feed.

Claw length at 19 wk was negatively associated with feeding behavior at 28, 48, and 66 wk. The connection between claw length and feeding behavior is unclear, and could be due to the hen's choice to engage in feeding from the feeder rather than foraging, which would involve scratching the claw on an abrasive surface. Yet the ability of the 19 wk claw length measurement to be consistently associated with feeding behavior throughout the hens' lay cycle illustrates how strong early life conditions and experiences can resonate throughout a hen's life. A negative association was observed between claw length and walking across all ages (Figure 9). Again, it is unclear as to whether hens with longer claws walked less because longer claws impacted their mobility or if hens had shorter claws because they engaged in more walking behavior. Regardless of which came first, the trend was consistent throughout the hen's lay cycle and persisted regardless of when the claw was measured.

Lower comb pecking scores at 66 wk could be associated with increased variability in nest box use at 66 wk. The hens may have stabilized their social and spatial distribution and therefore did not need to engage in agonistic behaviors. However, a negative relationship between comb score and dust bathing behavior was observed. Hens are highly motivated to dust bathe and will work at access substrate for dust bathing (Olsson & Keeling 2005). As dust bathing is a highly social behavior, hens that were socially subordinate may have been prevented from accessing dust bathing substrate or were disrupted during a dust bathing bout due to an agonistic interaction potentially resulting in a comb pecking wound.

Flocks with higher levels of activity are at a greater risk of having higher feather damage scores (Lee, et al. 2011), and hens from a high feather pecking line were more active than hens from a low feather pecking line (Kjaer 2009). These increased levels of activity associated with feather pecking may explain why higher levels of walking at 48 wk were associated with higher

plumage scores at 48 wk. However, higher plumage scores at 48 wk were associated with less time spent walking at 28 wk. This could be due to hens becoming subjected to feather pecking at 28 wk and those that were more stationary were victimized by individuals performing feather pecking because they were easy to access. However, the inverse relationship between plumage damage score and walking behavior at 28 wk and 48 wk could be interpreted as the hens choosing to make behavioral adjustments in their walking behavior and activity levels as to adapt to the persistent feather pecking behavior within the flock.

Hens with higher keel bone scores at 19 wk spent more time utilizing the nest box at 28 wk. As discussed previously, keel bone injury can potentially be painful, and the nest box is an isolated location for a hen with an injury to separate itself from the stochastic dynamic of the room. This suggests that the hens were using the nest box for reasons other than egg laying, and this occurrence has also been observed in other studies (Carmichael, et al. 1999, Channing, et al. 2001). The relationships observed from this study are strictly correlative. Therefore, concrete relationships were unable to be determined and would benefit from future study. Here, speculation surrounding these different relationships was presented to provide a launching pad for future investigation.

CONCLUSION

Consideration should be made with regard to the differences in amount of time required for different WQ parameters to change. An outbreak of feather pecking, an aggressive interaction, or a crash landing could respectively change the hen's feather condition, comb pecking or keel score within the same day. However, it may take several days for the

environmental conditions or hen choices to have an impact on physical parameters such as body weight and foot pad dermatitis. Therefore, some WQ parameters may be better suited to providing an assessment that provides a picture of the animal's state over a longer period of time.

Welfare assessment protocols must take a group approach due to practical constraints such as time, resources and the need to handle each individual. However, if some of the individuals assessed are found to have a high level of welfare, then the potential for animals to have a high level of welfare is present. And of course, the converse would be true if animals with low levels of welfare are found. Further, physical and management environments may provide an opportunity for welfare to be high, but we cannot guarantee good welfare for all animals in the environment without assessing all of the individuals housed within.

Essentially, animal welfare assessment programs that provide information at the group level are reporting either the average welfare of the group in that housing system, or the potential level of welfare the animals could have in that housing system. Group level welfare can give us an idea of what we should expect to find from the average individual in that group. Assessing welfare at the group level gives no insight into the welfare of the individual – and it can help us to make comparisons across groups as to whether the potential for good welfare is higher or lower among different groups.

Therefore, animal welfare assessment schemes should begin to promote the concept of *Animal Welfare Potential* that provides the average and the range of parameters observed to the farmer. This would provide useful information about the welfare state of the animals within while using language that would provide transparency about how all of the animals are performing, not just how the average animals are performing. These changes could identify

facilities where the potential for the animals to experience a high level of welfare is high, even though not all of the hens housed within have a guarantee of good welfare due to factors they impose upon themselves.

APPENDIX

Table 4.1: Ethogram of behaviors developed to identify posture, behavior and resource use. A hen was considered to be performing a new posture, behavior, or resource use when she stopped performing the previous behavior for >5 s or began performing a new behavior for > 5 s.

<i>Posture</i>	Description
walk	Walking more than 3 steps in succession with head up or when walking hen has not been standing, drinking, feeding, or foraging in litter for the previous 5 s
stand	Hen is upright and supported off of the ground or perch by legs
sit	Hen is upright with body touching the ground or perch
<i>Behavior</i>	
feed	Hen pecks at feed in the feeder. Recording starts at first peck
drink	Head is turned upwards towards water source, and hen uses beak to peck at one of the nipples, apparently consuming water
preen	Hen may be sitting or standing. Beak is used to manipulate, rearrange, pull, or smooth body feathers on self. Bill is often run along the length of the feather, starting at the base and moving out toward the tip of the feather
dust bathe	While squatting or lying, hen performs dust bathing activities including vertical wing shaking, bill raking, scratching, ground pecking, movements of the feet and wings to raise dust into the ruffled plumage, rubbing of head and sides in the dust, feather-ruffling and shaking dust out of the feathers. Starts with first wing shake
forage	Hen pecks at substrate while standing or stepping forward with head below rump level. Starts when hen makes >3 successive pecks at substrate, or when foraging hen has not been standing or walking with head up, or feeding, for the previous 5 s
rest	Starts when hen lies down (sternum resting on substrate) from an upright position or when lying bird has made no dust bathing or preening movements for the previous 8 s
<i>Resource</i>	
feeder	Hen has head in feeder and pecks at feed in the feeder
drinker	Head is turned upwards towards water source, and hen uses beak to peck at one of the nipples, apparently consuming water
perch	Hen is standing, walking, or resting on perch, the rail in front of the nest boxes, or black base of slats underneath raised perches
nest box	Hen is standing or resting inside a nest box

Table 4.2: Physical parameters measured as part of the Welfare Quality[®] (2009) assessment program for poultry.

Physical parameter ¹	Description
Body weight	Mass of hen (kg)
Claw length	Length from cuticle to tip of middle right claw (cm)
Comb pecking wound	0 – no evidence of pecking wounds 1 – fewer than three pecking wounds 2 – more than three pecking wounds <i>Three areas of body (head/neck, back/rump, belly) are given a score. For each body part a score is given on a 3-point scale</i> A – No or slight wear, (nearly) complete feathering (only single feathers lacking) B – Moderate wear, i.e. damaged feathers (worn, deformed) or ≥ 1 feathers-less areas $< 5\text{cm}$ in diameter C – > 1 featherless area $\geq 5\text{cm}$ in diameter at the largest extent
Plumage damage score	<i>Combine body part scores into single general score per bird</i> 0 – all body parts have score ‘a’ 1 – ≥ 1 body parts have score ‘b’, but no body part has score ‘c’ 2 – ≥ 1 body parts have score ‘c’
Keel bone score	0 – no deviations, deformations or thickened sections, keel bone completely straight 2 – deviation or deformation of keel bone (including thickened sections) observed
Foot health score	0 – feet intact, no or minimal proliferation of epithelium 1 – necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling 2 – swollen (dorsally visible)

¹Welfare Quality[®] assessment program for poultry (broilers, laying hens)

Table 4.3: Associations between WQ parameters measured at 19 wk of age and time performing behaviors at 19, 28, 48, and 66 wk of age.

19 wk WQ	Age (wk)	Behavior	R₁₅	P value
body weight	48	sit	0.512	0.051
claw length	28	stand	-0.708	0.003
	28	walk	-0.522	0.046
	28	feed	-0.692	0.004
	48	feed	-0.579	0.024
	66	feed	-0.63	0.012
	19	drink	-0.706	0.003
foot health	No significant associations observed			
plumage damage	66	stand	-0.614	0.015
	66	sit	0.784	0.001
	28	walk	-0.558	0.031
	66	feed	-0.527	0.044
comb pecking	48	preen	0.544	0.036
keel bone	28	sit	0.753	0.001
	19	feed	-0.567	0.028
	28	nest box	0.646	0.009

Table 4.4: Associations between WQ parameters measured at 28 wk of age and time performing behaviors at 19, 28, 48, and 66 wk of age.

28 wk WQ	Age (wk)	Behavior	R₁₅	P value
body weight	No significant associations observed			
claw length	28	stand	-0.753	0.001
	66	walk	-0.562	0.029
	28	feed	-0.661	0.007
foot health	28	sit	-0.55	0.034
	19	drink	0.668	0.007
	28	drink	0.586	0.022
	28	dust bathe	-0.596	0.019
	28	rest	-0.5	0.058
	48	feed	0.517	0.049
	66	feed	0.702	0.004
	66	drink	0.541	0.037
plumage damage	No significant associations observed			
comb pecking	28	rest	-0.595	0.019
	48	rest	-0.741	0.002
	66	dust bathe	-0.581	0.023
	66	forage	-0.687	0.005
keel bone	No significant associations observed			

Table 4.5: Associations between WQ parameters measured at 48 wk of age and time performing behaviors at 19, 28, 48, and 66 wk of age.

48 wk WQ	Age (wk)	Behavior	R	P value
body weight	48	stand	-0.659	0.008
	48	walk	0.609	0.016
	48	preen	0.52	0.047
claw length	66	preen	0.625	0.013
	28	walk	-0.917	0.014
	66	walk	-0.687	0.005
	28	stand	-0.709	0.003
	19	preen	-0.687	0.005
	28	dust bathe	0.544	0.036
	28	forage*	-0.659	0.01
foot health	19	sit	-0.584	0.022
	19	feed	0.606	0.017
	19	perch	-0.791	0
	28	forage*	-0.761	0.002
plumage damage	19	sit	-0.655	0.008
	48	stand	-0.679	0.005
	28	walk	-0.6	0.018
	48	walk	0.511	0.052
	19	rest	-0.65	0.009
comb pecking	48	forage	0.506	0.054
	66	drink	-0.571	0.026
	66	dust bathe	-0.75	0.001
	19	preen	0.557	0.031
keel bone	66	stand	-0.552	0.033
	28	walk	-0.57	0.027
	28	feed	-0.51	0.052
	66	feed	-0.631	0.012
	48	rest	0.609	0.016
	28	nest box	0.549	0.034
	66	perch	0.519	0.047

*sample size n = 14. Otherwise, n = 15

Table 4.6: Associations between WQ parameters measured at 66 wk of age and time performing behaviors at 19, 28, 48, and 66 wk of age.

66 wk WQ	Age (wk)	Behavior	R	P value
body weight	48	sit	0.516	0.049
	66	preen	0.733	0.002
claw length	28	stand	-0.723	0.002
	28	walk	-0.649	0.009
	66	walk	-0.648	0.009
	28	feed	-0.501	0.057
	66	feed	-0.523	0.046
	19	drink	-0.662	0.007
	28	drink	-0.706	0.003
	28	dust bathe	0.606	0.017
	28	forage*	-0.538	0.047
	66	rest	0.613	0.015
foot health	48	forage	-0.692	0.004
plumage damage	48	stand	-0.665	0.007
	48	sit	0.577	0.024
	48	preen	0.598	0.039
	48	forage	0.659	0.008
	66	forage	0.506	0.054
comb pecking	66	stand	-0.551	0.033
	66	sit	0.509	0.053
	48	dust bathe	-0.704	0.003
	66	dust bathe	-0.657	0.008
keel bone	48	feed	-0.725	0.002
	66	feed	-0.821	0
	19	dust bathe	-0.506	0.054

*sample size n = 14. Otherwise, n = 15

Figure 4.1: Boxplots of (a) standing, (b) sitting, (c) walking, (d) feeding, (e) drinking, (f) preening, (g) dust bathing, (h) foraging, (i) resting, (j) nest box use, and (k) perch use for non-cage laying hens at four different ages throughout the lay cycle. Mean differences ($P < 0.05$) are indicated with lowercase letters. Differences among standard deviations ($P < 0.05$) are indicated with symbols.

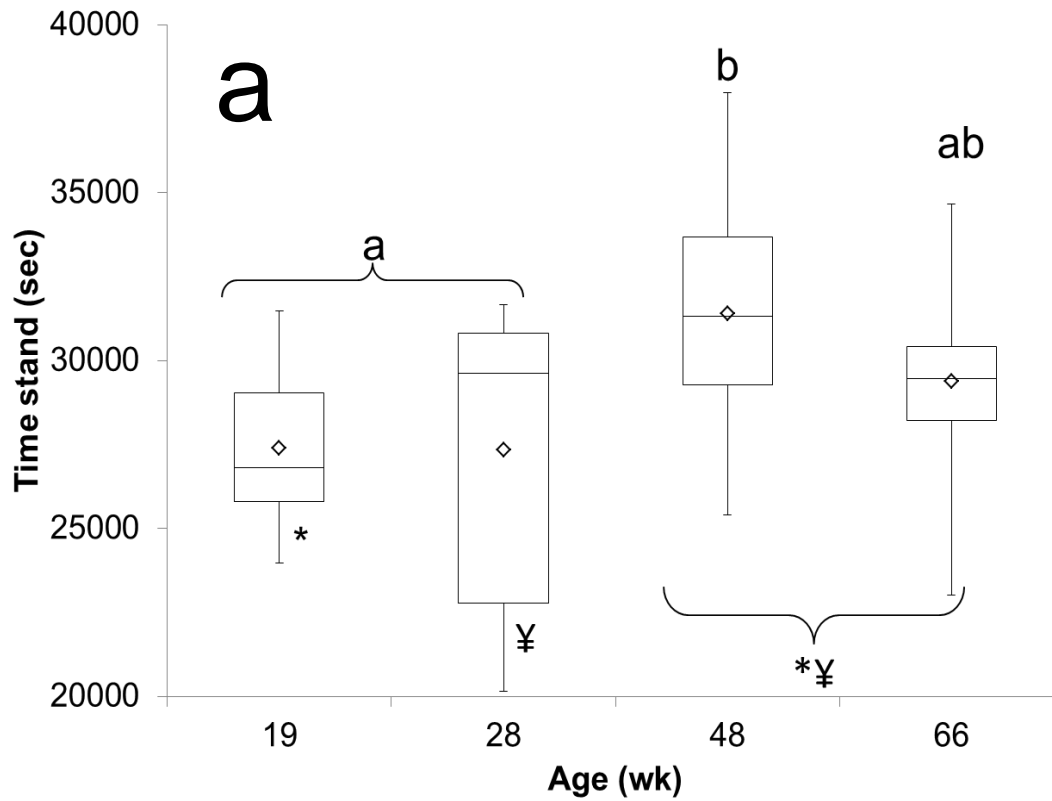


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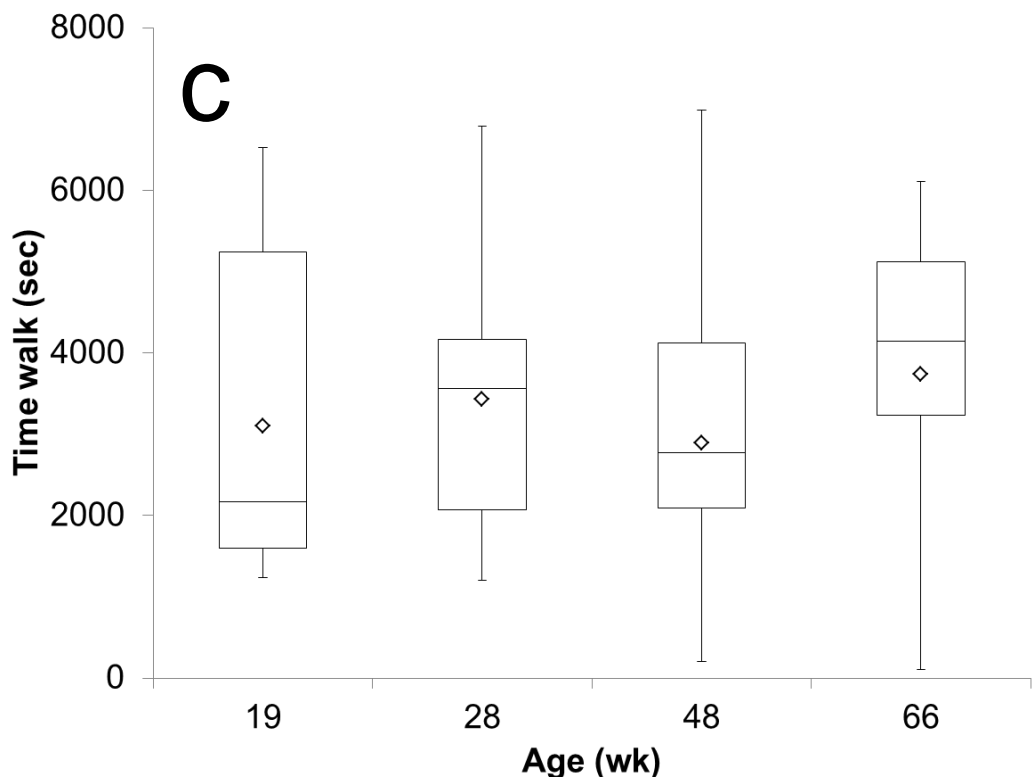
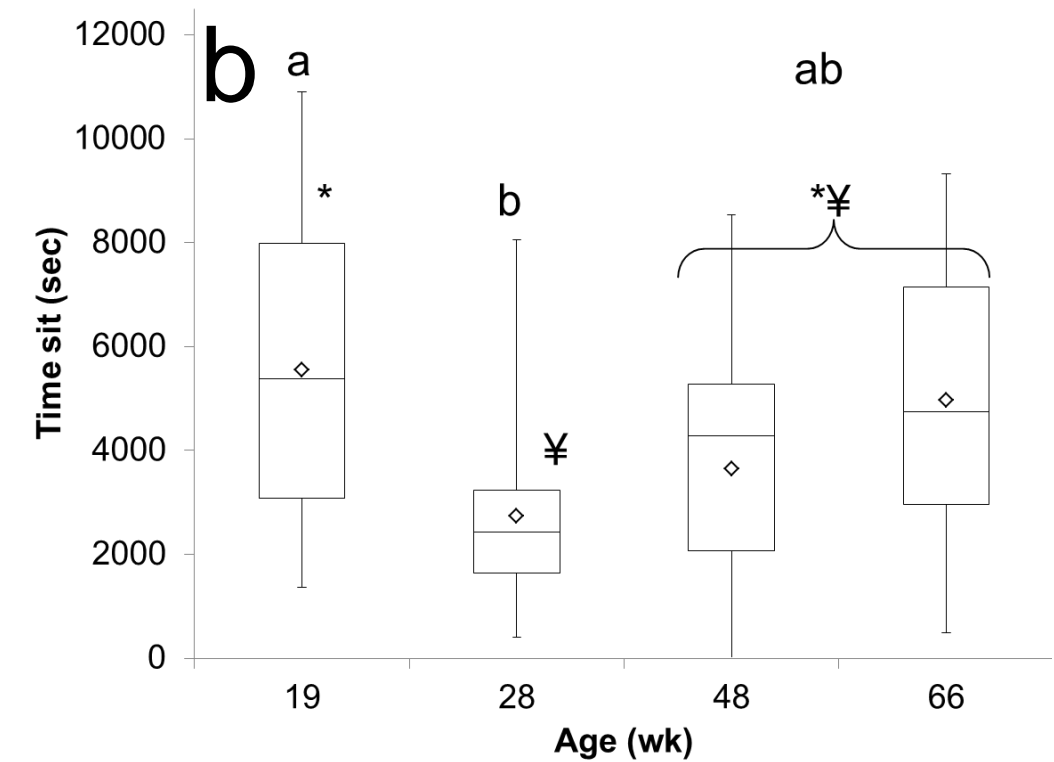


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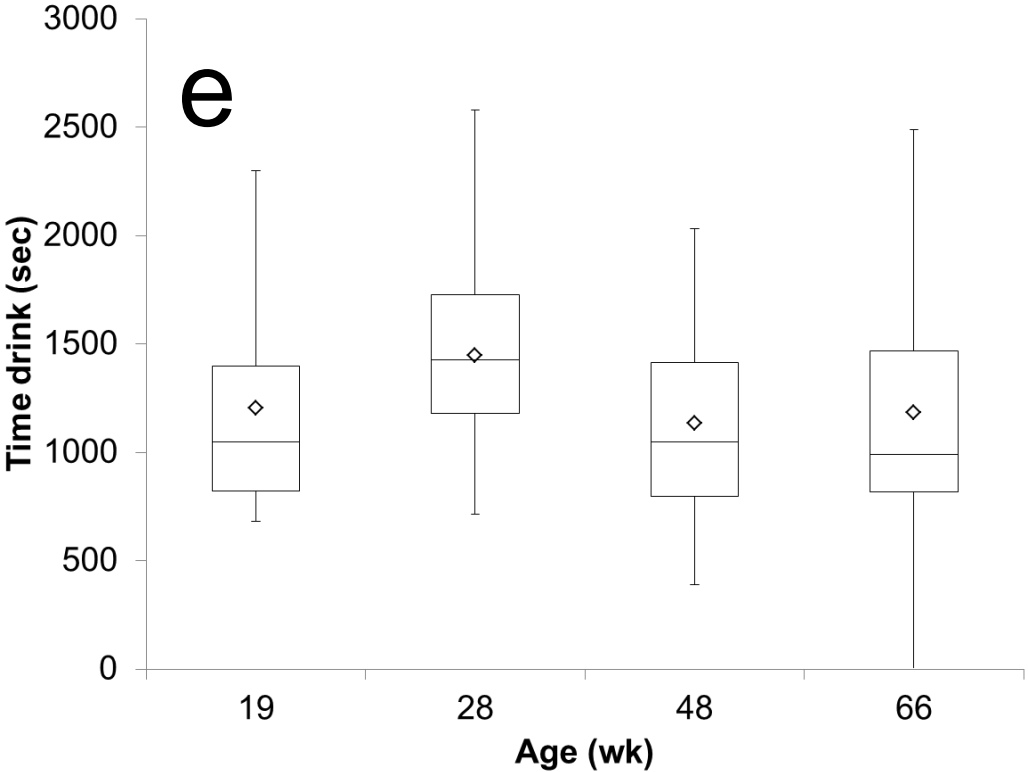
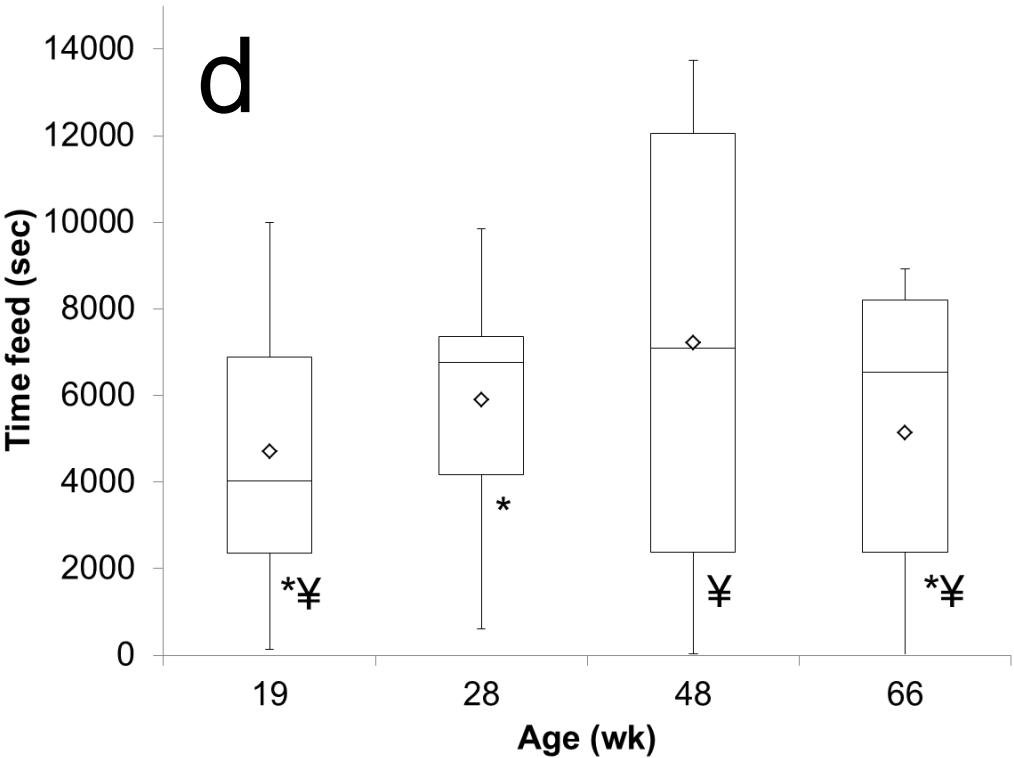


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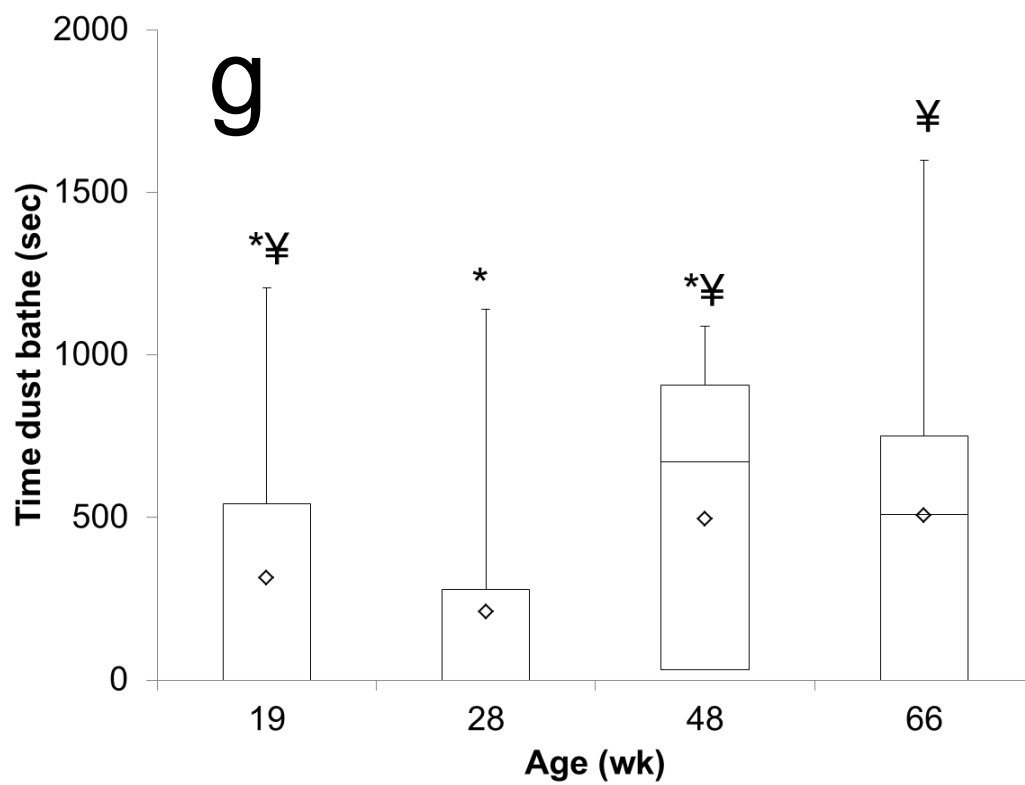
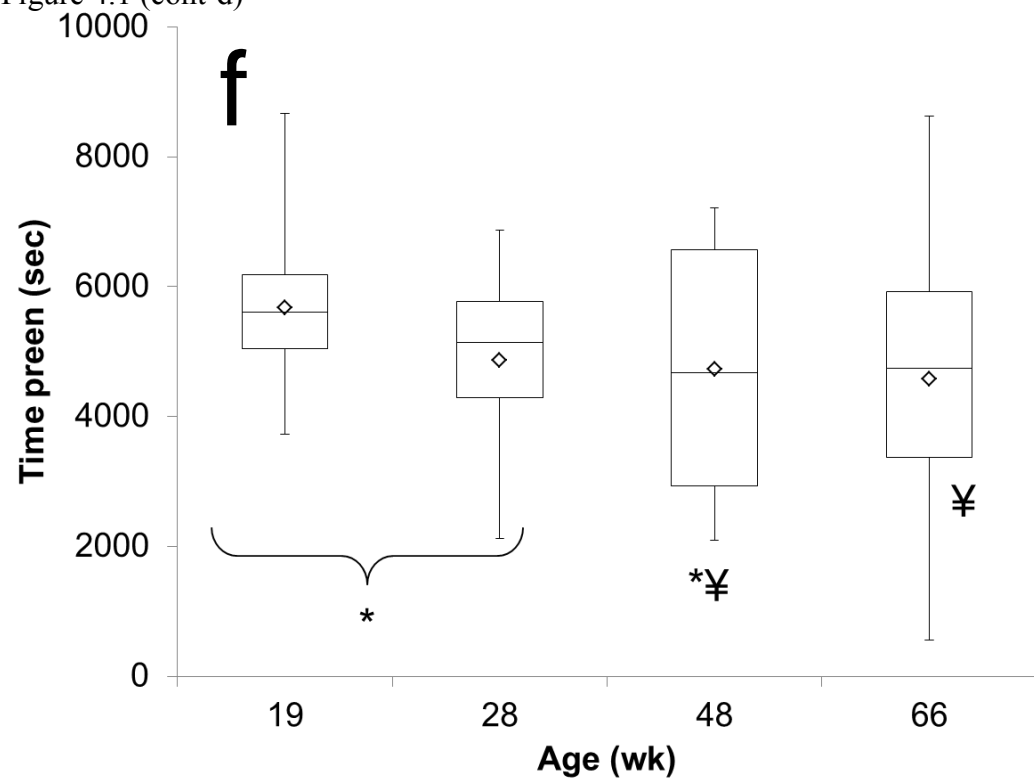


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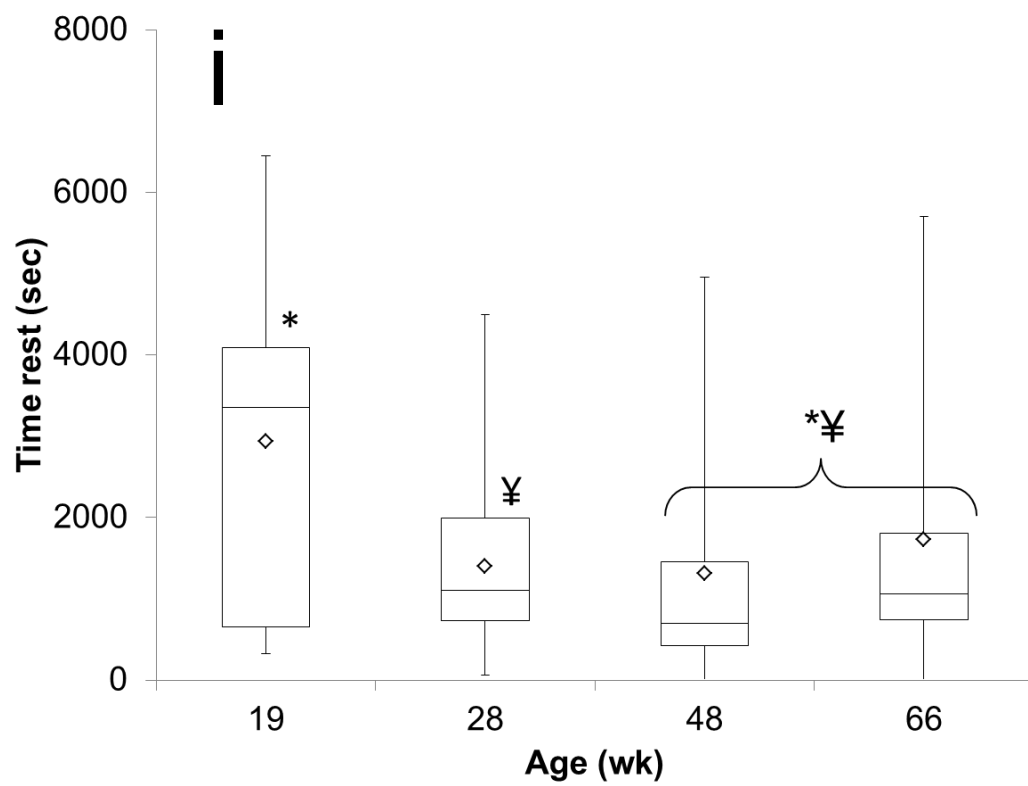
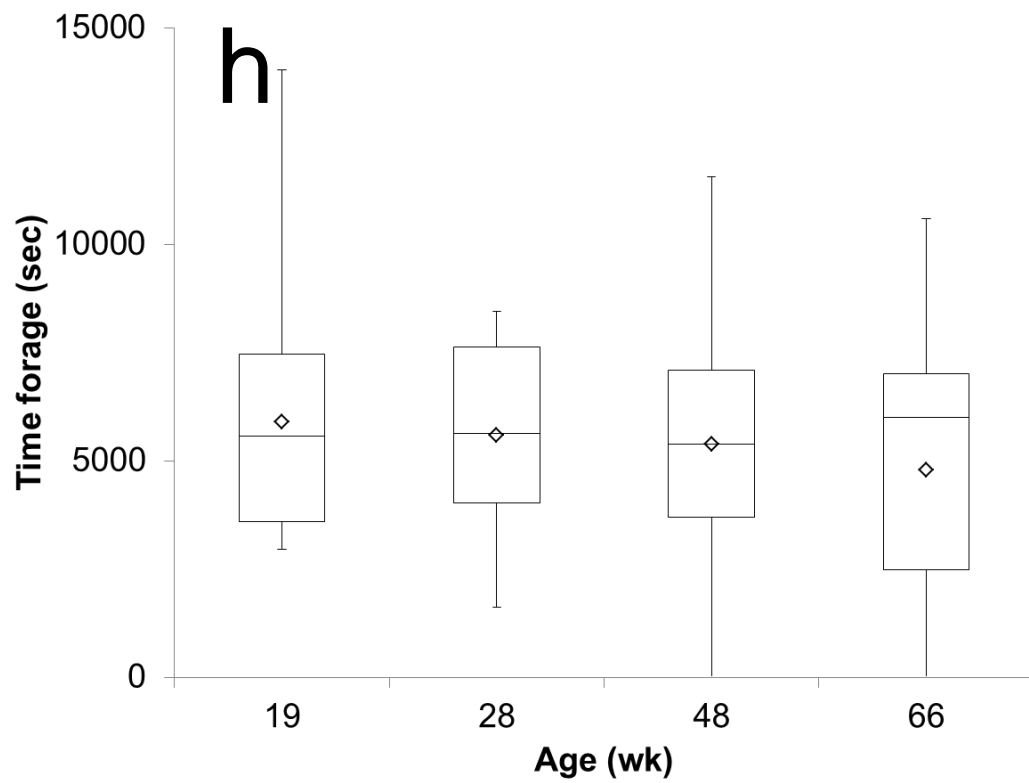


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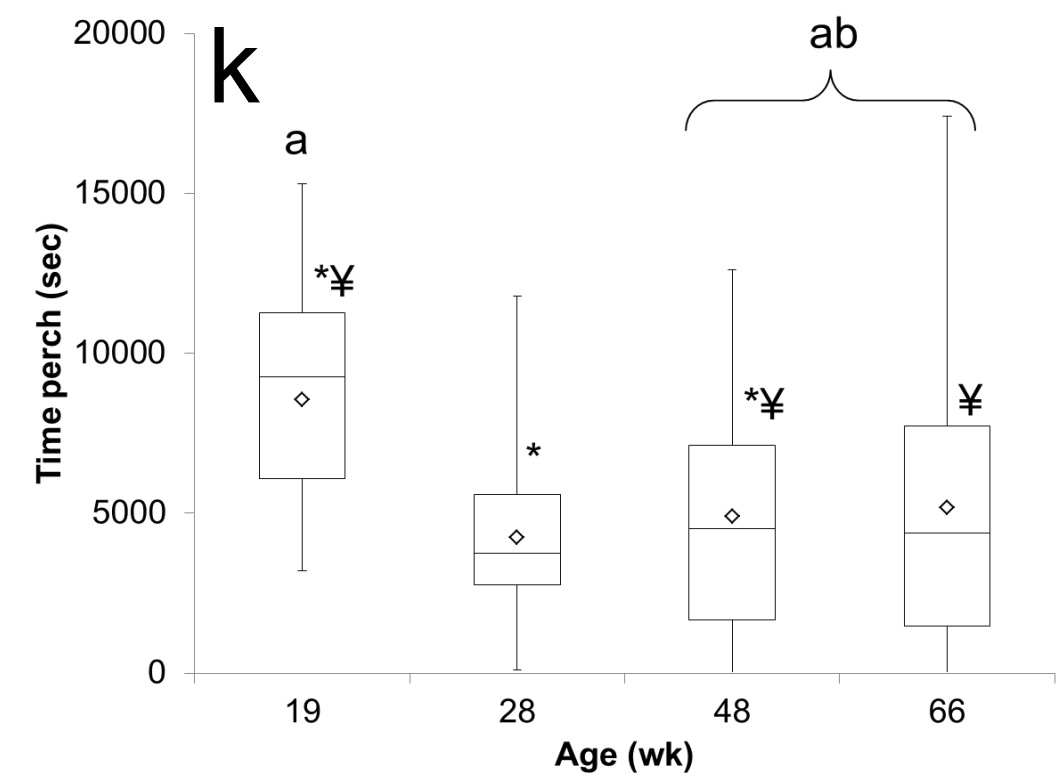
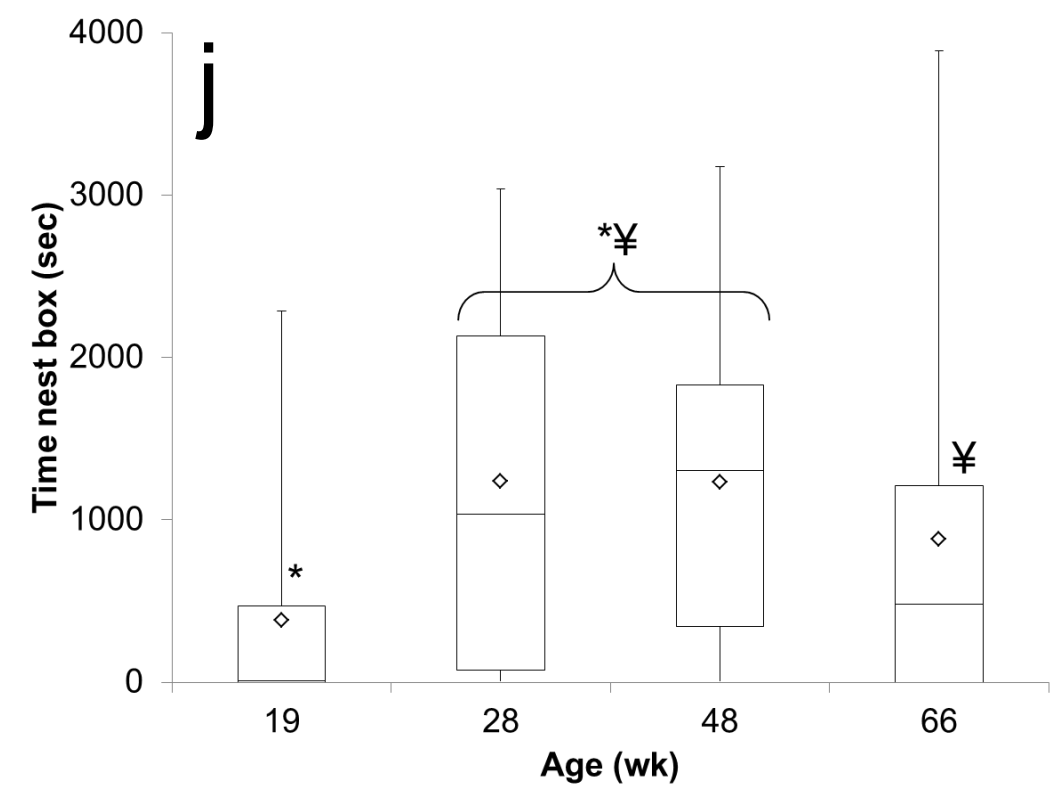


Figure 4.2: Boxplots of (a) body weight, (b) claw length, (c) plumage damage score, (d) comb pecking score, (e) keel bone score, and (f) foot health score from the Welfare Quality[®] assessment protocol for non-cage laying hens at four different ages throughout the lay cycle. Mean differences ($P < 0.05$) are indicated with lowercase letters. Differences among standard deviations ($P < 0.05$) are indicated with symbols.

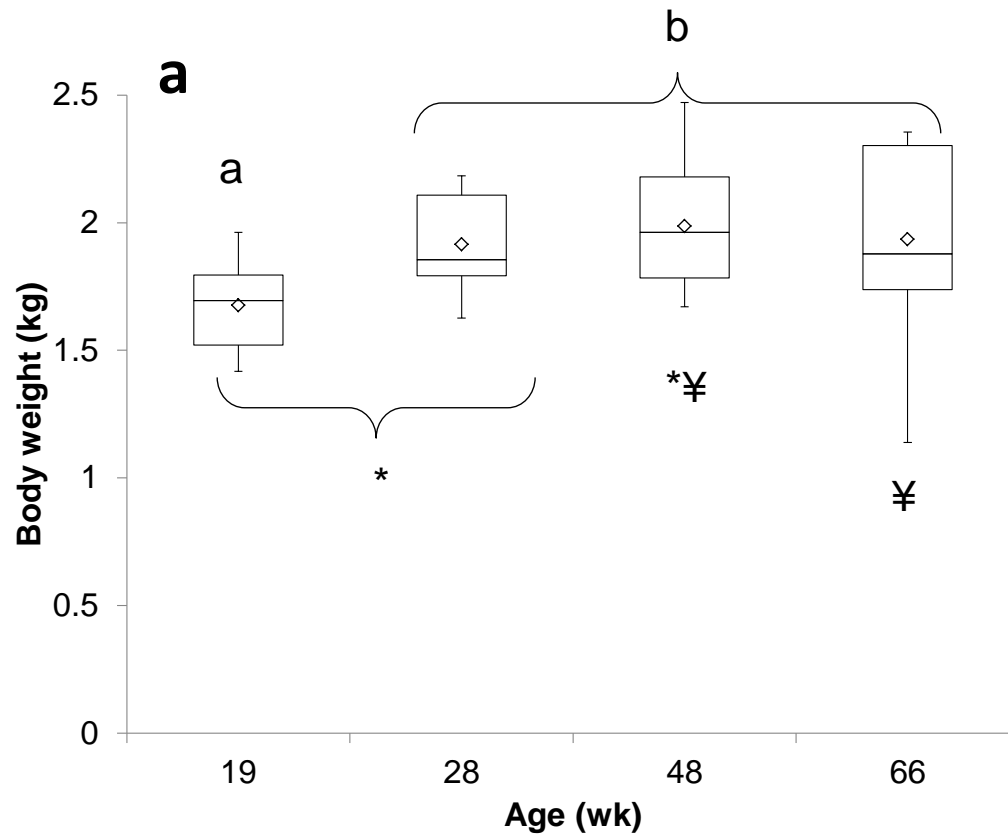


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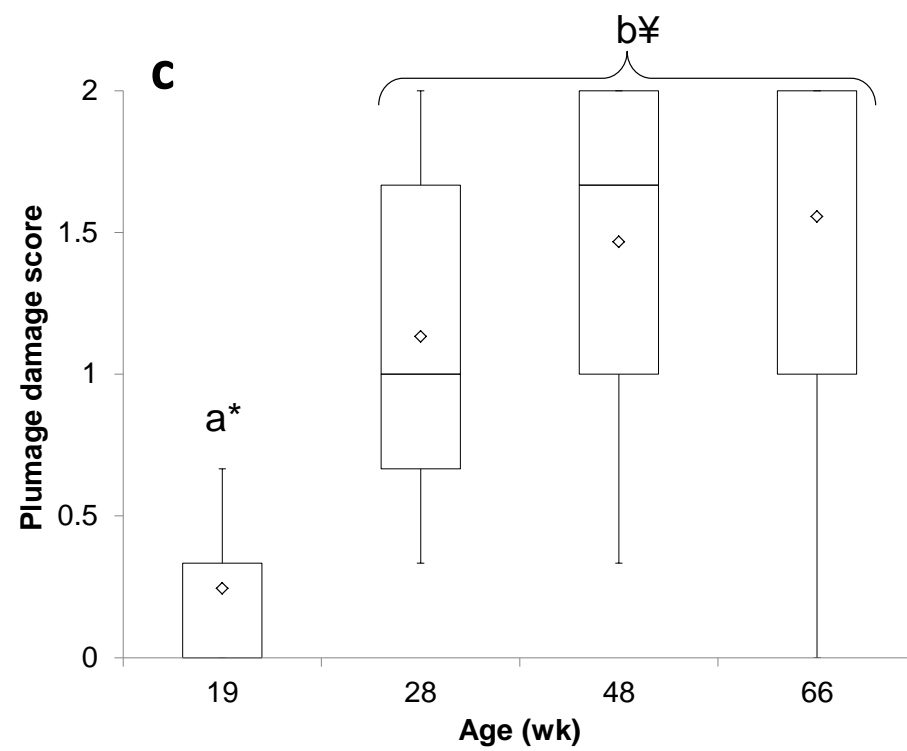
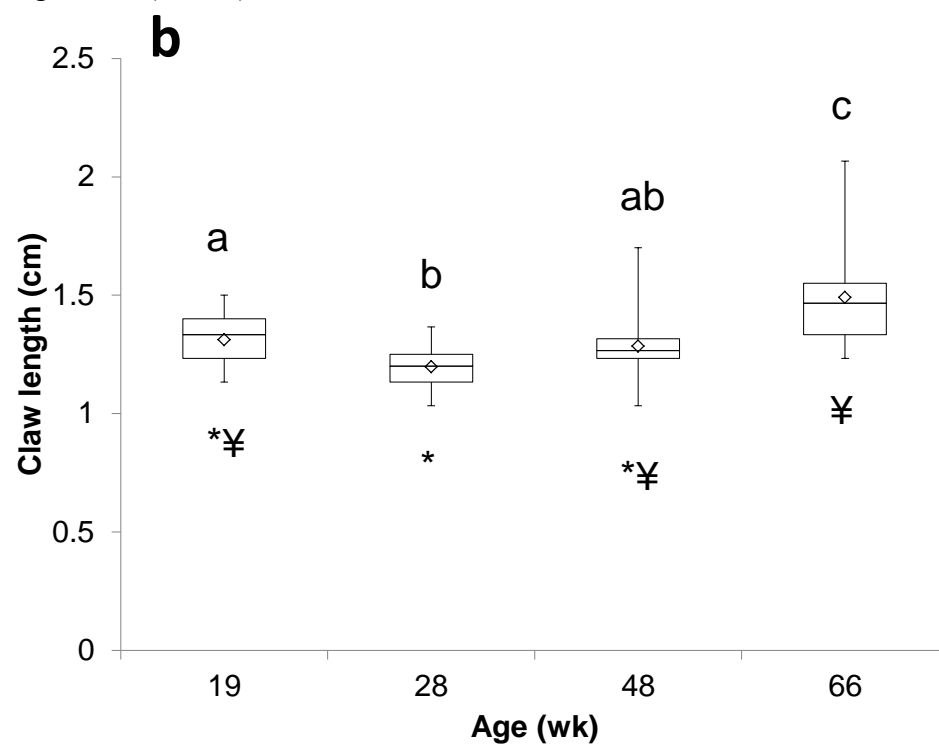


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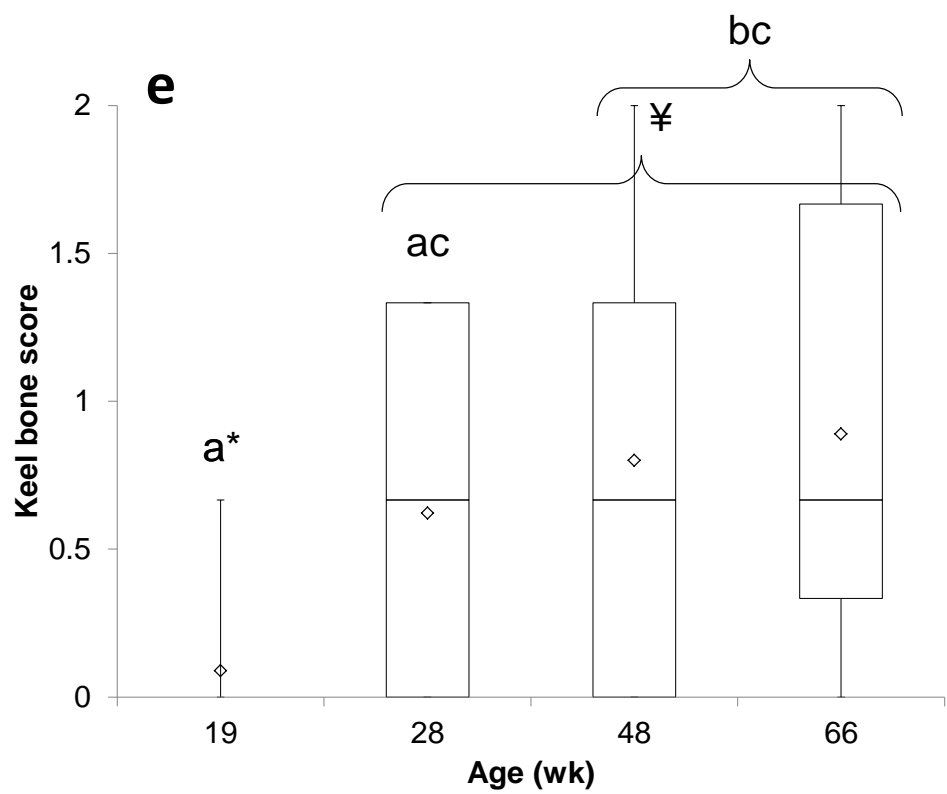
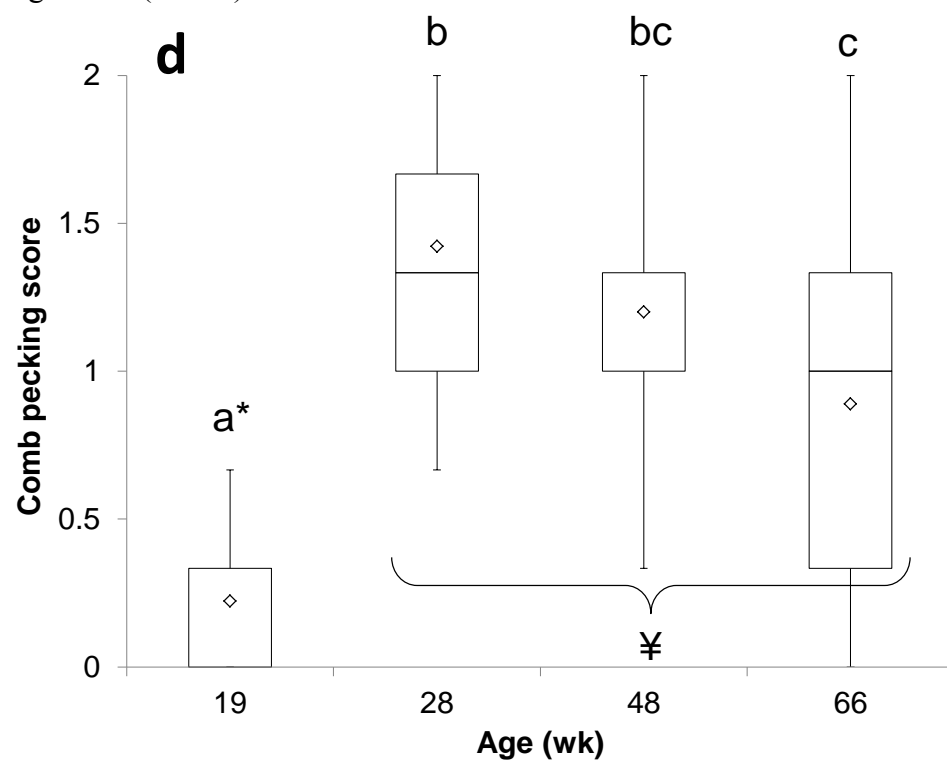


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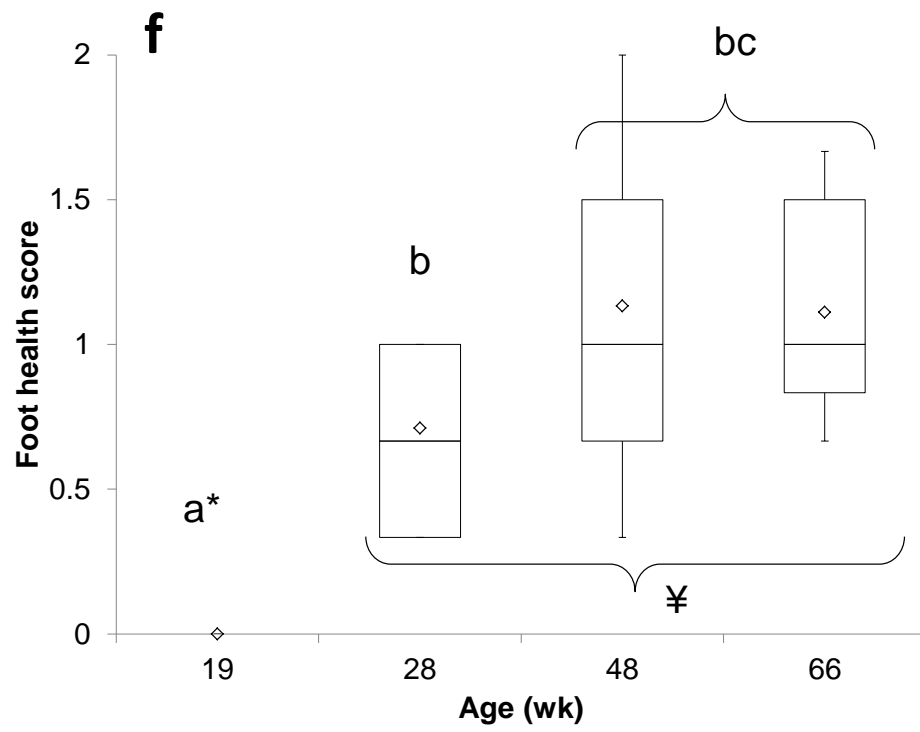


Figure 4.3: Scatterplot illustrating relationships between claw length measured at 19 wk and the amount of time the hen spent feeding at 28, 48, and 66 wk.

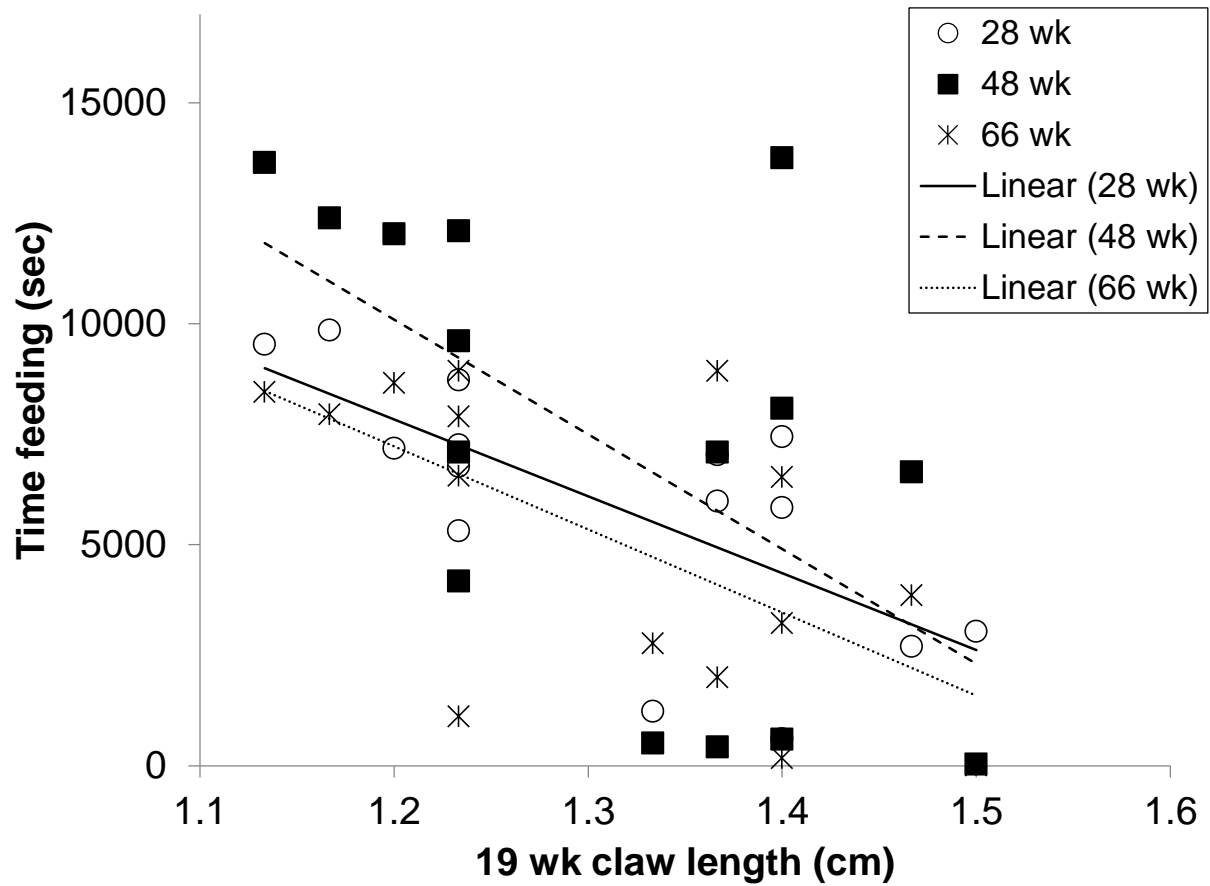


Figure 4.4: Scatterplot illustrating the relationship between foot health score at 28 wk and amount of time spent feeding at 48 and 66 wk.

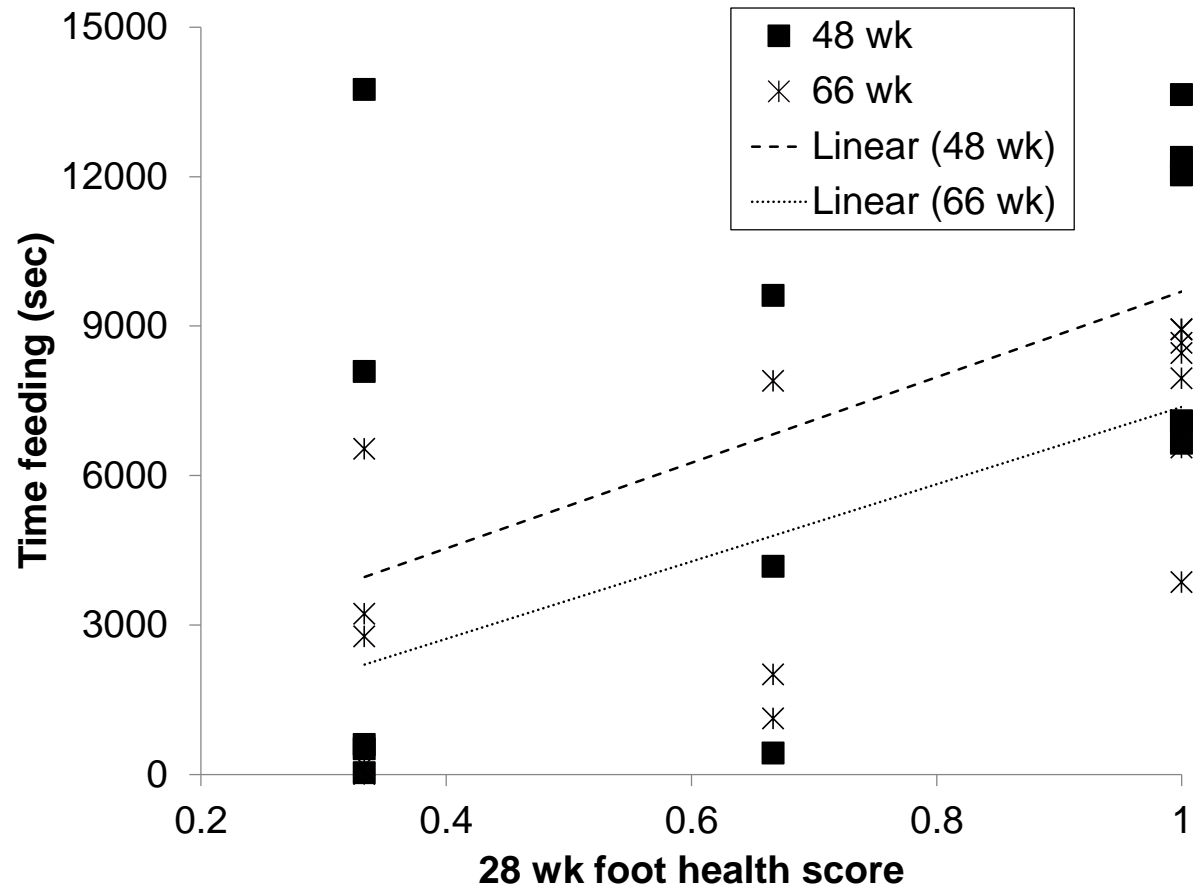


Figure 4.5: Scatterplot illustrating the relationship between foot health at 48 wk and amount of time using perch at 19 wk.

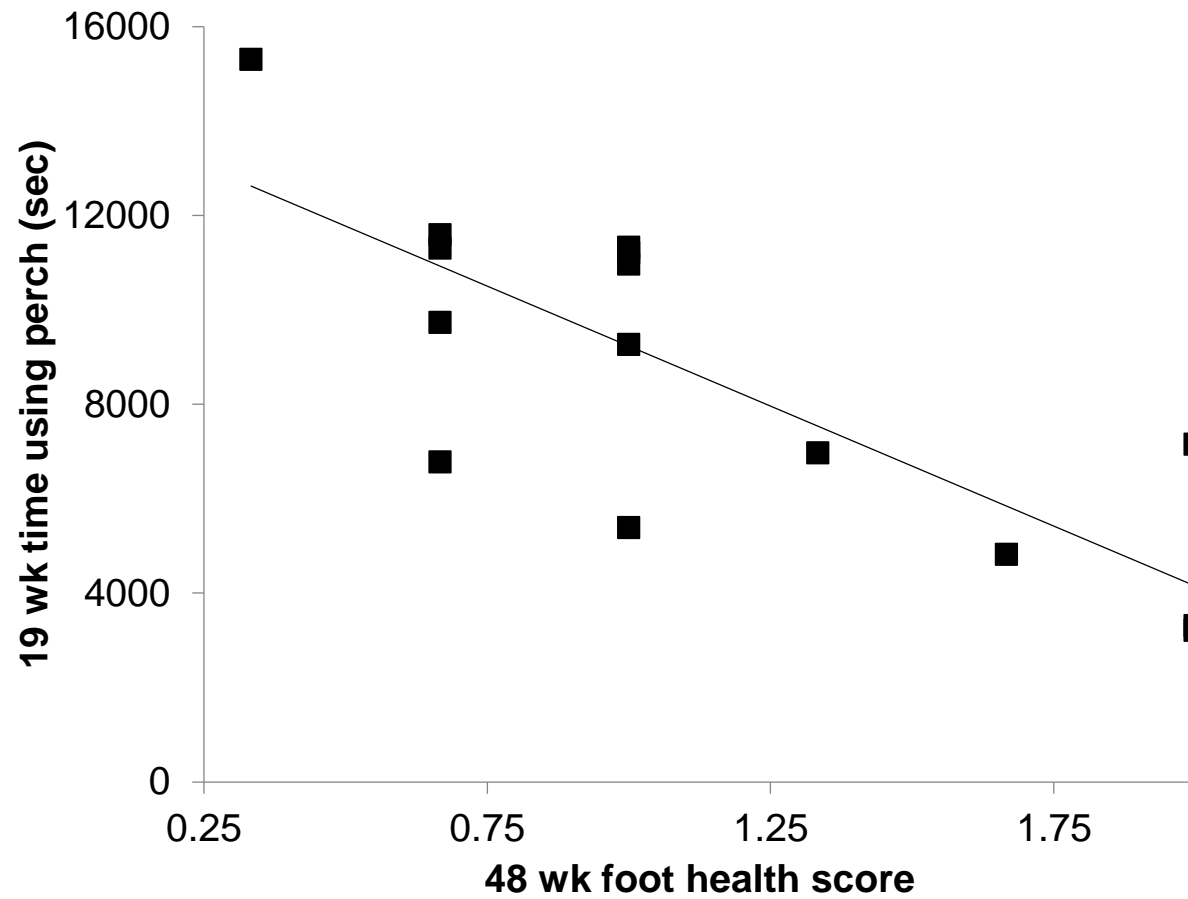


Figure 4.6: Keel bone scores for four randomly selected individual hens throughout the lay cycle.

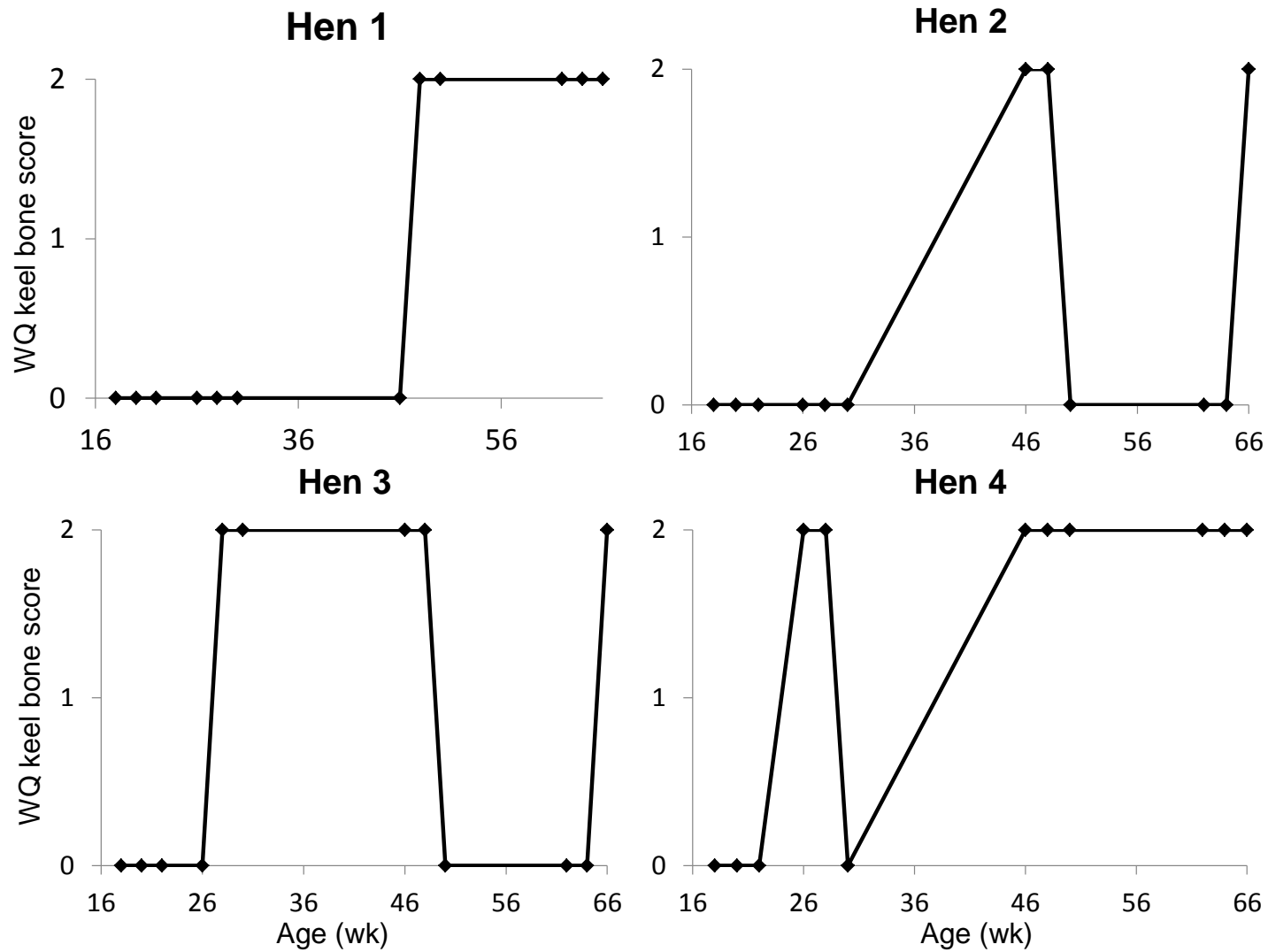


Figure 4.7: Foot pad dermatitis scores for four randomly selected individual hens throughout the lay cycle.

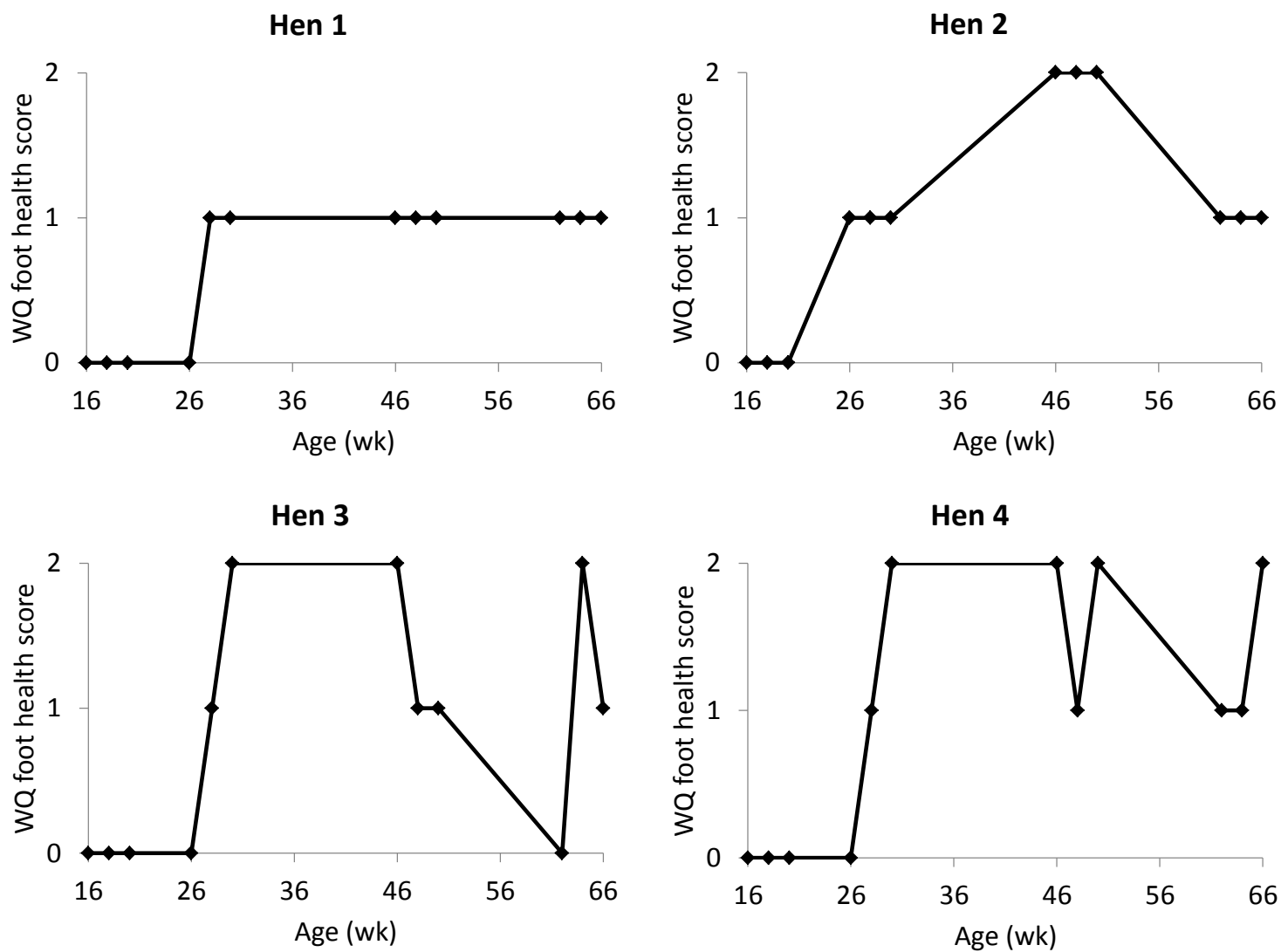


Figure 4.8: Scatterplots of keel bone score at 19wk, 48wk, and 66wk with relation to the duration of time spent feeding throughout the lay cycle.

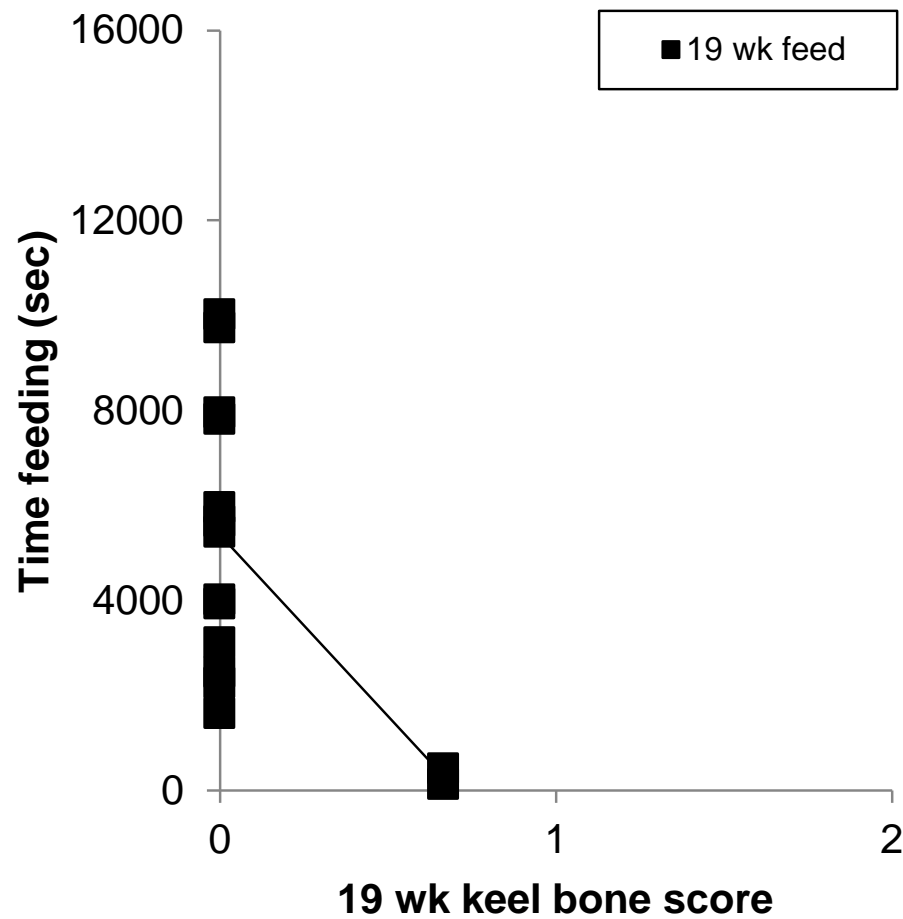


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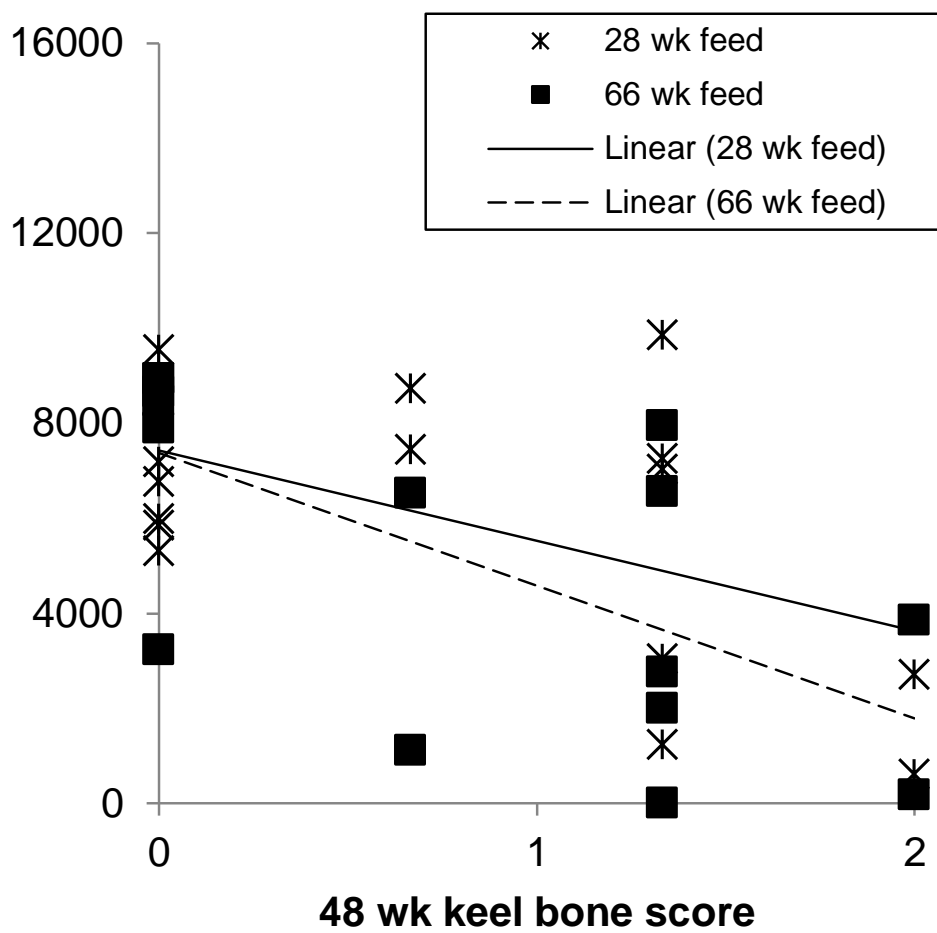


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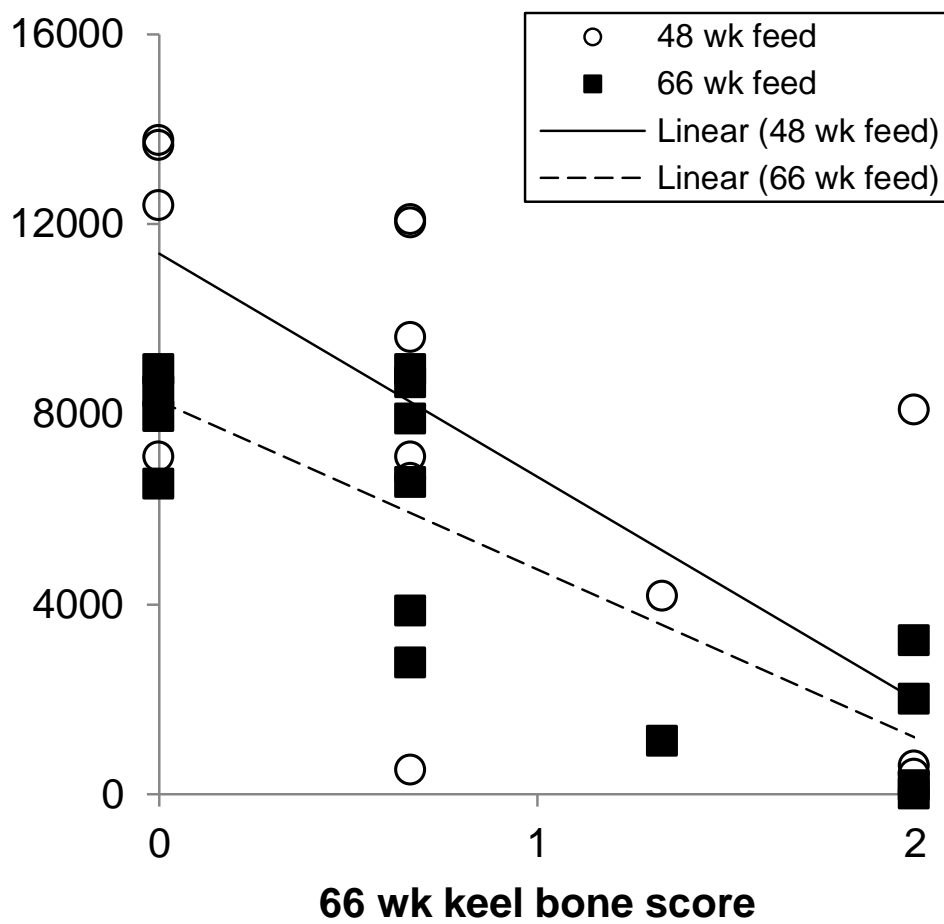


Figure 4.9: Scatterplots of claw length measurements at 19, 28, 48, and 66wk with relation to the duration of time spent walking throughout the lay cycle.

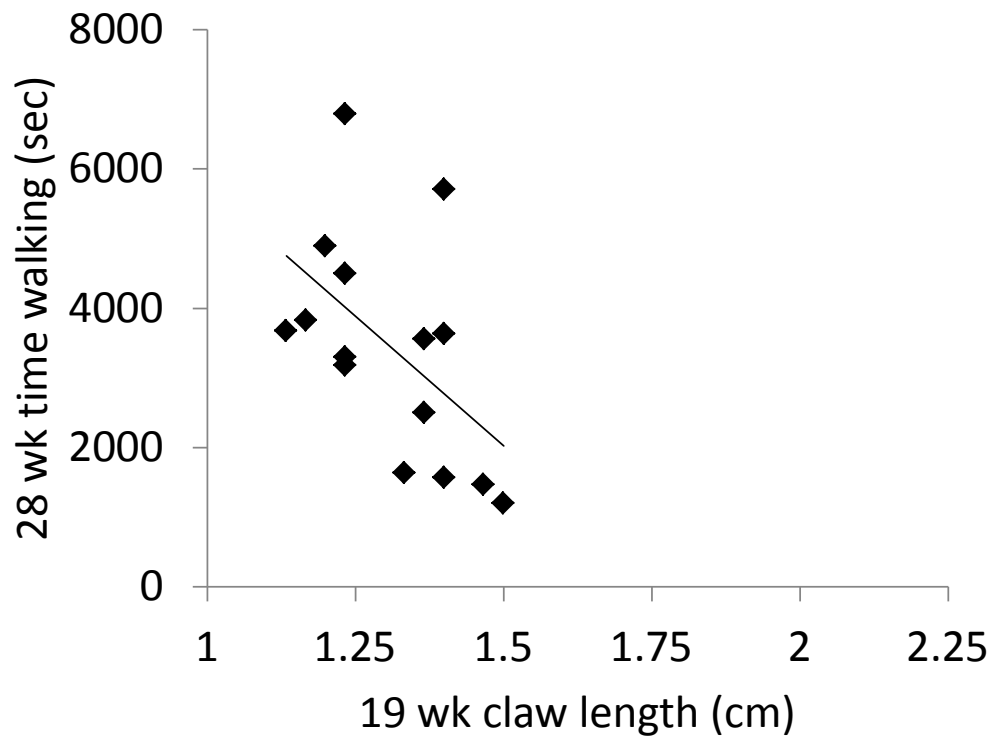


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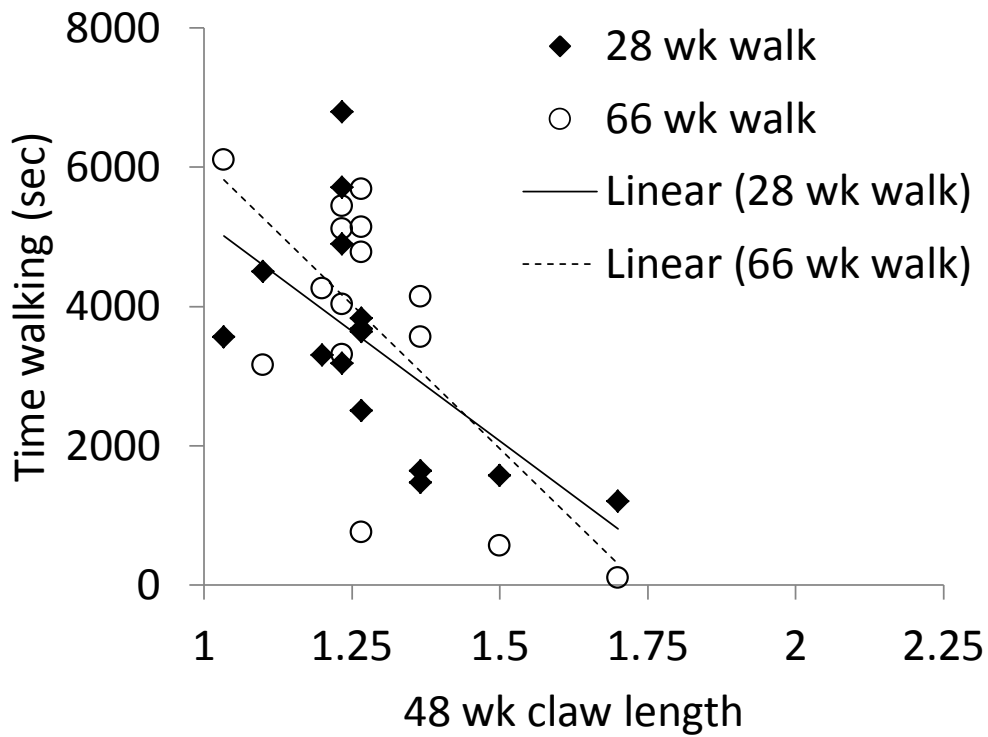
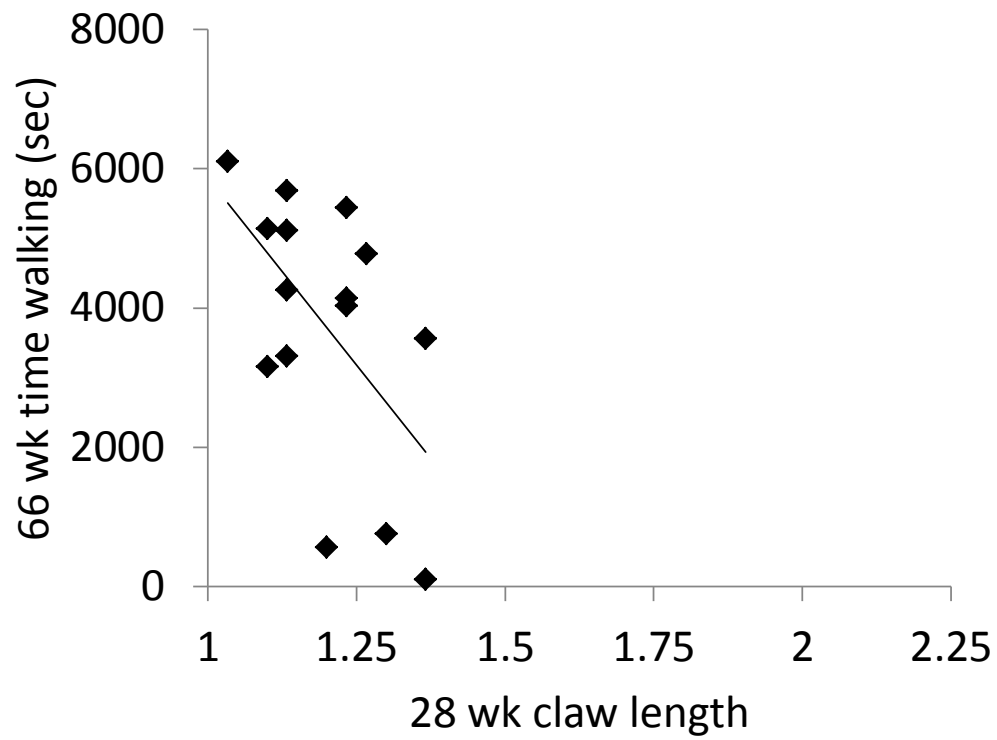
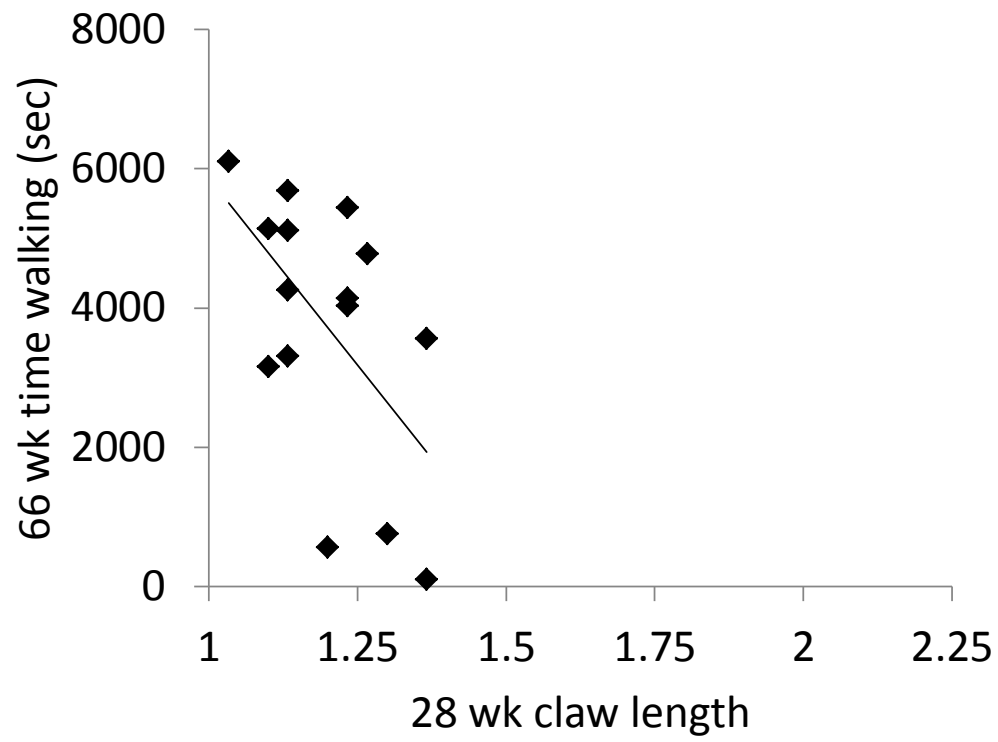


Figure 4.9 (cont'd)



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CHAPTER 5: INTEGRATING WIRELESS SENSOR TECHNOLOGY WITH NON CAGE HEN BEHAVIOR TO CREATE A SPATIOTEMPORAL REPRESENTATION OF SPACE USE WITH REGARDS TO WELFARE

ABSTRACT

A proof of concept approach for applying wildlife ecology techniques to welfare science in an agricultural environment was utilized with egg-laying chickens. A wireless body-worn sensor system was used to track the location of nine individual laying hens in a non-cage environment at two different ages. The output from this sensor system was collated with behavioral observation taken from video recorded at the same time, and both data were collated into Geographic Information System (GIS) to develop a spatiotemporal representation of hen behavior and space use. Home ranges, utilization distributions, hen movement overlap, and hotspot maps were created to illustrate hen behavior and space use. Diverse spatial and behavioral patterns were exhibited among different hens housed within the same environment, and some hens showed changes in their behavioral patterns across time. Here we show that hens, despite similar genetics and life experiences, exhibit diverse behavioral and spatial patterns. This proof of concept approach will allow us to better understand the welfare of individual animals housed in stochastic environments.

INTRODUCTION

Many of the fundamental concepts of animal welfare are rooted in an individual's response to a situation, thus the concept of animal welfare inherently implies the importance of the individual experience (Dawkins, 2003; Duncan and Mench, 1993; Fraser, 2008). Animal welfare is a subject of scientific study that encompasses the physical and psychological well-being of animals, measured with indicators including behavior, emotional state, physiology, health, and reproduction. Most theories of welfare apply to individuals because only individuals possess the characteristics (e.g., affective state, perception, needs, and motivation) that make lives better or worse; and individual variation with regards to genetics, experiences, and temperament can impact how the individual perceives its current situation, the choices it makes, and ultimately its welfare. The egg industry is transitioning from housing laying hens in groups of 6 – 10 hens in a single small conventional cage to housing hens in larger groups that can reach thousands in indoor non-cage environments. The hens housed in these alternative systems may face different welfare challenges from those historically faced by their conventionally caged counterparts. Yet, issues of practicality limit the ability of welfare assessors to gather behavior and resource use information on farms from individual hens. Therefore, measurements are usually taken at the group level. However, the average condition of the group of animals may not accurately reflect the condition of a specific individual animal as the condition of individuals within the same group may vary widely; and by using the average we may be ignoring the very poor welfare of individuals at the ends of the spectrum. Yet, by taking a multidisciplinary approach to implementing technological advancements we may be able to capture the ranges of conditions being experienced within the group.

Utilization distributions represent the probability distribution describing a collection of recorded spatial locations for an individual animal. Utilization distributions represent individual home ranges, habitat selection, and space use sharing across time and among different individuals, and are specifically defined as the probability distribution defining the animal's use of space (Fieberg et al., 2005; Winkle, 1975). Information about individual non-cage laying hens use their space is unknown. Furthermore, little is understood as to whether hens are consistent in their space and resource use patterns over time. Collecting individual spatial data is challenging as hens in non-cage systems are usually housed in groups that range from several hundred to several thousand and most of them are visually phenotypically similar. Furthermore, as laying hens are housed in groups that are larger than found in the wild, and larger than has been traditionally housed in commercial production, management approaches that incorporate concepts from wildlife behavioral ecology (e.g., optimal foraging theory, social network theory, kin recognition, sexual selection in breeding flocks, etc.) at the individual level should be incorporated into daily animal husbandry practices to enhance animal production and welfare. These techniques are needed because production animal systems are beginning to mirror large groups of animals found in natural environments and group housed hens in production environments may face similar challenges. Therefore management practices will need to accommodate these changes.

Feeding and foraging are appetitive behaviors that hens are motivated to perform and are required for survival (Nicol et al., 2011; Duncan and Wood-Gush, 1972a). Feeding behavior is defined as the hen consuming grain directly from the feeder. Hens are considered foraging when they use their feet to search the ground for food. When looking at hens in managed conditions, food is only provided at a feeder and therefore, the location of feeding behavior is restricted to

space in which a feeder is located. However, if multiple feeders are provided, it is unknown whether hens consistently use the same feeder; and some hens may choose to not use the feeder and acquire their nutrients from the feed that has been spilled on the ground by other hens. These hens may prefer to acquire their food via foraging, they may be thwarted from accessing the feeder due to social interactions, or they may forage due to inherent behavioral drives. Regardless of why the hens forage, this behavior still provides a nutritional reward but is not as spatially constrained by the presence or absence of a feeder.

Preening is a maintenance and can also be performed when hens are comfortable and in the presence of familiar conspecifics (Nicol, 1989). Preening is a grooming behaviour where the bird cleans and straightens its feathers with its beak. However, preening can also be considered part of a stress response (Williams et al., 1984) or be performed as a displacement behavior when hens are in frustrating situations (Duncan and Wood-Gush, 1972b). Preening can occur at any time of the day and is not constrained by posture (e.g., sitting or standing) or location (Mishra et al., 2005). Therefore, variations observed in preening behavior may be indicative of differences in health, maintenance, stress response, preference, or comfort among different hens. Changes in individual preening behavior may also highlight whether conditions for specific individuals have changed or whether welfare concerns have arisen.

A wireless body-worn sensor has been developed to track the location of individual non-cage laying hens. This sensor was attached to a single hen using a nylon figure-eight harness and communicated with a network of sensors located in strategic points throughout the hen's environment. Valuable welfare-relevant information can be collected at the individual level with this sensor. The sensor collected data on the hen's location (proximity to stationary sensor). Feeding, foraging, and preening behavioral observations were combined with locational data

produced from this sensor network – all of which are important in understanding welfare from a health and emotional perspective.

Behavioral data and sensor network output were collated in Geographic Information Systems (GIS) to construct a utilization distribution for each hen and provide spatiotemporal representation of her behavior and space use. This location and behavioral data were collected from the same hens at two different ages to identify relationships between their location and behavior and whether these patterns remained consistent over time. Specifically, these data were used to determine general ranging patterns within the non-cage environment, identify the size and amount of overlap in the home ranges, and identify whether there was spatial segregation by behavior. This project is the first integration of wireless sensor technology and GIS as applied to poultry in an indoor agricultural setting, the first characterization of individual hen home ranges in a non-cage environment across time, and the first illustration of individual hen variability as represented by their spatiotemporal behavior. This research provides a proof of concept for applying wildlife ecology techniques to animal welfare science in an agricultural environment.

MATERIALS AND METHODS

Animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Teaching and Research Center (MSU-PTRC). Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University Institutional Animal Care and Use Committee. Two identical rooms (6 m x 4.5 m) at MSU-PTRC were used. Each room was furnished in the same configuration with nest boxes, perches,

tube feeders, and a water line with nipples. Sixteen nest boxes (each 0.4 m long x 0.3 m wide x 0.3 m high) in an 8 x 2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1 m x 6 m slatted area at a height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8 cm of wood shavings at time of data collection. Food and water were provided daily *ad libitum*. Daily care, including egg collection, feeding, and hen inspection, occurred at least once a day. Two incandescent light bulbs (60 lux at bulb level) on an automatic timer provided light 15 h per day in each room. Temperature was maintained between 16°C -22°C using a ventilation fan and forced air heating.

Hy-Line Brown laying hen pullets (n = 270, 135 hens/room) were reared in each of the rooms as described above with accommodation made for their size (i.e., smaller perches, which were removed at 6 wk) and immaturity (i.e., access to nest boxes was not granted until 10 wk of age). Each room provided 0.21 m² floor space, 17.8 cm of perch space, 0.01 m² of nest box space, and 4.83 cm feeder space per mature hen. Thirteen nipples provided drinking space for 10.3 hens per nipple. All room parameters met or exceeded United Egg Producers and Federation of Animal Science Society housing requirements for non-cage laying hens.

At 10 wk, all hens were weighed and fitted with uniquely numbered leg bands. At 11 wk, 10 hens per room were selected and fitted with a sensor. Sensor-wearing hens were selected from across the range of body weights obtained at 10 wk in an attempt to select for hens of varying social ranks (Cloutier and Newberry, 2000). Collection of data from the sensor to monitor the

hen's behavior prior to and immediately after the onset of lay as part of a separate experiment was the motivation for fitting the hens with sensors prior to comb development. Therefore, even though combs are important to hen social hierarchy, it was not possible to include comb size as a parameter indicative of social status to select hens to wear sensors as described by O'Connor et al. (2011). The remaining 125 hens in each room did not receive sensors. Previous research indicated that wearing the sensor did not have any long-lasting effects on hen resource use or agonistic interactions (Daigle et al., 2012).

Behavioral data collection

Hens in each room were videotaped for 48 h at 48 and 66 wk of age using ceiling-mounted cameras. Though 10 hens per room were fitted with sensors, video data were only decoded for the five individual hens per room that were actively transmitting sensor data. Therefore, the individual behavioral data collection for this research was restricted to the same five individuals per room. One individual died between the two data collection points, so the final analysis includes data from nine individual hens.

Continuous observation of individual hen posture, behavior, and resource use (Table 1) was made over a 30 min period every 90 min (06:00-06:30, 07:30-08:00, 09:00-09:30, 10:30-11:00, 12:00-12:30, 13:30-14:00, 15:00-15:30, 16:30-17:00, 18:00-18:30, and 19:30-20:00) during the lights on period (Daigle and Siegford, submitted). Data were collected across a 48 h time period with the dark period (21:00-06:00) omitted because even though infrared cameras allowed observation of behaviour at night, substantial amounts of movement were not observed (most movement during lights off was due to hens transitioning between standing and sitting as

they readjusted during the night). Therefore data collection efforts were focused on the lights on period only. Posture, behavior, and resource use were recorded in mutually exclusive categories and are reported in duration of time spent (sec) in that state across the 48 h period.

Wireless sensor network

The sensor system consisted of three components – (i) a Mica2Dot mote radio mobile mounted on hens, (ii) Mica2 mote stationary radio nodes acting as beacons for the proximity detection process and (iii) a base station running TinyOS operating system that collected the data wirelessly from stationary nodes and stored the data on a laptop PC. Each component consisted of a processor and a radio subsystem, running a TinyOS operating system. All network components operated via a 900 MHz wireless radio link.

The sensor system consisted of ten stationary nodes strategically placed throughout the room (Figure 1). To minimize communication interference between stationary and mobile nodes, the stationary nodes were hung 1 m above the ground with ceiling-mounted PVC pipes to point their antennas downwards. Each mobile node (~10 g) was placed inside a plastic casing and mounted on a hen's back with figure eight nylon harness. The casing was colored to match feather color and painted with a unique number for easy visual identification (Figure 2). After experimentation, mounting the sensor on the back of the hen was determined to yield maximum sensor stability while maintaining sufficient signal quality for proper wireless communications with stationary nodes and avoiding tissue damage to the hen. Hens wore the sensor casing throughout the lay cycle. The base station was strategically placed outside of the room to maintain communication with all stationary nodes.

A polling-based time division multiple access (TDMA) protocol (Figure 3) administered communication between the nodes in the wireless network. One of the stationary nodes acted as a polling node and sent periodic polling packets to each node in the network. When a mobile node received its polling packet, the mobile node transmitted a radio signal at an output transmission power of -10dBm, and the sampling rate was 0.125Hz (8 sec sampling interval). A low transmission power was used so that only the stationary nodes within one meter of the mobile node received the signal. Nodes were polled every 8 seconds because: the position of the hens did not drastically change during that period, this interval prevented drift between clocks in the different components, and lesser polling frequency resulted in greater energy consumption. When a stationary node received a signal from a mobile node, it noted the mobile node ID, as well as the received signal strength. On being polled, a stationary node sent a data packet to the base station. This data packet contained the received signal strength information (RSSI) of each mobile node in the current polling period. If a stationary node did not receive any signal from a particular mobile node, the received signal strength was set to 0 for that mobile node.

To minimize battery consumption and extend the mobile node operating life, an energy-aware sleep schedule created a pattern where mobile nodes slept during idle-time, i.e., when they were not receiving or transmitting. This energy conservation schedule allowed the system to run for 48-50 h compared to the 8 h observed when energy conservation was not used. The base station, upon reception of data packets from all stationary nodes in a polling period, saved the polling data on the laptop.

Prior to deployment, the system was tested in the hen housing facility. Proper calibration was necessary before deployment as the non-cage laying hen environment is complex and stochastic. The mobile nodes were programmed with different transmission powers ranging from

-18dBm to -2 dBm and signal characteristics tested. The transmission power (-10dBm) was determined to be the optimal power that would minimize over- or under-representation of hen location.

Sensor output was processed and analyzed using custom software written in Java. For each sampling interval, the two stationary nodes that were nearest to each mobile node were identified. The nearest stations were determined using the RSSI transmitted by each mobile node and reported by the stationary nodes. For every mobile node, the stationary nodes with the two strongest signals were selected to be the nearest stations. Resource use was identified as the resource that was closest to the two nearest stationary nodes, with a higher weight assigned to the stationary node with the stronger signal.

Combining sensor network output and behavioral observations into GIS

First, the room where the hens were housed was georectified to the exact dimensions in ArcMap 10.0 (Environmental Systems Research Institute, Redlands, CA).

Gaps in the sensor network due to uneven node distribution and packet loss required that probabilistic movement paths be interpolated between missing locations. Therefore, a continuous-time correlated random walk model was applied to the sensor data (Johnson et al., 2008). This model was fit in R (R statistical software version 2.15.1, <www.cran.r-project.org>, accessed 1 May 2013) using the package CRAWL, which employs a Kalman-Filter to predict locations from the existing point pattern based on a continuous-time stochastic movement process. In this way, locations that were missed because of the location of the stationary sensor within the network were imputed to make the database of chicken locations complete.

This locational database was used to delineate home ranges for each individual chicken in both rooms by time period (48 and 66 wk). In this capacity, utilization distributions (UDs; Kernohan et al. 2001) were developed in R using the Kernel Density Estimator library. These UD were fit using a bivariate plug-in matrix that calculated bandwidth along rotated axes for each individual chicken (Gitzen and Millspaugh, 2003; Kernohan et al., 2001; Gitzen et al., 2006). Subsequently, UD and amount of overlap among the chickens were calculated for each time period.

Next, hotspot maps were created representing the spatial distribution of foraging, preening, and feeding behaviors. The categorical behavioral data was converted into a binary form (representing the presence or absence of the behavior of interest) to portray the spatial distribution of these behaviors. Thiessen polygons, which are well-suited to binary data (Farris et al., 2010), were utilized to develop the spatially-explicit maps of these behaviors. The precision of the sensor network (8 sec intervals) meant that chickens were recorded performing multiple behaviors in the same location. Thus, the Thiessen polygons present the mean count of the behaviors of interest within each individual's home range. For presentation purposes, these data are divided into equal intervals representing space use for foraging, preening, and feeding in increments of high, medium, low, and none.

RESULTS

Individual hen home ranges and conspecific overlap

A wireless sensor network was installed at the MSU Poultry Teaching and Research Center to monitor individual hen movement within a non-cage system. Sensors (~ 10 g) were

worn on the back of nine HyLine Brown hens from two identical rooms with a nylon figure eight harness (Figure 2). The sensor system consisted of three components – (i) five Mica2Dot mote radio mobile nodes mounted on hens, (ii) ten stationary Mica2Mote radio nodes strategically placed throughout the room (Figure 1) acting as beacons for the proximity detection process and (iii) a single base station to wirelessly collect data from stationary nodes and store the data in a laptop PC. A polling-based time division multiple access (TDMA) protocol (Figure 3), administered communication between the nodes in the wireless network. One of the stationary nodes acted as a polling node and sent periodic polling packets to each node in the network. When a mobile node received its polling packet, the mobile node transmitted a radio signal at an output transmission power of -10 dBm. A low transmission power was used so that only the stationary nodes within one meter of the mobile node received the signal. When a stationary node received a signal from a mobile node, it noted the mobile node ID, as well as the received signal strength. On being polled, a stationary node sent a data packet to the base station. This data packet contained the received signal strength information (RSSI) of each mobile node in the current polling period. If a stationary node did not receive any signal from a particular mobile node, the received signal strength was set to 0 for that mobile node, and the sampling rate was 0.125 Hz (8 sec sampling interval). Sensor output was processed and analyzed using custom software written in Java. For each sampling interval, the two stationary nodes that were nearest to each mobile node were identified. The nearest stations were determined by using the RSSI transmitted by each mobile node and reported by the stationary nodes. For every mobile node, the stationary nodes with the two strongest signals were selected to be the nearest stations.

Sensor and video data were collected for a continuous 48 h at 48 and 66 wk and collated into ArcMap 10.0 (Environmental Systems Research Institute, Redlands, CA) from two (Blue

room and Yellow room) separate identical rooms. The sensor system recorded the proximity of the hen to a known location in the room every 8 s. Gaps in the sensor network were interpolated using a continuous-time random walk model. This model was fit in R (R statistical software version 2.15.1, <www.cran.r-project.org>, accessed 1 May 2013) using the package CRAWL which employs a Kalman-Filter to predict locations from the existing point pattern based on a continuous-time stochastic movement process. Therefore, locations missed in the sensor were imputed to make the database of hen location complete.

This locational database identified large differences home range size and amount of overlap with conspecifics (Table 1). On average, home range size (m^2) at 48 wk was larger (5.48 ± 1.13) than at 66 wk (5.17 ± 1.48), while the proportion of overlap (%) with conspecifics decreased from 0.88 ± 0.05 at 48 wk to 0.84 ± 0.07 at 66 wk. Hens that used a smaller proportion of the room were observed to have more of their home range overlap with their conspecifics, while hens with larger home ranges had less home range overlap. Substantial variation was observed among the hens with regard to overlapping home ranges. Five hens showed a relative decrease in the amount of overlap as they aged, while two showed an increase and another two remained relatively consistent (Figure 4). Consequently, this was reflected in changes observed in pairwise associations (m^2) between two hens (Table 2).

Overall utilization distributions were developed for all hens in the Yellow room at 48 (Figure 5a) and 66 wk (Figure 5b). This image illustrates the areas in which hens spent their time, and the intensity of the color corresponds to how much time they spent in each respective area and how their space use changed as they aged. Hens within this room were variable in regard to where they spent their time, and how much time they spent in each area.

Feeding, foraging, and preening

Hotspot mapping was used to represent the spatial distribution of foraging, preening, and feeding behaviors of all nine hens at both ages. Thiessen polygons were used to develop spatially-explicit maps illustrating where each individual hen would likely be found feeding, foraging, and preening at two different ages. These Thiessen polygons present the mean counts of the behavior of interest within each individual's home range. For presentation purposes, these data are divided into equal intervals representing space use for foraging, preening, and feeding in increments of high, medium, low, and none. A wide variety of feeding, foraging, and preening patterns were observed among the nine hens across time. The average proportion of the hen's time devoted to these behaviors decreased with age. Several hens appeared to perform feeding behavior in a single area, while other hens distributed their feeding behavior evenly throughout the room in which feeders were located (Figures 6 and 9). Some hens compensated for the lack of feeding behavior by increasing the amount of time spent foraging (Figures 7 and 10), while others did not. One hen was reported to never forage and spent very little time feeding.

Preening was observed to occur in a variety of locations throughout the room (Figures 8 and 11). There was also a wide range of how much preening was occurring. Furthermore, two hens within the same room were observed to exhibit similar preening and foraging patterns across time, which could be indicative of either an affiliative or aggressive relationship.

DISCUSSION

As agricultural animals are housed in larger groups, and the public is increasingly concerned about animal welfare, there is a need to develop tools to understand individual animal behavior in larger groups. The wireless sensor network used in this study is one such tool and this research shows how data from this tool combined with behavioral observations can be used to identify spatiotemporal differences among individual laying hens in a non-cage environment across time. Furthermore, these results highlight the individual variation exhibited among laying hens with regard to their overall and behavior-specific space use. Changes in the location and frequency of behaviors could provide insight into individual hen condition, while understanding the variability among hens within the same environment can identify the areas important to hens for performing specific behaviors. This information can facilitate improved housing design and animal management practices.

Foraging by chickens is a behavior that engages the entire body when performed. Foraging requires mobility and is not constrained by the presence or absence of specific resources. Hens must balance on one leg and use the other leg to scratch the ground, and subsequently use their beak to manipulate the substrate as they search for food. Therefore, changes in the amount of time spent foraging or the area in which foraging occurs could be indicative of several health problems including illness, bumble foot (Hester, 1994), neuromas resulting from improper beak trimming (Kuenzel, 2007), broken claws, or broken bones – all of which are relevant to welfare. The location of foraging may also help egg farmers identify environmental conditions that are favorable to hens for foraging – which can aid in understanding hen perception of favorable conditions.

Feeder use requires access to a highly valued and spatially concentrated resource. Some hens may be restricted from accessing the feeder due to social interactions, or they may prefer to acquire their feed by foraging for feed that has dropped into the litter. Similar to foraging behavior, changes in feeding behavior could be indicative of illness, injury, or change in social dynamics, which could be better understood with this type of data. Furthermore, understanding the variability in how hens in non-cage systems use the resources provided to them will allow housing design development that best meets hen spatial and behavioral needs.

Patterns of preening behavior can provide insight into where hens choose to rest, groom, and spend time with familiar conspecifics, and may provide more insight into the hen's mood or emotional state. Hens that exhibited similar spatiotemporal preening patterns to those shown by other hens may be exhibiting affiliative behaviors; conversely, they may be avoiding one another. Two hens may choose to spend time together preening, or they may choose to use the same space for preening, but use it at different times thus avoiding one another. This analysis can only identify whether individuals use the space similarly and cannot identify if hens were performing behaviors and using space simultaneously, therefore, future investigations using dynamic modeling may be able to highlight social-spatiotemporal relationships.

Utilization distributions have traditionally been used to illustrate behavioral patterns for wild animals that have relatively unlimited space but limited resources, while in this context these hens have limited space but seemingly unlimited resources. Thus, though this analysis tool is used in the present context in similar ways, it is being used to gather different information. The animals in these different environments are subjected to different pressures and make different decisions accordingly. Hen movement within the non-cage system is restricted by the confines of the room, and their movement within is probably based more on social or immediate

interactions and related motivations rather than the drive to locate optimal habitats and establish home ranges. However, further investigation into hen movement patterns is necessary to determine underlying motivations and affective states.

Utilization distributions in wildlife contexts represent the probability that an animal will be within a certain region in order to illustrate relationships between animal movement and site fidelity, resource availability, and habitat selection (Millspaugh et al., 2006). When several individuals exhibit overlapping utilization distributions, these overlaps can identify areas of optimal habitat for reproduction, prey location, or foraging. However, overlaps for hens in a closed system more likely provide non-traditional overlap information. Hens may exhibit more or less fidelity to specific areas of the room based upon personal preferences, and several hens may have a high degree of overlap due to social interactions or resource availability. However, hens may also have a high degree of overlap simply due to density and have no choice but to inhabit one another's space.

Here, a proof of concept approach to understanding the relationship between non-cage laying hen behavior and space use is presented. Commercially-housed laying hens have been genetically selected to be similar with regards to growth, nutritional needs, productivity, and longevity. Despite this genetic similarity and even identical rearing environments, mature hens housed within the same environment exhibited very different patterns of space use and had home ranges of varying sizes. Furthermore, some hens exhibited changes in their behavioral patterns over time, while others remained relatively consistent – which may be reflective of different levels of behavioral plasticity or changes in social status or health. This work emphasizes that even though hens may be genetically similar, they still exhibit individual preferences and

behavioral patterns. Therefore, there is a need to provide environmental conditions that can accommodate the diverse spatial needs and preferences of laying hens in non-cage environments.

APPENDIX

Table 5.1: Home range area (m^2), proportion of the room in which the home range covers, proportion of individual hen's home range that overlaps with her conspecifics, and individual hen behavioral time budgets (%). Results are presented for hens from two separate rooms (Blue, as indicated with a B and Yellow as indicated with a Y) at two different ages (48 and 66 wk).

Animal ID	Age (wk)	Range size (m^2)	Proportion of room	Proportion of overlap with conspecifics	Behavioral time budet (%)						
					feed	drink	preen	dust bathe	forage	rest	other
B01	48	4.82	0.17	0.97	35.81	11.83	7.58	0	14.13	1.57	29.07
B02	48	1.52	0.05	1	31.46	1.85	12.15	2.73	9.51	11.09	31.21
B05	48	6	0.22	0.89	32.35	4.92	9.18	2.32	15.11	4.22	31.9
B08	48	6.83	0.25	0.94	1.71	3.54	19.82	2.63	22.45	14.14	35.71
B10	48	11.28	0.41	0.76	12.1	2.69	19.36	0.18	15.47	1.98	48.23
Y05	48	2.97	0.11	1	18.45	2.19	16.78	1.8	17.68	0.75	42.34
Y07	48	2.8	0.1	0.9	0.09	1.44	5.45	0	0	0	93.02
Y08	48	10.04	0.36	0.5	1.33	2.68	18.75	0.24	30.05	3.39	43.57
Y10	48	3.14	0.11	0.96	17.28	4.27	7.72	0	20.77	6.55	43.4
B01	66	1.65	0.06	1	22	6.48	7.93	2.61	15.62	2.87	42.49
B02	66	7.64	0.27	0.94	17.07	3.87	12.91	1.69	17.48	1.95	45.04
B05	66	9.11	0.33	0.84	20.77	3.79	9.66	1.33	17.03	1.93	45.49
B08	66	7.68	0.28	0.85	0.45	3.09	15.39	2.18	27.53	0	51.36
B10	66	1.66	0.06	0.99	2.91	3.42	1.44	0	2.79	0	89.44
Y05	66	0.37	0.01	1	24.1	2.46	16.02	4.31	8	4.06	41.05
Y07	66	1.82	0.07	0.76	0.02	1.08	2.09	0	0	18.9	77.91
Y08	66	13.35	0.48	0.27	7.46	2.27	23.29	0	19.01	11.55	36.42
Y10	66	3.26	0.12	0.9	10.41	6.22	21.36	4.22	19.14	4.89	33.77

Table 5.2: Total area (m^2) of overlap between two hens at two different ages (48 and 66 wk).

Pairwise overlap between	48 wk	66 wk
B10 - B01	4.39	0.51
B10 - B02	1.49	1.53
B10 - B05	5.09	1.59
B10 - B08	6.21	1.15
B01 - B02	1.27	1.64
B01 - B05	3.65	1.64
B01 - B08	3.10	1.57
B02 - B05	1.09	6.57
B02 - B08	1.43	5.49
B05 - B08	2.98	5.87
Y05 – Y07	0.08	1.01
Y05 – Y08	0.33	2.87
Y05 – Y10	0.03	2.07
Y07 – Y08	1.37	2.48
Y07 – Y10	0.77	1.22
Y08 – Y10	2.88	2.88

Figure 5.1: Diagram of room set up and stationary node placement. F represents a feeder. The water line is represented by the thick solid line in the middle of the room.

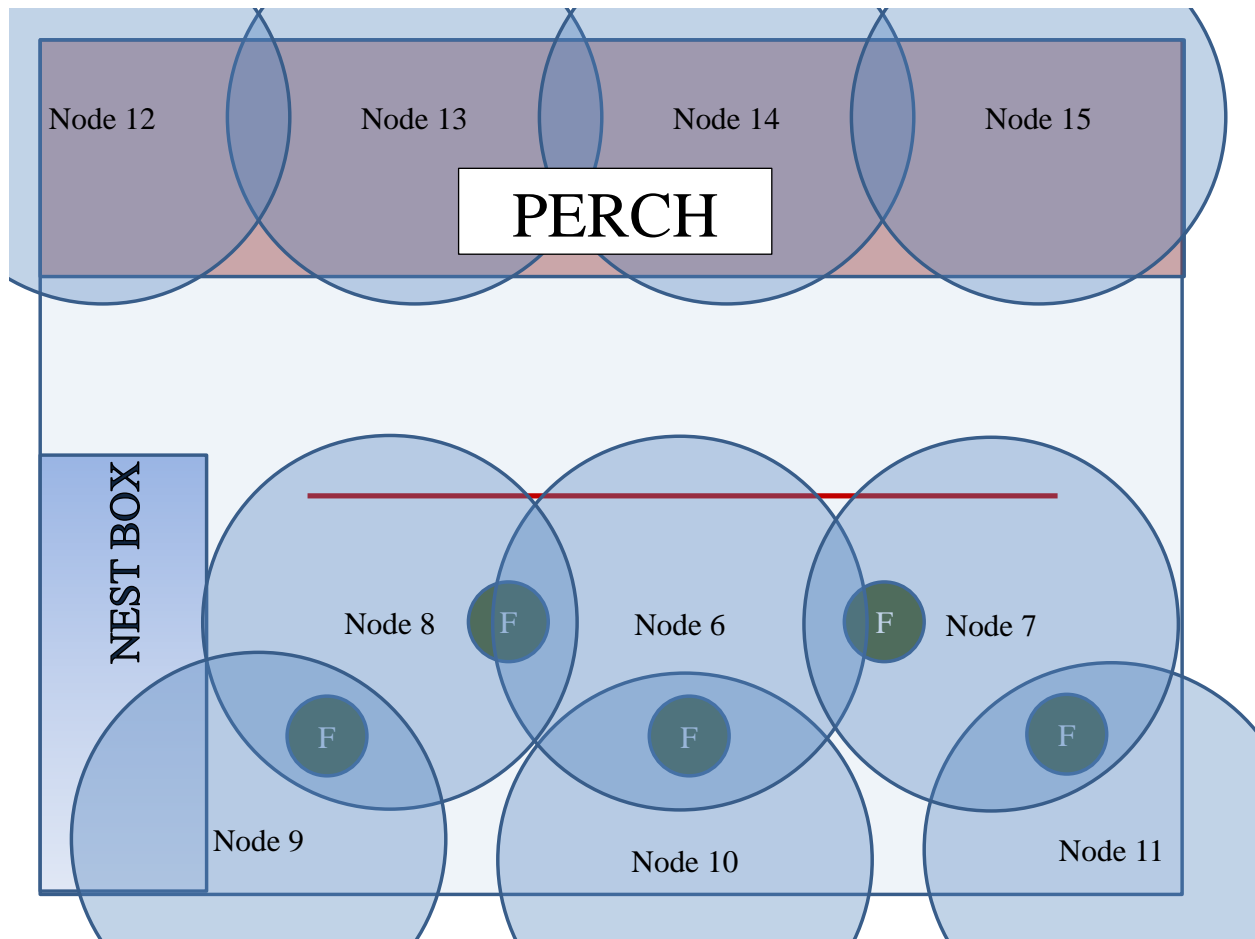


Figure 5.2: Photograph of a laying hen wearing a wireless sensor. Sensors were packaged in a plastic case to prevent entry of dust and moisture. The sensor was mounted on the back of the hen using a figure-eight nylon harness. Both the sensor case and the harness were colored to blend in with the hen's feathers to avoid attracting attention of other hens in the experimental room. This picture was taken several days after the hen was fitted with the sensor.

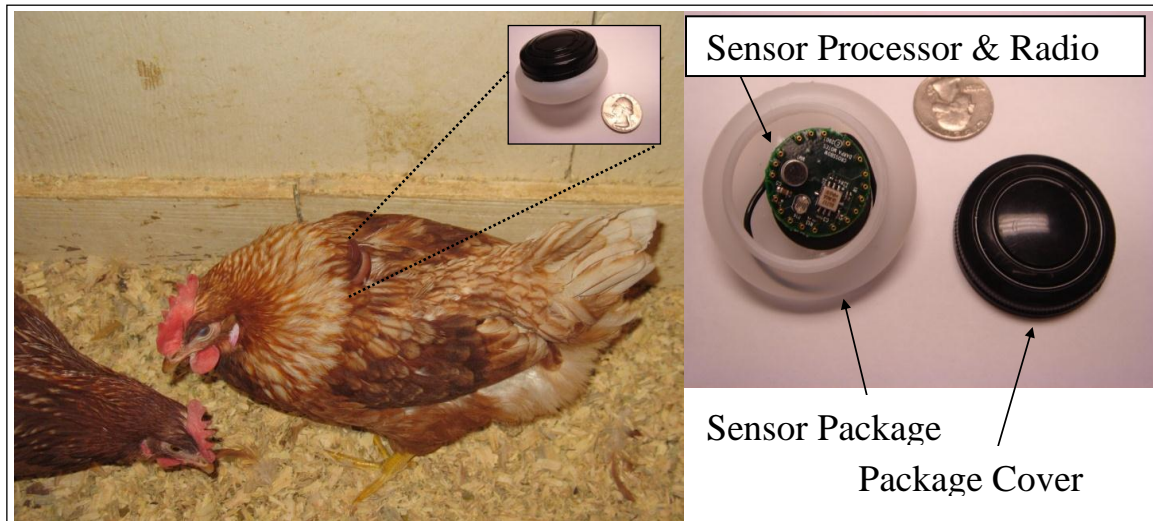


Figure 5.3: Illustration of the Polling-based time division multiple access (TDMA) protocol implemented to operate the wireless sensor system.

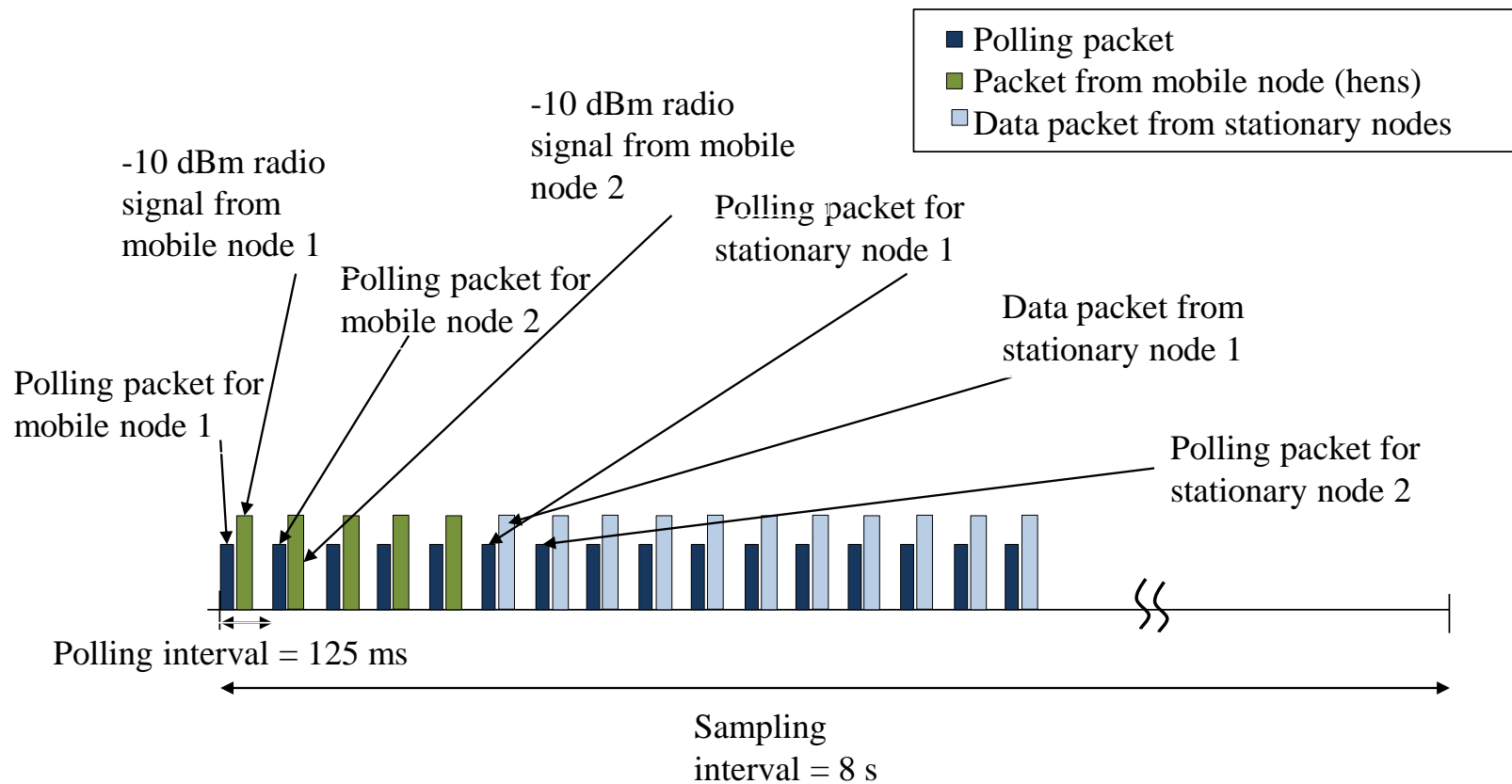


Figure 5.4: Amount of home range overlap (m^2) for each hen across time.

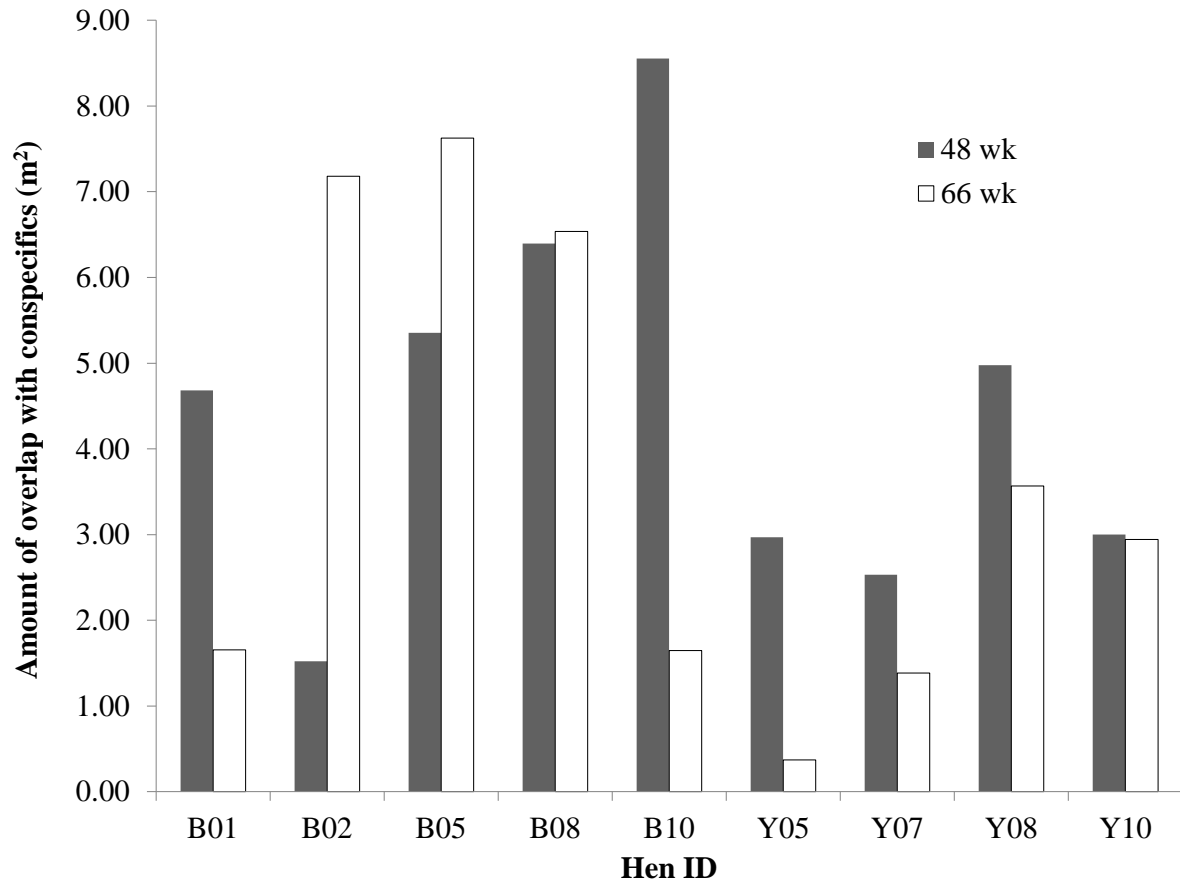


Figure 5.5: Overall utilization distribution for hens housed within the yellow room at (a) 48 and (b) 66 wk. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

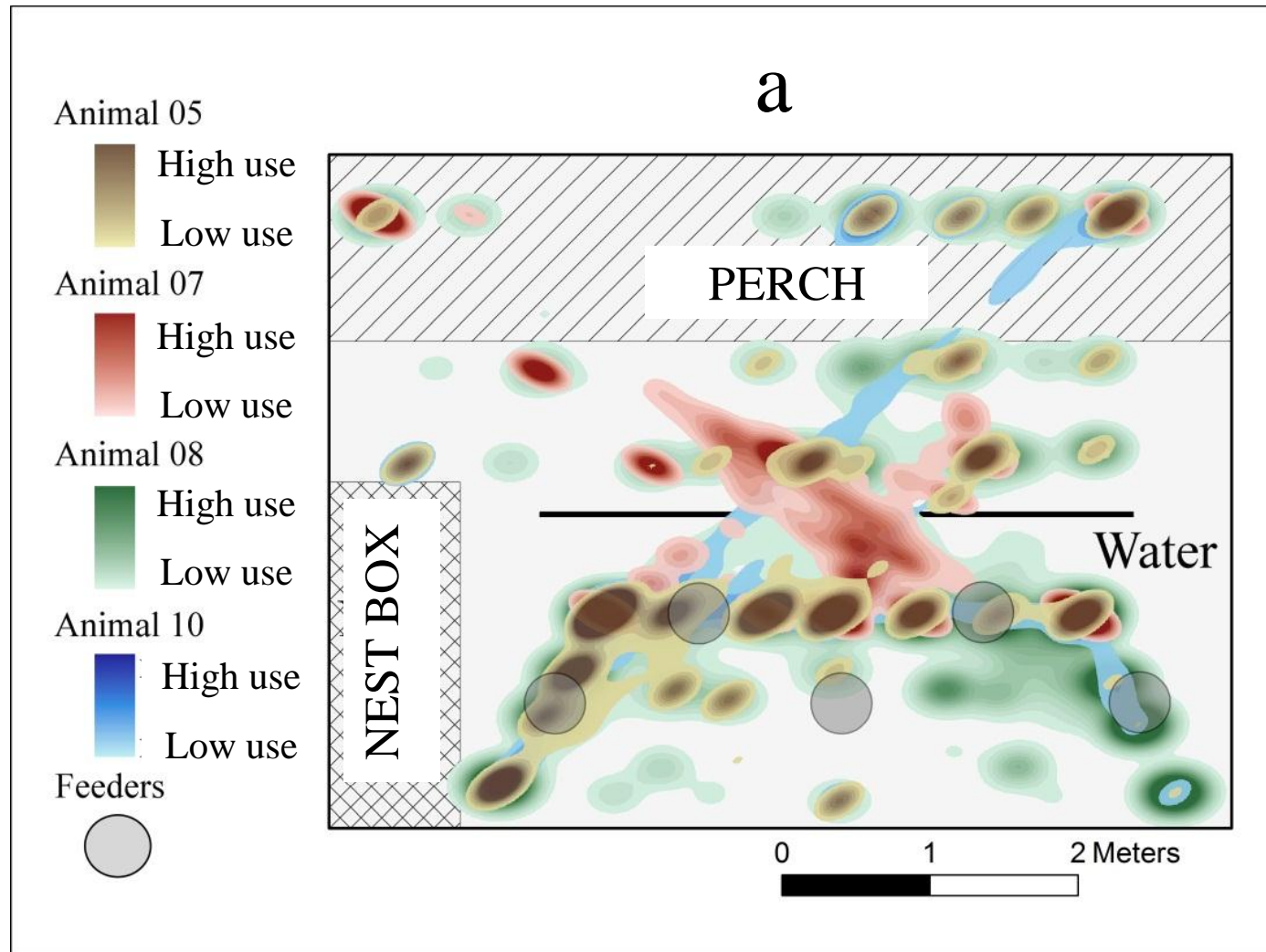


Figure 5.5 (cont'd)

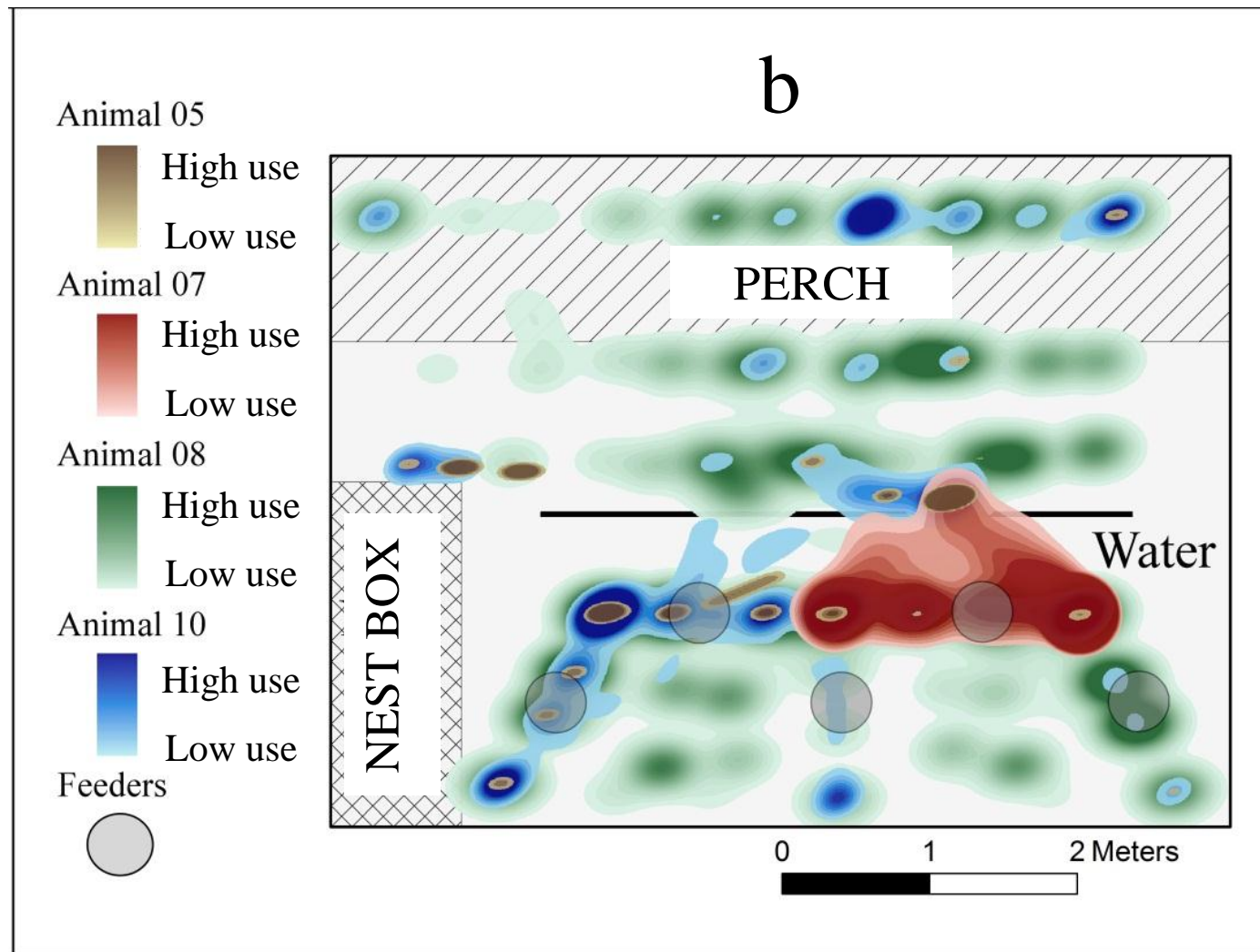


Figure 5.6: Hotspot maps of feeding behavior for hens housed within the Blue Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing feeding behavior in that area.

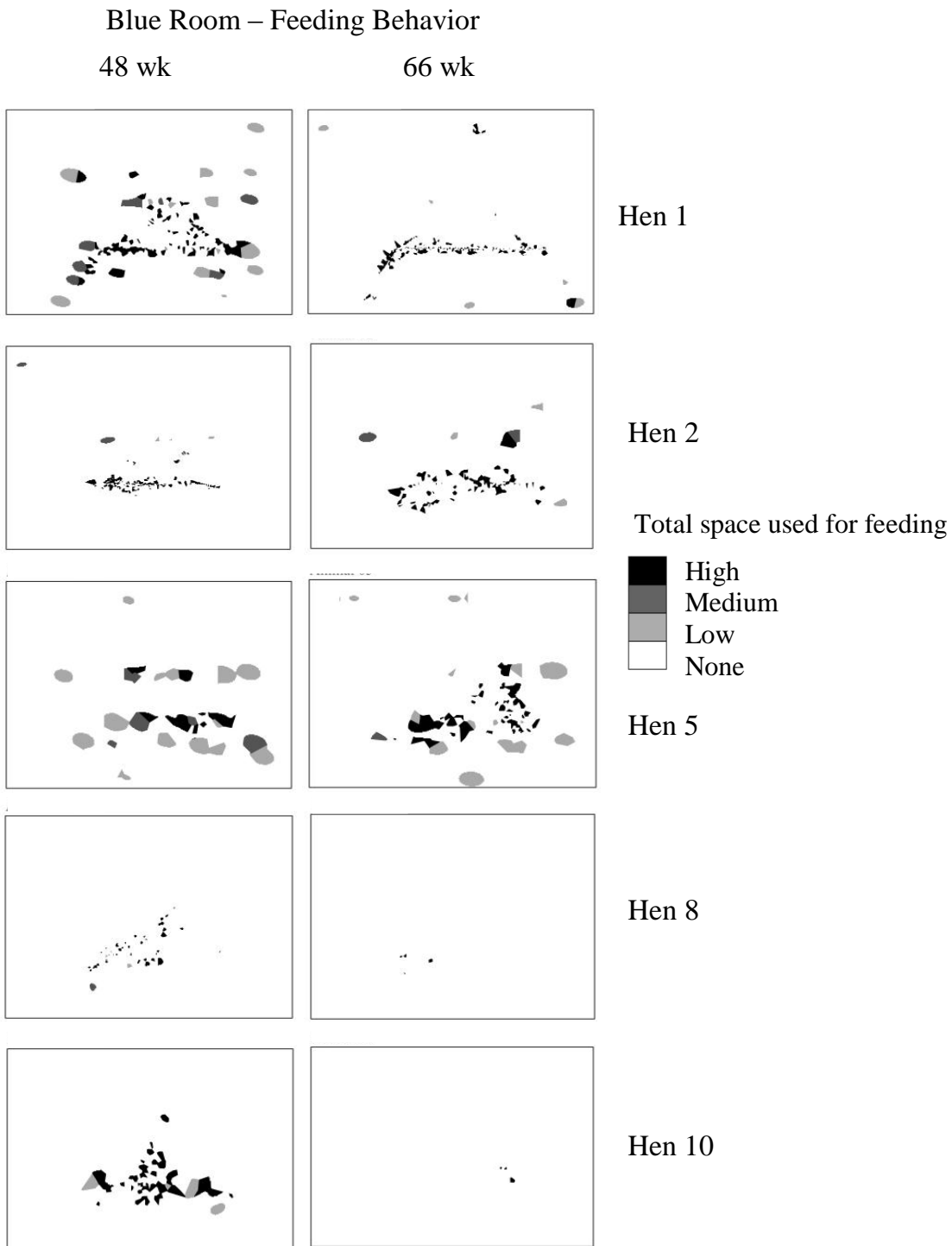


Figure 5.7: Hotspot maps of foraging behavior for hens housed within the Blue Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing foraging behavior in that area.

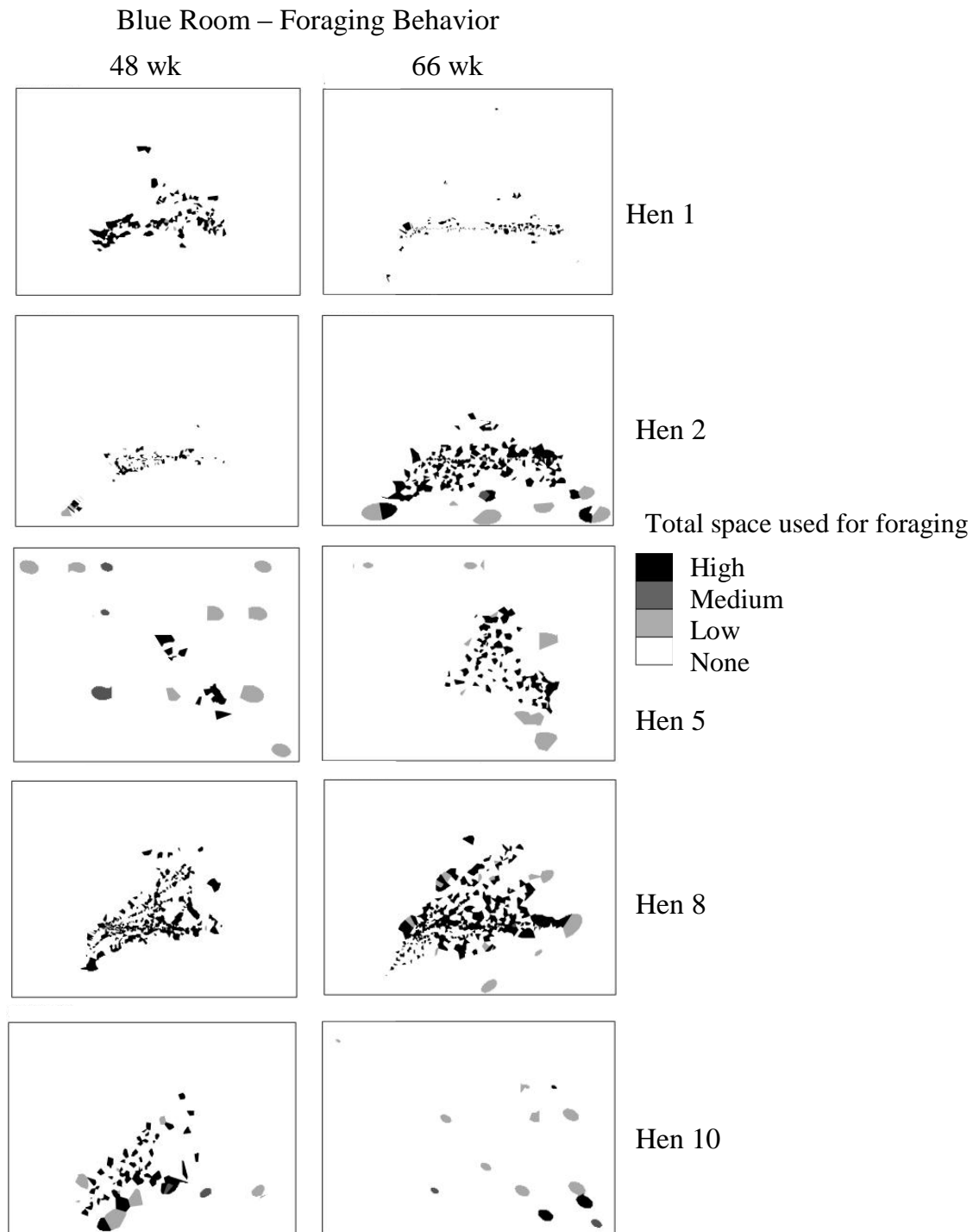


Figure 5.8: Hotspot maps of preening behavior for hens housed within the Blue Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing preening behavior in that area.

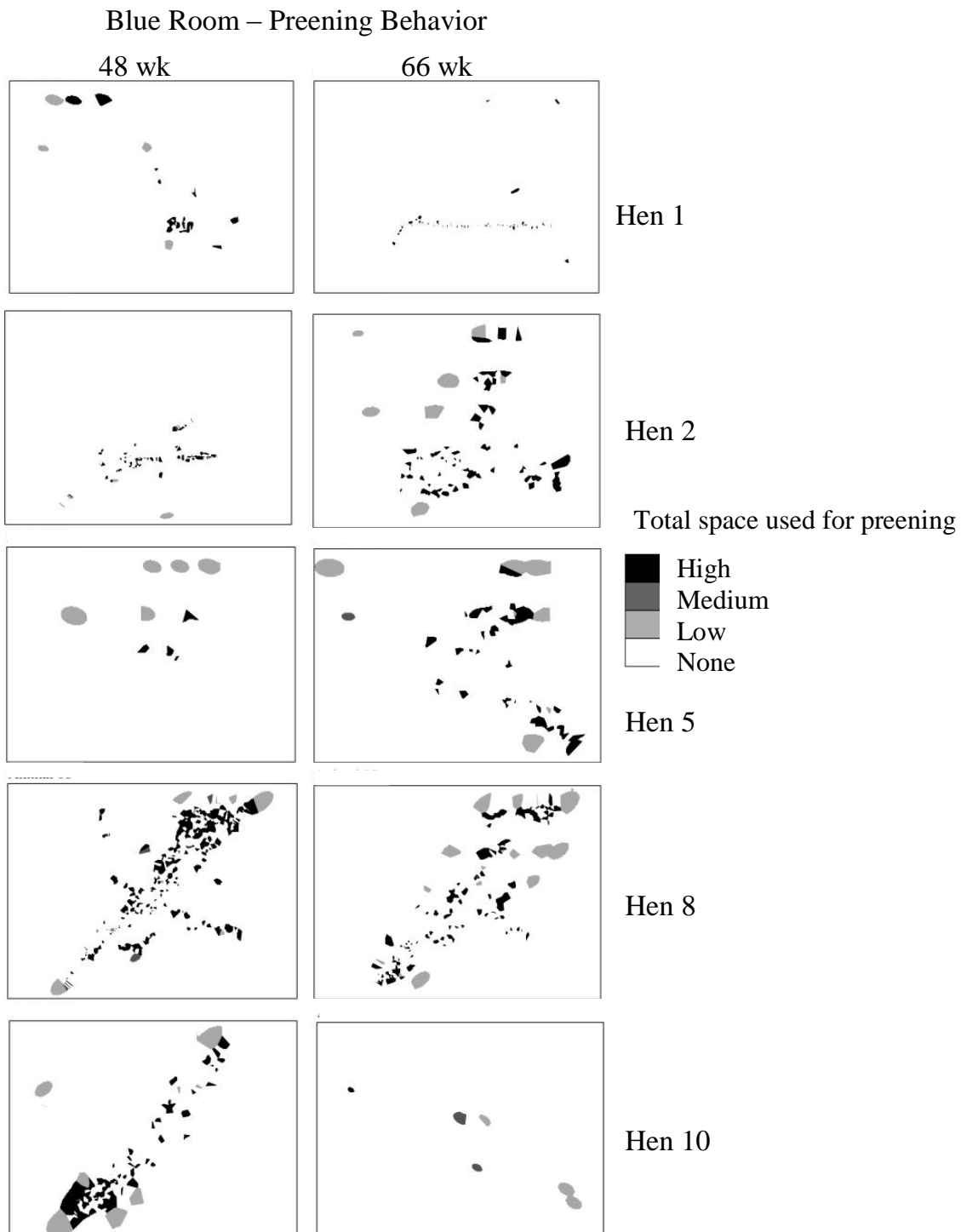


Figure 5.9: Hotspot maps of feeding behavior for hens housed within the Yellow Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing feeding behavior in that area.

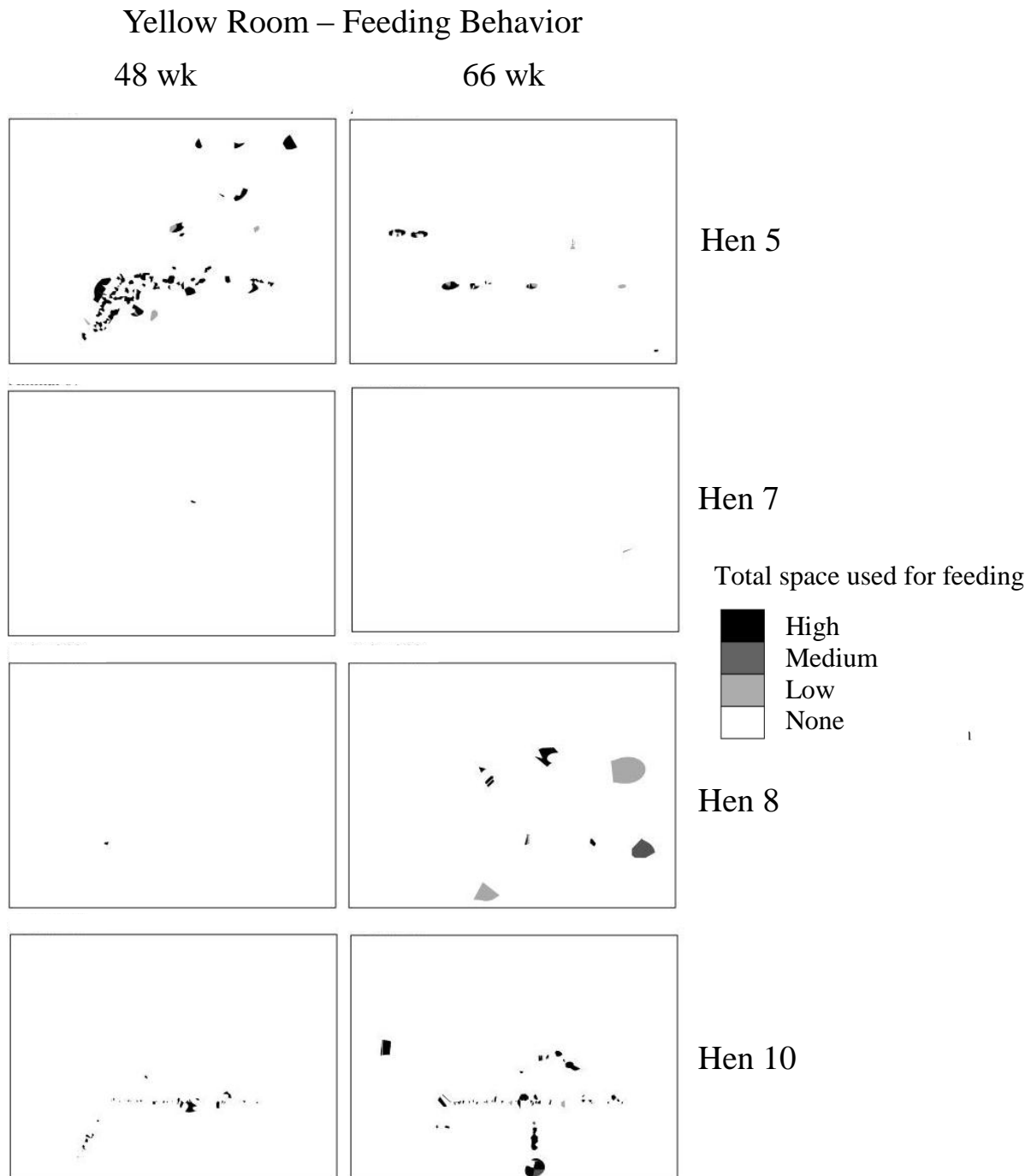


Figure 5.10: Hotspot maps of foraging behavior for hens housed within the Yellow Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing foraging behavior in that area.

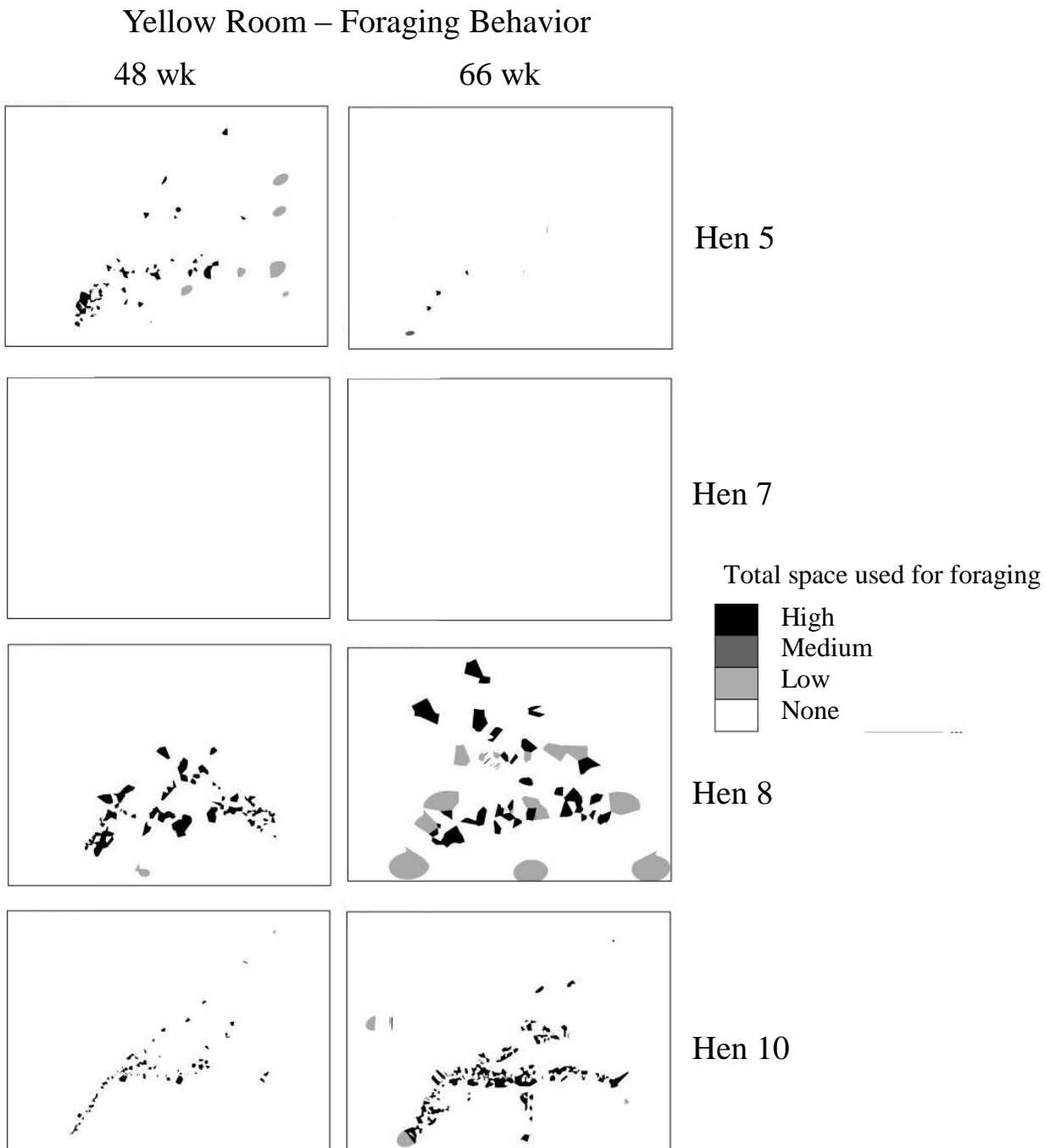
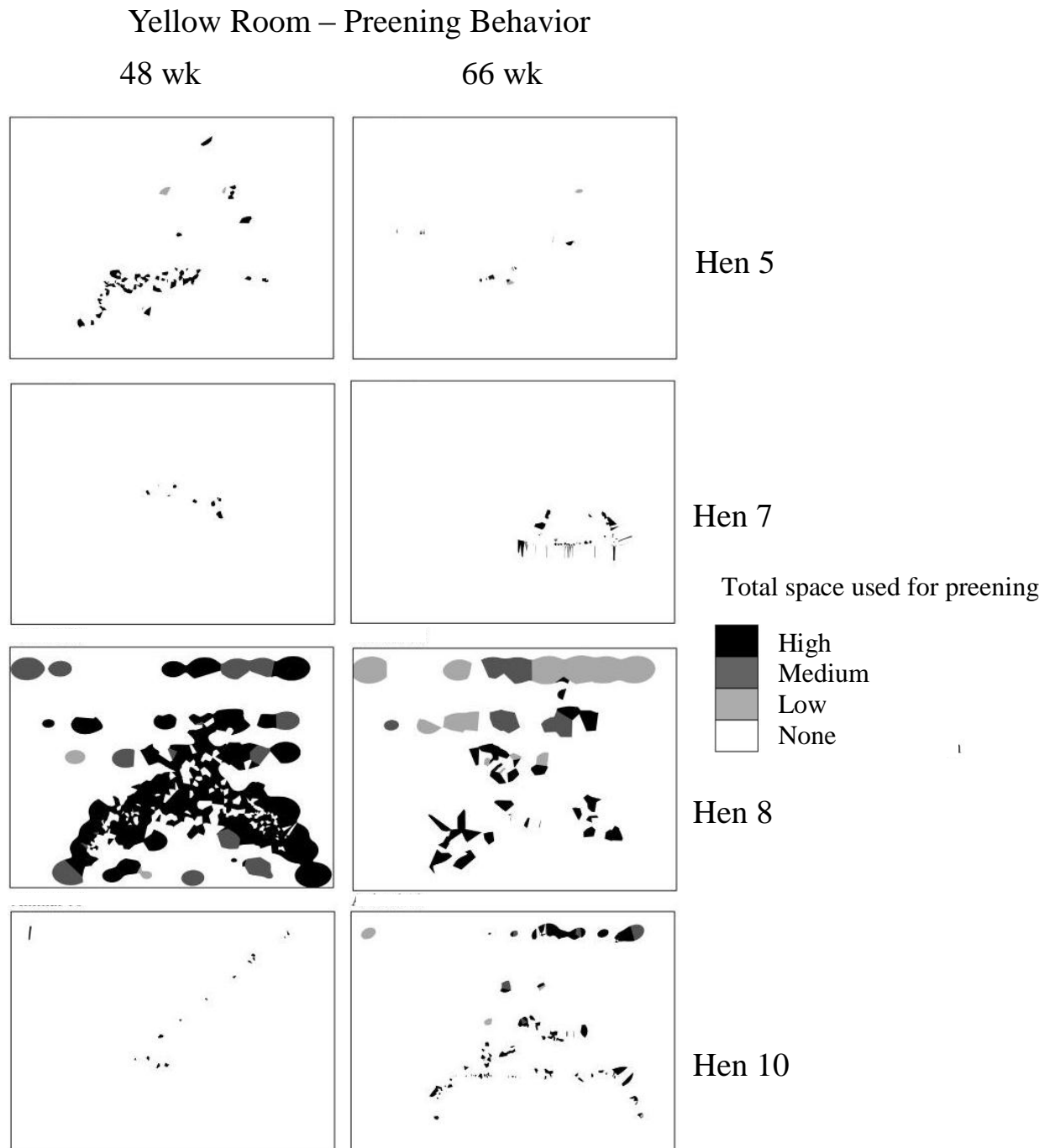


Figure 11: Hotspot maps of preening behavior for hens housed within the Yellow Room at 48 and 66 wk. The labels at the top of each panel indicate which hen is represented. The legend to the right illustrated the amount of time (high, medium, low, none) the hen spent performing preening behavior in that area.



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DISCUSSION

DISCUSSION

The findings from this dissertation are multi-faceted. A newly developed wireless body-mounted sensor to track individual hen location in a non-cage environment was successfully implemented and used to identify movement patterns of individual hens housed within the same environment. Since the impact of the sensor on hen resource use and agonistic interactions was minimal and short lived, the results of the sensor output provide an unbiased representation of individual hen spatiotemporal behavioral variability across time.

Behavioral data collection for this research was labor intensive and time consuming. Therefore, a tool was developed to identify parsimonious sampling techniques for different types of behaviors (e.g., static, dynamic, etc.) that were subsequently applied in later data collections. Though this tool was developed for research described here, other researchers may be able to benefit from the same knowledge to facilitate their data collection. Not only was individual hen variability highlighted in this research, but the relationship between the hen's behavior and her physical body illustrated as well. Tracking individual behavior in a non-cage system is challenging; therefore, by identifying links between the hen's physical body and her behavioral choices, animal managers and animal welfare assessors may be able to better understand hen behavior without visual observation.

As the egg industry transitions to housing laying hens in large groups, the management approaches to their welfare must also transition accordingly. The research from this dissertation emphasizes that even though laying hens with similar genetic backgrounds and rearing experiences were housed in uniform non-cage environments, causing them to produce and grow in similar patterns; they behaved very distinctly from one another. Furthermore, these non-cage

environments could be embraced as single species ecological units. In wildlife ecology, emphasis is usually placed on retaining biodiversity and ensuring that all species within the ecological web are interacting appropriately. However, in an agricultural setting, groups of animals of the same species are housed together. Therefore, management techniques may not be focused so much on retaining biodiversity, and more focused on ensuring that all individuals within this dynamic and constantly changing group are receiving the necessary care and resource access. Therefore, this requires acknowledging the individuality of the hens within each unit and making accommodations for all needs to be met.

Without utilizing new approaches to assessing non-cage laying hen behavior, the behavioral profile of the individual will remain elusive. New sampling techniques and technological advancements can facilitate efficient data collection. Furthermore, new technologies, when used appropriately, can provide information about a hen's experience. Humans are equipped with a different set of sensory mechanisms than animals, animals perceive the world very differently from humans and may make choices based upon information we cannot or do not perceive (Sherwin 2007). By developing technology that can help humans understand the world from the hen's perspective, best practices can be implemented to provide optimal environmental conditions from the hen's perspective.

When individual hen behavior and movement can be assessed, a new genre of agricultural research questions can begin to be asked related to the internal subjective experience of the animal. An animal's coping style (or personality, temperament, axes, constructs, or behavioral syndrome) has been linked to behavioral and physiological characteristics such as exploratory behavior and boldness (Stowe et al. 2010; van Oers et al. 2011) and be consistent

over time and across contexts (Re'ale et al. 2007; Sih et al. 2004). Personality differences can be reflected by the different choices animals make or the behaviors they perform.

Hens in non-cage environments have more choice with regard to which behaviors they perform and where they choose to perform them. Individual choices and behaviors are based upon different experiences, motivations, and internal drives (e.g., genotype, physiological), all of which can be affected by early experiences. So, by understanding where an individual chooses to spend its time and perform specific behaviors, we may be able to identify the link between temperament, choice, and the animal's welfare.

Motivational drive influences these choices, and based upon the results of this research; different hens may be motivated differently in where they choose to perform behaviors, and spend their time. Using individual hen movement patterns, researchers can start to apply concepts such as optimal foraging theory to coping styles and resource placement within the housing environment. Understanding how far a hen is willing to move to access food, water, and nest boxes is based upon its predisposition for coping with stress and can facilitate appropriate and efficient placement of these resources within the housing environment.

Yet, hens may have different degrees of internal motivational drive. The strength of feeding motivation can be influenced by differences in physiology (e.g., level of hunger or pain) that can be observed in the amount of time spent feeding relative to their conspecifics. Previous experience or familiarity can also influence animal choices because they may show a temporary avoidance of, or attraction to, unfamiliar options, not because it is preferred, but simply because it is novel (Phillips, Fraser & Thompson 1996). By focusing on specific factors underlying the preference (e.g., characteristics of the items tested for preference such as traction, heat retention,

etc.), features that are important to the animals that were not part of the initial research may be better understood (e.g., perch temperature for laying hens as described in (Pickel, Scholz & Schrader 2011)). However, the impact of social facilitation should not be ignored – as personality type may affect the choice of an individual to perform a specific behavior (e.g., in geese where the focal individual – regardless of individual personality type – spent more time feeding with bold companions than shy; see Kurver et al. (2012)), and individuals may be more or less inclined to make a certain decision depending on their social role, personality, or the choices of their conspecifics (Pedersen, Heiskanen & Damm 2003).

However, a hen's choices may also be influenced by her own physical condition. Physiological challenges, such as a bone break or bumble foot, can influence how a hen chooses to or is able to behave. Hens with poor foot condition have been observed to walk less (Hester 1994), and hens with known keel bone fractures take more time to jump from a perch than hens without fractures (Nasr, Nicol & Murrell 2012). By identifying links between the hen's body and behavior, animal managers can begin to understand how the hens in their system are behaving without direct behavioral observations. This can increase the depth of information gained through a physical assessment during either veterinary exams or welfare assessments.

Recently, the link between physiological parameters and positive welfare and emotional states has come under investigation (Boissy et al. 2007; Wemelsfelder 2007), but a clear discussion linking behavior and the neuropsychological state has not yet been incorporated into animal welfare scientific thinking (Mellor 2012). Mellor (2012) also recommends that researchers work less on bringing a poor welfare state to neutral and focus more on reaching a net good welfare state.

If personality and affective state can affect animal behavior, and behavior is expressed through animals' choices, then individuals with different personalities could make different choices as to what is important to their specific welfare. Because animals can exhibit a wide variety of preferences, and we use animal preferences to guide animal housing decisions, animal personality may play a role in how housing systems are designed. As animal choice is increasingly considered to be a welfare proxy in animal housing design, we should ensure that the choices provided represent the factors we are aiming to assess, the results are interpreted in context of the test, and the choices reflect the variety or continuum of preferences expressed from all personalities – all of which can begin to be addressed with individual hen information.

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CONCLUSION

CONCLUSION

Non-cage laying hens are variable in their space use and behavioral patterns. Yet, these differences remain consistent for the individual hen over time, indicating that hens may behave differently based upon differences in their temperament or social situation. Connecting animal behavior and movement in non-cage systems with coping style should be further investigated, as should the relationship between hen movement patterns and resource placement. By acknowledging what can and cannot be gained by using new technologies, and applying them in appropriate contexts, individual hen responses to environmental conditions can begin to be explored.

The link between the body and behavior is complex and multifaceted (Daigle and Siegford, in preparation), and understanding both is integral to understanding animal welfare. Identifying the impact of one on the other can also facilitate understanding of hen welfare in situations where only one parameter (either behavior or physical condition of the hen) can be measured as part of a welfare assessment. Ultimately, promoting captive animal welfare must begin with the individual. Without understanding the individual in its environmental context, a true understanding of animal behavior and welfare will continue to be a mystery.

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