OBJECTIVE GAIT ANALYSIS IN HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*)
AND DOMESTIC DUCKS (*CAIRINA MOSCHATA DOMESTICA*) USING A PRESSURE
SENSITIVE WALKWAY

BY

JULIE D. SHELDON

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in VSM - Veterinary Clinical Medicine
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2019

Urbana, Illinois

Masters Committee:

Assistant Professor Matthew C. Allender, Chair
Adjunct Clinical Assistant Professor Sathya K. Chinnadurai
Clinical Professor Jennifer N. Langan
Adjunct Clinical Assistant Professor Michael J. Adkesson
ABSTRACT

Lameness due to osteoarthritis and/or pododermatitis is one of the most common problems encountered with aquatic birds such as ducks and penguins under professional care. Successful treatment and management is variable and often incomplete or transient. There is a lack of a validated objective tool for identification of gait abnormalities and for monitoring progress during or after treatment. This project objectively characterized the gait of Humboldt penguins (Spheniscus humboldti) and domestic ducks (Cairina moschata domestica) using a pressure sensitive walkway (PSW), and developed an experimental lameness induction model in ducks that will facilitate analgesic efficacy studies for lameness therapy in these species. Among normal penguins (n=16), there were no significant differences between feet or sex in any temporospatial gait parameter measured. Abnormal penguins (n=5) with historical right-sided (n=3) or bilateral lameness (n=2) had significantly shorter left step width compared to normal penguins. While the abnormal penguin data set was not controlled or of adequate sample size in this study to stand alone, these preliminary findings indicate that contralateral step width may be a sensitive marker of lameness, that there is partial to adequate pain management in this group, and/or that lesions were not significant enough to cause more statistically significant gait changes at the time of the study. Normal ducks (n=18) also had no difference between right and left feet in any parameter as measured by the PSW. Transient unilateral tarsal arthritis was induced in 6 randomly selected, anesthetized ducks using a monosodium urate solution injection. Serial PSW trials up to 24 hours post-injection identified that maximum force and impulse were significantly lower for the affected limb at the 3- and 4-hour time points. This model allowed for repeatable objective assessment of lameness in domestic ducks with maximum force and impulse
serving as the most sensitive gait parameters for lameness detection. This method has potential to assess analgesic efficacy for other avian species. Normal values will be used to objectively monitor progression and response to therapy of current and future cases of lameness in penguins and ducks under human care and set the groundwork for investigating this methodology in other species.
ACKNOWLEDGEMENTS

The primary author thanks her Master’s advisor, Dr. Matthew C. Allender, and the Master’s committee, Drs. Sathya Chinnadurai, Jennifer Langan, and Michael Adkesson for their strong ongoing support and guidance with this project. The authors thank Dr. Ryan Bailey for his expertise and significant contributions during the data collection of this project, and Dr. Julie Balko for her initiative and help learning and teaching the logistics of the pressure sensitive walkway. We also thank John Pauley and the carpentry department of the Chicago Zoological Society Brookfield Zoo for help with transport, development and design of custom additions to the pressure sensitive walkway, and veterinary student, Ria Sari, for assistance with data organization and analysis. We gratefully recognize the Aurelio M. Caccamo Foundation and the Grainger Foundation for their support of this project.
# TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION AND JUSTIFICATION .................................................. 1

CHAPTER 2: LITERATURE REVIEW ........................................................................ 4

CHAPTER 3: OBJECTIVE GAIT ANALYSIS IN HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*) USING A PRESSURE SENSITIVE WALKWAY ........................................ 28

CHAPTER 4: EVALUATION OF A PRESSURE SENSITIVE WALKWAY FOR OBJECTIVE GAIT ANALYSIS IN NORMAL AND ARTHRITIC DOMESTIC DUCKS (*CAIRINA MOSCHATA DOMESTICA*) ................................................................. 42

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS ................................. 58

CHAPTER 6: BIBLIOGRAPHY ................................................................................. 60
CHAPTER 1:
INTRODUCTION AND JUSTIFICATION

Aquatic birds make up a large and charismatic portion of animals housed in zoos for educational and conservation purposes. Under professional care, aquatic birds often live extended life expectancies compared to wild counterparts; as a result, some health management challenges arise due to degenerative, infectious and environmental processes. Aquatic birds originate from a wide range of habitats worldwide, and spend various amounts of time flying, swimming, and walking or running. For example, just the Spheniscidae family (penguins) has 18 extant species ranging from New Zealand temperate rainforests down to Antarctic ice and snow (AZA Penguin TAG 2014), and the Anatidae family (ducks, geese, swans) has over 170 species living on every continent except Antarctica (Carboneras 1992).

Lameness due to osteoarthritis and/or pododermatitis is one of the most common problems encountered with aquatic birds such as ducks and penguins in human care, and prevalence in free-ranging birds is largely unknown (Backues 2015, Erlacher-Reid 2012, Reidarson 1999, Reisfeld 2013, Wyss 2015). Causes of osteoarthritis can be multifactorial but include aging, genetics, damage from trauma and/or infection, developmental abnormalities, and obesity (Buckle 2011, Dernenn 2011, Dias 1994, Horvai 2015). Causes of pododermatitis include trauma, obesity, inappropriate substrate, perching, hygiene, temperature, or water availability, overgrown claws, and nutritional disease (Ehlacher-Reid 2012, Wakenell 2016). Diagnosis is usually made on physical examination with or without radiographs to characterize bony involvement.
Several medical and surgical treatment modalities exist to address these problems, but success is variable and usually incomplete or transient. One important aspect of therapy is appropriate analgesia to help the bird recover to a normal gait. Advances have been made in the field of avian analgesia using objective efficacy measurement tools such as the incapacitance perch and the thermal threshold model (Cole 2009, Ceulemans 2014, Desmarchelier 2012, Guzman 2011, Guzman 2012, Guzman 2013, Guzman 2014a, Guzman 2014b; Paul-Murphy 2009a, Paul-Murphy 2009b). However, these methods measure weight placed on the limbs only in a perching/standing position.

The PSW has yet to be used for objective gait analysis in zoo-housed aquatic birds.

The following objectives of this project were investigated and are presented in this thesis:

1. To objectively characterize the gait of normal Humboldt penguins (*Spheniscus humboldti*) and those with historical lameness-causing disease using a pressure sensitive walkway.

2. To objectively characterize the gait of normal domestic ducks (*Cairina moschata domestica*) using a pressure sensitive walkway.

3. To develop and validate an experimental lameness induction model in domestic ducks that will facilitate analgesic efficacy studies for lameness therapy in these species.
CHAPTER 2:
LITERATURE REVIEW

The Humboldt Penguin (*Spheniscus humboldti*)

The Humboldt penguin (*Spheniscus humboldti*) belongs to Order Sphenisciformes and Family Spheniscidae. They are classified as vulnerable by the International Union of Conservation of Nature (IUCN) and are found along the western coast of South America in Peru and Chile. This species has suffered population declines from initial population estimates of over 100,000 birds to the current rangewide population estimate of approximately 32,000. Population declines at Punta San Juan, Peru (a major rookery for this species) specifically have been attributed to guano harvesting in the 1800s and El Niño events in the 1980s, causing the population to drop to about 6,000 (Birdlife International 2018 *Spheniscus humboldti*). Humboldt penguins nest on slopes of rocky coastlines in dirt and guano burrows, rock cervices, sea caves, and surface nests (Birdlife International 2018 *Spheniscus humboldti*). They breed year-round, but mostly in autumn-winter and spring (Birdlife International 2018 *Spheniscus humboldti*). Free-ranging diet consists of fish species such as anchovies, herring, silversides, hake, scad, pilchard, garfish, and squid (Birdlife International 2018 *Spheniscus humboldti*). While rearing chicks, adults forage within 35 km of the colony; however, during incubation, they will travel up to 72 km for food (Birdlife International 2018 *Spheniscus humboldti*). The major threats to this species in the wild include El Niño events that limit prey availability, interactions with fisheries (gill nets, explosives), predation by invasive rats, cats, dogs, native Andean foxes, gulls and vultures, human interaction (tourists) and consumption, and habitat loss mainly due to over-exploitation of guano and industrial development (Birdlife International 2018 *Spheniscus humboldti*).
Humboldt penguins are medium sized black and white birds with a pink patch of skin on their face that is thought to allow them to adapt in warmer climates. Zoo-housed Humboldt penguins have a lifespan of over 20 years (Gailey-Phipps 1978), while free-ranging life expectancy is largely unknown but is suspected to be shorter (Birdlife International 2018 *Spheniscus humboldti*). They are generally sociable and housed in zoos worldwide, serving as charismatic ambassadors to their wild counterparts. In the United States, the Humboldt penguin population is managed by the Species Survival Program and Penguin Taxon Advisory Group. Under professional care, common disease problems include plasmodium, aspergillosis, West Nile Virus, avian poxvirus, pododermatitis, osteoarthritis, uropygial gland infections, gastrointestinal foreign bodies, and neoplasia (Wallace 2015).

**The Domestic Muscovy Duck (Cairina moschota domestica)**

The Muscovy duck (*Cairina moschata*) belongs to Class Aves, Order Anseriformes, and Family Anatidae. It is classified as Least Concern by the IUCN; however, its global free-ranging population is suspected to be decreasing due to hunting and habitat destruction (Baldassarre 2014, BirdLife International 2018 *Cairina moschata*). This species is native to the United States, Central and South America, and lives in a very wide range of habitats (BirdLife International 2018 *Cairina moschata*). Muscovy ducks are known for a large body, with males much larger than females, and fleshy facial caruncles extending around the orbit to the base of the bill. They are omnivorous, feeding on fish, reptiles, invertebrates, crustaceans, insects, termites, water plants, seeds, and crops. Domesticated Muscovy ducks were brought to Europe, Africa, Asia, and Australia, due to their lean meat and low fat content (compared to the Asian Pekin duck), and have been bred for more varied colorations and increased size (Stahl 2005).
Overall, this species is hardy and can survive in many habitats; however, they are susceptible to diseases such as avian influenza (Stallknecht 2007), avian cholera (Botzler 1991), avian bornavirus (Hoppes 2010), duck viral enteritis (Campagnolo 2001), and aspergillosis (Wobeser 1997) among other infectious and parasitic diseases (Backues 2015). Like other waterfowl, they are susceptible to heavy metal toxicity, pododermatitis, osteoarthritis, algal toxins, oil spills, and amyloidosis (Backues 2015).

**Osteoarthritis in Birds**

Zoo and wildlife rehabilitation veterinarians are frequently challenged with cases of osteoarthritis and pododermatitis, especially in aquatic avian species, such as sphenisciformes, anseriformes, and phoenicopteriformes (Backues 2015, Erlacher-Reid 2012, Reidarson 1999, Reisfeld 2013, Wyss 2015). Osteoarthritis, also known as degenerative joint disease, is defined as degeneration of articular cartilage and the changes that occur during the bodies’ attempt to repair the damage, including bony remodeling of synovial joints, formation of bony proliferations called osteophytes, fibrosis of the joint capsule, and inflammation (Horvai 2015). Osteoarthritis occurs across vertebrate taxa and the disease is most commonly associated with aging; however, it can also be due to genetics, secondary to trauma, damage from infectious arthritis, joint or bone deformities in young animals, or underlying systemic disease such as obesity or diabetes mellitus (Buckle 2011, Degernes 2011, Dias 1994, Horvai 2015).

Information on the prevalence of osteoarthritis in free-ranging birds is scarce, likely due to the difficulty of screening and diagnosing large numbers in field settings. Retrospectively investigating museum specimens, Rothschild et al. (2006) identified that 3% of over 2,000 red-tailed hawks (*Buteo jamaicensis*) and 9.8% of almost 3,000 pigeons had osteoarthritis. In all
cases, only the tarsus was affected. An overall prevalence of 5% was found in over 40,000 non-passerine museum specimens (Rothschild 2005). This group contained free-ranging and zoo-housed avian species in many taxa. Ibises, spoonbills, raptors, stilts, and pigeons had higher frequencies of osteoarthritis lesions than ratites, tinamous, anseriformes, pelicans, and owls (Rothschild 2005).

Prevalence of osteoarthritis in zoo-housed birds is also minimally reported. Waterfowl were the taxonomic group with the highest quantity of osteoarthritis cases in one retrospective study (Degernes 2011). Degernes et al. (2011) retrospectively reported that 48% of zoo waterfowl (n = 33) that died or were euthanized between 2001 and 2005 had osteoarthritis in one or both stifles (n=13), tarsi (n=4), tarsometatarsal joints, or toe joints (n=2) based on gross, histologic, and/or radiographic confirmation. Thirteen birds had ≥ 2 joints affected. No lesions were located in the wings, no infectious pathogens were isolated from joints, and there were no associations with sex or age (Degernes 2011). Lameness was reported in 13 and pododermatitis was reported in 8 of 16 ducks in this study (Degernes 2011). There are no published reports of population prevalence of osteoarthritis in free-ranging penguins, but it is reported anecdotally (Vivanco-Prengaman 2017). Buckle et al. (2011) reported one case of a free-ranging juvenile yellow-eyed penguin (Megadyptes antipodes) with bilateral coxofemoral osteoarthritis, likely due to aseptic necrosis or chondrosis.

Obtaining a definitive antemortem diagnosis of osteoarthritis requires imaging with radiographs or CT scan. Often, lesions can be palpated via decreased range of motion or crepitus in the joint. Severity of clinical signs does not always correlate with severity of lesions found on imaging or gross examination postmortem (Kim 2015a, Sarikaya 2015). In humans with coxofemoral osteoarthritis, only reduced joint space correlated with severity of pain, while
grading of osteophytes, sclerosis, cysts, and deformities did not (Sarıkaya 2015). Furthermore, only 9.1% of hips in humans with frequent pain had radiographic changes, and 23.8% of patients with radiographic hip osteoarthritis had frequent pain (Kim 2015a). Pain from stifle arthritis in people does not correlate with radiographic findings, and treatments are thus catered to the patient’s pain score (Cubukcu 2012, Jordan 1996, McAlindon 1993). This is consistent in animals, but the methodology becomes more complicated due to the inability of animal patients to describe their level of pain (Lascelles 2012). For example, there was no correlation between pain response and radiographic findings in cats with osteoarthritis using subjective behavioral pain score and objective measurement of joint range of motion (goniometry) (Lascelles 2012). In animals, subjective and objective measures of lameness and pain are utilized (Carr 2016).

Treatment of osteoarthritis across taxa varies, but is based on pain management using medications (systemic and local injections), joint supplements, laser therapy, acupuncture, and physical therapy (Goodrich 2006, Paul-Murphy 2001, Sandersoln 2009). Efficacy of therapy in veterinary medicine is most commonly based on subjective evaluation of the patients’ gait and behavior.

**Pododermatitis in Birds**

Pododermatitis, also known as bumblefoot, is the inflammation of the plantar surface of metatarsal, metacarpal, or digital pads of the feet. It is characterized by erythema, swelling, ulceration, infection, and even osteomyelitis in severe cases (Blair 2013). The plantar surfaces of feet of birds have rough skin or pads with fibrous connective and adipose tissue between the dermis and tendons (Blair 2013). Despite this adaptation for perching, there is a very small amount of tissue between the perch, tendons, and bones (Blair 2013). The etiology of
Pododermatitis is usually primary foot trauma such as a puncture or avascular necrosis from pressure. Risk factors include obesity, poor perching or inappropriate substrate, overgrown claws, injury to the contralateral leg, or nutritional disease such as hypovitaminosis A. *Escherichia coli* and *Staphylococcus spp.* are the most common bacterial isolates (Wakenell 2016). In African penguins (*Spheniscus demersus*), being male, larger body weight, spending less time swimming and more time standing, and occupying more time on concrete vs. grated plastic, all were associated with higher prevalence of pododermatitis lesions (Ehrlacher-Reid 2012).

The prevalence of pododermatitis varies among species and populations. For example, 95-100% of zoo-housed flamingos (Nielsen 2010, Wyss 2015) and 64% of Adelie penguins (*n*=107) (*Pygoscelis adeliae*) in a professional care collection were affected (Reidarson 1999). There was no correlation with age, sex, or whether they were hand-reared, parent-reared, or wild-caught. In African penguins (*n*=31), 59% of the males and 46% of the females were treated for pododermatitis each year (Erlacher-Reid 2012). Free-ranging birds of any species are rarely reported to be affected by pododermatitis (Gentz 1996, Herman 1962, Kummmrov 2010, Wyss 2015).

Diagnosis of pododermatitis is made upon physical examination of the plantar surfaces of the feet (Blair 2013). Several scoring and classification systems have been developed. For example, the University of Minnesota Raptor Center uses a 1-5 system depending on the depth of tissue affected and prognosis, with I as mildest, affecting only integument, and V as the worst with bony involvement and loss of full pedal function (Remple 1993). Another system for flamingos uses a score of 0-2 (no lesions to severe lesions) for four different types of pododermatitis lesions that flamingos develop (hyperkeratosis, nodular, fissures, and
papillomatous growths) (Nielsen 2010). Regardless of the system, progression of lameness, depth of tissue affected, and presence of infection indicate severe disease and poor prognosis, including end-stage sequelae of osteomyelitis and loss of foot function, usually warranting humane euthanasia (Blair 2013, Nielsen 2010, Remple 1993). Further diagnostics such as a complete blood count and chemistry are used to rule out systemic disease (Blair 2013). Radiographs help determine if the lesion extends to the internal skeletal tissues, causing osteomyelitis (Blair 2013). Bacterial cultures are also frequently used to isolate organisms and help direct antibiotic therapy (Blair 2013).

Treatment methods vary and depend greatly on species and severity (Blair 2013, Wakenell 2016). First, the underlying cause should be corrected, which usually begins with adjusting perching and/or substrate appropriately, providing enrichment to allow for natural behaviors and exercise, and providing a balanced diet to avoid obesity (Blair 2013). Psittacines and raptors should be offered clean, dry perches of various size, shape, and texture. Waterfowl and other aquatic birds require access to clean water for swimming and land for resting (Blair 2013). After substrate was renovated from concrete to cobblestone for a group of aquarium-housed African penguins, prevalence of plantar foot lesions began to decrease (Ehrlacher-Reid 2012). In addition, lesions were reduced to resolved in a group of aquarium-housed Magellanic penguins (Spheniscus magellanicus) after daily time swimming was increased by one hour using environmental enrichment such as live fish and toys (Reisfeld 2013).

The basics of wound care are to keep the lesion(s) clean and dry which may require flushing, hydrotherapy, disinfectants and/or topical medications with or without bandaging—all depending on the clinician, severity of lesion, species, and ability to handle the animal (Blair 2013). Analgesics are warranted in almost all cases of pododermatitis and are discussed later in
this review. Systemic antibiotics are warranted in cases with evidence of persistent or deep infections that are not benefiting from local management (Blair 2013). Intravenous regional limb perfusion with ampicillin/sublactam has successfully treated pododermatitis in brown pelicans rehabilitating from an oil spill (Fiorello 2017). In cases with necrotic tissue and/or abscesses that need draining, surgical debridement is indicated and must be combined with other previously listed therapies in order to be successful and allow blood-flow to the affected area for healing (Blair 2013). Some alternative treatments include laser therapy, photodynamic therapy (use of a photosensitizer drug, light, and molecular oxygen to generate reactive oxygen species that destroy microbes), and acupuncture (Blair 2013, Sellera 2014, Wakenell 2016). A single treatment for pododermatitis is seldom successful on its own; almost all cases require a combination of husbandry adjustments and direct therapy to the bird depending on etiology, lesion severity, and species (Blair 2013). Progression of disease despite treatment warrants humane euthanasia in severe cases (Blair 2013).

**Lameness in Poultry**

Lameness is responsible for significant economic loss in young broilers, ducks, and turkeys due to rapid growth, trauma, infectious arthritis, developmental disorders such as angular limb deformities, husbandry problems that cause pododermatitis, systemic abnormalities such as articular gout, or nutritional deficiencies leading to fractures (Bisgaard 1981a, Bisgaard 1981b, Gentle 2011, Kestin 1992, Wilcox 2009). Thermography revealed that 45% of hens were either clinical or mildly clinical for pododermatitis (Wilcox 2009). Additionally, 16-92% of broilers were observed with pododermatitis (Pagazautundua 2006).
In response to these significant problems threatening welfare in the poultry industry, many studies evaluating pain associated with inflammatory joint disease in broiler chickens (*Gallus gallus domesticus*) have been performed. Nociceptors in the tarsal joint capsule in broilers have been sensitized using injections of adjuvant to mimic bacterial arthritis, and sodium urate solution to mimic gout (Gentle 1992, Gentle 1994, Gentle 1997). Behavioral experiments show pronounced pain-coping behaviors such as lameness, standing on one leg, and sitting after tarsal sodium urate injections (Gentle 1995). Walking difficulties in modern broilers were identified by subjective scoring systems and found that over 27.6% of birds had poor locomotion and 3.3% were almost non-ambulatory (Kestin 1992, Knowles 2008).

**Subjective Gait Analysis**

Subjective gait analysis, or visual observation of gait, is performed on a daily basis in many human and veterinary medical settings and is useful due to its practicality to identify lameness. Systematic approaches to semi-quantify lameness using a numerical rating scale or a visual analog scale are commonly utilized in the clinical setting (Figure 2.1) (Carr 2016).

However, it is difficult to be reliable, consistent and accurate due to lower sensitivity, observer variability, placebo effects, and species variability (Campbell 2014, Evans 2005, Keegan 2013, Williams 2009). Only 11% of observers were able to subjectively identify lame Labradors following cranial cruciate ligament repair, while an objective force plate identified 75% of the abnormal dogs (Evans 2005). Comparison of objective gait analysis and visual observation using a standardized assessment scale by physicians of humans with traumatic brain injury showed low accuracy (30-50%) and high variability between clinicians (Williams 2009). There was significant but weak association ($R^2 = 0.347 – 0.511$) between three experienced
equine veterinarians and objective analysis using an inertial sensor system for detection of lameness in privately owned horses of various breeds, ages, and purposes being examined for lameness or pre-purchase exam at a veterinary teaching hospital (Keegan 2013).

A placebo effect is a perceived improvement in a patient’s health condition after a sham treatment that the patient believed was a true treatment (Conzemius 2012, Turner 1994). This occurs frequently in human medicine, with the patient’s own perception of feeling better, but a placebo effect can also occur in animal caregivers and can further complicate subjective gait analysis (Turner 1994). In a prospective, randomized, double-blinded, placebo-controlled multi-center clinical trial, during subjective evaluation of dogs with osteoarthritis, there was a placebo effect present by owners 39.7% of the time, and by veterinarians 44.8% of the time at a walk, 44.8% at a trot, and 43.1% upon palpation of the joint (Conzemius 2012). Caregiver placebo effects have yet to be investigated in zoo settings but is a possibility based on these findings.

In addition to observer variability, species gait variability likely contributes to the challenges of gait analysis. Especially for zoo veterinarians and zookeepers, the variety of gaits observed daily is substantial—from bipedal primates, quadripedal carnivores and hoofstock, saltatorial (leaping) amphibians, lagomorphs, and marsupials, to flighted and non-flighted bipedal birds. Even within the world of birds, some species hop such as small passerines, some run such as roadrunners and ratites, and some swim and walk or waddle such as ducks and penguins. As a result, the animal’s strategy to compensate for lameness would therefore depend on its type of gait. Besides gaining experience around normal animals of each species, studies of normal gait for each species, or type of gait, are needed to bridge the gap between reliable subjective and objective gait analysis (Campbell 2014, Caplen 2012).
The gaits of Adelie (*Pygoscelis adeliae*), emperor (*Aptenodytes forsteri*), and white-flippered penguins (*Eudyptula albosignata*) were observed and a simultaneous measurement of oxygen consumption during locomoting on a treadmill was collected (Pinshow 1977). The authors concluded that it costs more energy to waddle than other bird species of similar body weights that walk or run such as rheas and turkeys (Pinshow 1977). Force platform analysis in emperor penguins concluded that penguins generate the same amount of force as a rhea and that waddling actually helps to conserve mechanical energy compared to what would be required for the penguins to walk (Griffin 2000). Furthermore, penguins have more frontal than lateral plane motion variability in the waddle, which is the opposite of walking animals such as humans (Kurz 2008). These studies emphasize foundational differences in gait between taxa and gait types due to natural history and anatomical adaptations. Thus it would be inappropriate to compare the gaits of such different bird species directly.

**Objective Gait Analysis**

Due to previously discussed studies that highlight discrepancies between subjective and objective gait analysis, multiple tools have been developed to consistently and accurately measure different aspects of gait in humans, domestic, and production animals. These tools are used to describe normal gait, identify lameness and disease, monitor progression of disease and efficacy of therapies, and identify production animal welfare problems. There is scant information regarding the use of these tools in a zoo animal setting. Available objective gait analysis methods used for animals include kinematics, force plates, and the focus of this thesis—pressure sensitive walkways (Table 2.1) (Agostinho 2012, Bennett 1996, Bescanon 2003, Besancon 2004, Caplen 2012, Caplen 2013, Carr 2016, Clark 1979, DeCocq 2004, Duggan 2016,

Kinematics gait analysis uses reflective, colored, or light-emitting diode markers placed on specific anatomic positions (i.e. limb joints) that are tracked by cameras in order to measure positions, velocities, angles, and acceleration or deceleration of those body parts in space (Carr 2016, Kim 2011, Torres 2015). The main advantage of this method is that it provides both two- and three-dimensional information about bone and joint movement, velocities, and range of motion (Carr 2016, Kim 2011, Torres 2015). Disadvantages of this method include animal variability in tolerance of the equipment (video cameras and markers that have to be placed on shaved skin cleaned with alcohol), human variability in marker placement, species or breed variability due to differences in body structure and lack of normal references, increased movement of the skin, and cost of the equipment (Carr 2016, Kim 2011, Torres 2015). Kinematic gait analysis has been used frequently in canine and equine orthopedic medicine and research for evaluation of dogs with hip dysplasia, treatment for dogs with experimentally-induced sodium urate synovitis, and gait effects of certain shoe-types and saddle equipment for horses (Bennett 1996, DeCocq 2004, Punke 2007, Scheffer 2001). Kinematics has also been used in production animal settings (Caplen 2013, Flower 2005). It was successful in identifying and describing lameness characteristics in cows with sole ulcers and was able to differentiate them from healthy cows (Flower 2005). Kinematics was also able to identify evolutionary gait differences between ancestral jungle fowl and selected broiler chickens (Caplen 2012), and
differentiate lame broiler chickens treated with non-steroidal anti-inflammatory from those treated with a placebo (Caplen 2013).

Kinematics using high-speed videography has also been used in biology and engineering fields to describe and compare avian flight between different species with different wing shapes, to compare use of wings of penguins to pectoral fins of fish, and to describe wing motion of bats (Clark 1979, Tian 2006, Tobalske 2003). Kinematics has also been used to describe three-dimensional characteristics of bipedal gait of ostriches using hind limb specimens (Rubenson 2007). While this methodology has been successful in describing gait parameters across taxa, it requires a large amount of equipment and tolerance by the animal and has yet to be explored in zoo species for medical purposes.

Force plate analysis measures ground reaction forces in three dimensions (vertical, medial/lateral, and anterior/posterior) via metal plates mounted on the ground. The plate is solid on top with three orthogonal piezoelectric sensors underneath the corners (Tekscan™ 2015). The most common indices used to identify lameness are peak vertical force, which is the largest force at a single point during a stance phase, and vertical impulse, which is calculated using the area under the vertical force curve, taking time into account (Besancon 2004, Carr 2016). Disadvantages include not being able to measure step and stride length, needing a long walkway with consistent velocity for multiple trials, relatively non-portable equipment, and low practicality for common practice (Carr 2016). To date, the force plate has been the most widely used and validated method in veterinary objective gait analysis; however, this method is not ideal for nondomestic species that are not trained to walk a certain speed over marked plates on the ground consistently and repeatedly (Carr 2016). Cheetah and greyhound gaits at high speeds were compared using force plates and kinematics, however this required a custom designed 90m-
long track (Hudson 2012). In poultry, both subjective gait scoring and force plate analysis did not predict leg health based on post-mortem findings (Sandilands 2011).

A pressure sensitive walkway (PSW) provides temporospatial information using a pressure-sensing mat that communicates with a computer software system to calculate several gait parameters (Tekscan™ 2015). The mat contains a matrix of resistive thin force sensors that gather information on the vertical ground reaction forces and contact area of the weight bearing-foot as the human or animal walks across the mat (Tekscan™ 2015). This is different from the force plate because the force plate only measures the total force on the top plate, giving one force measurement; however, the PSW obtains a force measurement from each high resolution sensor, providing more information on the entire foot contact area and different pressures within the plantar surface (Tekscan™ 2015). When the foot puts pressure on the sensor, it causes contact and compression between two layers of flexible piezoresistive (change in electrical resistance) ink, which then leads to a change in electrical signal and is calibrated into force units (Tekscan™ Load cell vs force sensor).

Advantages of the PSW include portability, ability to measure multiple steps in a single trial (as opposed to static step analysis with the force plate), ability to easily evaluate gait symmetry, and flexibility in patient size (Carr 2016). The PSW provides the following: number of foot strikes; cadence (steps/min); gait time, distance and velocity; stance and swing time; step time, distance (length and width), and velocity; stride time, length and velocity; maximum force in kilograms and percent of body weight; impulse (force per second); and maximum peak pressure. Gait is defined as one full cycle of the same event (i.e. the left foot heel touching the ground) and is made up of the stance phase (when the foot is in contact with the ground) and the swing phase (when the foot is not in contact with the ground). A stride is defined as the distance
that either the right and left foot moves forward during the gait cycle, while a step is the distance only the right or left foot moves forward (i.e. the right step length plus the left step length should equal the stride length). Step width is the lateral distance between the right and left foot strike (Figure 2.2) (Whittle 1993).

Pressure sensitive walkways have been validated for use in humans and dogs, but have been utilized for objective gait analysis in other domestic and several nondomestic species (Faria 2015). PSWs were validated for human gait analysis in the early 2000s (McDonough 2001) and have gained many different utilities such as evaluating therapies for post-stroke hemiplegia (Yang 2014) and evaluating obstacle-crossing deficits in elderly women (Kim 2015b).

Shortly after this system was validated for humans, PSW gait analysis in domestic animals, mainly dogs, was frequently published (Besancon 2003, Besancon 2004, Lascelles 2006, Lascelles 2012). The most commonly used PSW is the Tekscan™ system. The PSW identified improvements in dogs with cranial cruciate injuries treated with carprofen compared to untreated dogs, based on differences in ground reaction forces between groups (Horstman 2004). In dogs with spinal cord trauma (T3-L3 spinal cord lesions), combining the coefficients of variation for stride length, stride time, and swing time distinguished neurologic from non-neurologic dogs with 89% accuracy (Gordon-Evans 2009a). Neurologic dogs had significantly decreased stride time, stance time and stride length than clinically normal dogs (Gordon-Evans 2009b). Spatial and temporal parameters (stride time and length, stance and swing time) vary greatly in neurologic and ataxic dogs, making the PSW a superior objective gait analysis methodology compared to force plates or kinematics (Gordon-Evans 2009b). The PSW has also been used to characterize gait in domestic cats, finding no significant differences between males and females, or fore limbs and hind limbs (Verdugo 2013). Data obtained from force plate and
PSW analysis among various dog breeds was consistent and can each evaluate gait over time in the same dog (Besancon 2003, Lascelle 2006).

In a study evaluating gait parameters in normal female Santa Ines sheep, individuals were walked down the walkway using a halter and lead in each direction with food as a motivator. Trials were counted if they walked within a specific velocity and acceleration range and a total of five trials were performed (Agostinho 2012). No asymmetries were found between left and right limbs; however, young sheep (8-12 months old) had higher peak vertical force as percent of body weight in fore and hind limbs and higher vertical impulse as percent of body weight in forelimbs when compared to sheep over 5 years old (Agostinho 2012).

Several PSW studies with pigs investigating effects of age and growth, lameness, and analgesic treatments. First, growing weaned piglets were trained and allowed to walk across the PSW weekly from 5 – 15 weeks of age. Results showed that all parameters varied by week during growth, and that peak vertical force and pressure, load rate, and vertical impulse were higher in forelimbs than in hind limbs. This variation was concluded to be due to growth. Similar to the aforementioned dog study, higher velocities led to higher peak vertical force, pressure, and load rate. Asymmetry indices were calculated to derive absolute value, unit-less, weight-independent numbers to compare right and left temporospatial parameters. The equation for asymmetry index is \[(L-R)/(0.5(L+R))]\*100. Velocity and time did not affect the asymmetry index of any parameter, so this was determined to be the preferred tool for analysis in settings where velocity cannot be controlled (Meijer 2014a). PSW data of pigs diagnosed with lameness of one limb to normal pigs and found that lame animals were consistently asymmetric in peak vertical force and pressure, load rate, and vertical impulse. Cut-off values were made based on receiver operator curve (ROC) analysis of asymmetry indices and lame pigs could be objectively
identified. When lame pigs were treated with buprenorphine (0.04mg/kg IM) or a placebo, pigs that received buprenorphine showed objective decreased asymmetry in load rate, vertical impulse and peak vertical pressure. In addition, subjective observations of activity in an open field setting showed improved activity in animals that received buprenorphine compared to placebo (Meijer 2015). These studies collectively showed that a PSW can serve as a useful tool for objective gait analysis to confirm subjective analysis in animals that are not easily handled, are frequently afflicted with lameness affecting their welfare, and can benefit from effective analgesic therapy when indicated.

Pressure sensitive walkways have also been used for objective gait analysis in a few avian species—mainly production chickens, turkeys, and ducks (Corr 2003, Duggan 2016, Naas 2010, Naas 2012, Pickel 2011, Wyneken 2015). Broiler chickens commonly have lameness problems secondary to rapid growth and large pectoral muscle mass (Naas 2010). The gait of 28-49 day-old broiler chickens was evaluated using both a gait scoring system and a PSW (Naas 2010). Subjective gait scores increased with age and weight of the birds, objective peak forces of the right and left feet were asymmetric and slow regardless of age or weight, and walking deficiencies were more pronounced in older birds (Naas 2010). Peak vertical force differed in right and left feet, which was not identified by subjective visual evaluation (Naas 2010). Images of the walking pressure area of the plantar surface of the feet of birds with higher scores (more lame) had abnormal patterns due to the animal laying down often on the walkway (Naas 2010). The peak vertical pressures of broiler chickens were compared between individuals with and without dietary vitamin D supplementation (Naas 2012). Individuals without supplementation were observed with asymmetric pressures between right and left feet. Additionally, all animals of older age groups regardless of treatment had gait asymmetries, indicating that the PSW identified
evidence of disease associated with aging in addition to nutritional disease (Naas 2012). Based on postmortem analysis, the chickens without vitamin D supplementation had more musculoskeletal lesions (Naas 2012). The PSW confirmed the hypothesis and subjective observations that vitamin D supplementation improved gait and health of chickens using objective data, adding strength to welfare recommendations.

Objective gait analysis using a PSW can help characterize the causes of lameness in production poultry. High growth rate breeding lines of broiler chickens and ducks and those fed an ad libitum diet walked slower with feet wider apart and spent more time with two feet supporting the body than low growth rate lines and those fed a restricted diet. This evidence that higher growth rate lines lead to more mobility issues will help select for more effective breeding and feeding strategies (Duggan 2016).

Pressure sensitive walkway sensor technology has not only been used to analyze gait during walking, but also to analyze pressures of the keel and foot pads on different types of perches in order to determine which sizes and materials were most beneficial for the welfare of laying hens (Pickel 2011).

Turkey poults are known to develop footpad dermatitis when kept on wet litter, compared to dry (Mayne 2007). Two turkey breeds kept on dry or wet litter were treated with or without analgesia (betamethasone or bupivacaine). Gait differences were found between breeds in stride and stance time and single support time (time standing on one limb); gait of birds kept on wet litter consisted of decreased velocity, increased double support time (time putting weight on both legs at the same time), decreased stride length, and increased stance time, suggestive of pain. However, significant evidence of analgesia was not detected with either betamethasone- or bupivacaine-treated birds (Wyneken 2015). The reason for the lack of analgesia efficacy in this
study was unknown but pharmacokinetic and behavioral factors were suspected (Wyneken 2015).

**Pain and Lameness Models in Birds**

In order to improve welfare of birds in homes, zoos, and production settings, it is important to be able to objectively analyze gait, effectively treat problems, and objectively monitor progression or improvement. This requires developing tools used to perform pharmacodynamic studies for measuring effectiveness of medical therapies. For example, multiple pain models have been used in avian species to help determine effective medications for treating different types of pain in different avian taxa.

To start, the thermal threshold pain model has been used in many pharmacodynamic studies in a couple avian model species. For this method, a bird stands on a perch designed to deliver a heating stimulus underneath one foot. The perch increases in temperature, providing a noxious stimulus, causing the bird to lift its foot to escape the stimulus. With effective nociceptive treatment, the bird will require a stronger stimulus to lift its foot (increased thermal threshold). This model has been used to evaluate efficacy of tramadol and nalbuphine in Hispaniolan Amazon parrots (*Amazona ventralis*) (Guzman 2012, Guzman 2011), and tramadol (Guzman 2014), hydromorphone (Guzman 2013), buprenorphine (Ceulemans 2014), and butorphanol (Guzman 2014) in American kestrels (*Falco sparverius*). In addition, an incapacitance meter was modified for birds (originally used for laboratory rodents) as another avian pain model to measure analgesic efficacy of high- and low-dose meloxicam for orthopedic pain in pigeons post femoral fracture repair (Desmarchelier 2012). This objective incapacitance meter was able to detect significant differences in weight-bearing between the fractured and
normal limb 4 days after surgery, compared to subjective pain scores and ethograms that were able to detect a difference only until 2 days after surgery (Desmarchelier 2012). While this method requires more equipment than subjective scoring, it is more sensitive and robust at detecting evidence of nociception and analgesic efficacy. These thermal threshold and orthopedic pain models have contributed greatly to guiding appropriate clinical practice in avian medicine; however, the specific type of pain induced in these studies and lack of dynamic gait evaluation may be less relevant in solving issues regarding dynamic lameness due to osteoarthritis and pododermatitis in birds.

A third pain model used in animals including birds is arthritis-induction using microcrystalline sodium urate (MSU) injections. In chickens, an intra-articular injection of sodium urate crystals has been shown to induce acute inflammatory pain by sensitizing C fiber (unmyelinated, small diameter, low conductivity) nociceptors and depleting substance P (a neuropeptide) in the joint capsule, mimicking gout arthritis (Gentle 1992, Gentle 1994, Gentle 1995, Gentle 1997, Lunam 2004). Gout is a naturally occurring disease in both mammals and birds that is partly due to hyperuricemia leading to precipitation of MSU crystals within and around joints (Horvai 2015). Environmental factors (alcohol, sugar, high protein diets), genetics, and comorbidities (renal disease, metabolic syndrome) also contribute to the development of gout in humans and animals (Horvai 2015). Presence of crystals in the joint triggers release of cytokines, macrophage and neutrophil recruitment, free-radical release, complement activation, and further cytokine and arachidonic acid production (Horvai 2015). In chickens, prostaglandin D2 is the predominant prostaglandin in the intraarticular exudate after MSU injections and is a likely mediator of inflammation (Anhut 1979). This arthritis induction method has been utilized in chickens to determine the optimum analgesic dose of intra-articular bupivacaine to alleviate
pain caused by the sodium urate injections using behavioral profiles (Hocking 1997). Intra-
articular anti-inflammatory steroids (betamethasone, dexamethasone, and methylprednisolone),
but not µ-opioids (morphine, fentanyl and buprenorphine), were effective at treating pain
induced by MSU intra-articular injections in chickens using behavior profile analysis (Gentle

MSU arthritis induction was also used in psittacines as a method to determine analgesic
efficacy (Paul-Murphy 2009a, Paul-Murphy 2009b). In green-cheeked conures (*Pyrrhura
molinae*), MSU injections caused decreased weight bearing in the affected limb as measured via
the incapacitance meter perch, and subcutaneous injections of liposome-encapsulated
butorphanol tartrate (LEBT) were effective at increasing weight bearing. Interestingly, the
subjective analysis of activity and feeding behaviors was not able to identify a difference
between conures with and without induced arthritis (Paul-Murphy 2009). A similar experiment
was performed on Hispaniolan parrots to compare analgesic efficacy of intramuscular carprofen,
LEBT and carprofen + LEBT (Paul-Murphy 2009b). Carprofen alone was ineffective at
increasing weight bearing on the induced limb, but LEBT and LEBT + carprofen were effective
(Paul-Murphy 2009b). A follow-up study determined that meloxicam at 1mg/kg IM (but not
lower doses) was also effective at relieving pain from MSU injections, using the objective
analysis of the incapacitance perch (Cole 2009).

There is a gap in the literature for dynamic objective gait analysis using a PSW in
production and zoo-housed waterfowl to determine efficacy of commonly used analgesic
medications. Based on the above review, avian species vary greatly as to which analgesics are
effective in each taxa and for each type of pain investigated. Therefore, our objectives are to 1)
develop the PSW as a tool to identify lameness in normal zoo-housed Humboldt penguins and
those with naturally occurring arthritis and/or pododermatitis, and 2) to use the PSW to analyze normal gait of domestic Muscovy ducks with and without experimentally induced arthritis using MSU injections. The goal will be to use the PSW in the future for analgesic efficacy studies, similar to the incapacitance perch described previously.
<table>
<thead>
<tr>
<th>Table 2.1 Comparison of objective gait analysis methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinematics</td>
</tr>
<tr>
<td>Mechanism</td>
</tr>
<tr>
<td>Information obtained</td>
</tr>
<tr>
<td>Information not obtained</td>
</tr>
<tr>
<td>Species flexibility</td>
</tr>
<tr>
<td>Disadvantages</td>
</tr>
<tr>
<td>Photo</td>
</tr>
</tbody>
</table>
**Fig. 2.1** Example of visual analog scale and numerical rating scale used in subjective gait analysis (Carr 2016).

**Fig. 2.2** Diagram depicting the spatial parameters of gait measured by a pressure sensitive walkway (R = right, L = left).
CHAPTER 3:

OBJECTIVE GAIT ANALYSIS IN HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*) USING A PRESSURE SENSITIVE WALKWAY

**ABSTRACT:** Assessment of pododermatitis, osteoarthritis, and other causes of lameness in penguins can be challenging. Subjective gait analysis using visual observation and response to analgesic therapy can be affected by observer variation and caregiver placebo bias. A pressure sensitive walkway (PSW), however, allows for objective gait analysis and assessment of analgesic therapeutic response. In this study, a 3-meter long PSW was used to analyze gait in 21 adult Humboldt penguins (*Spheniscus humboldti*). Medical record reviews and comprehensive examinations were performed on all penguins; five penguins were considered abnormal (either right-sided (n=3) or bilateral historical lameness-causing disease (n=2) and analyzed separately from the normal data set). All penguins walked across the PSW four times and gait parameters (step and stride distances and velocities, maximum force, impulse, and peak pressure) were calculated for each foot in each penguin. Statistical comparisons were made between right and left feet, sexes, and normal and abnormal penguins for each gait parameter. Among normal penguins, there were no significant differences between feet or sex. Left step width was shorter in abnormal penguins than that of normal penguins. Study results established baseline values for Humboldt penguins. This will allow objective monitoring of progression and/or response to therapy in penguin lameness cases, both current and future. The data also provides a foundation to compare to gait parameters with other penguin populations and species.
INTRODUCTION

Pododermatitis and osteoarthritis are common causes of lameness in penguins under professional care. Multifactorial etiologies are common, with substrate, temperature, time spent swimming, obesity, trauma, and age-related joint degeneration as contributing factors (Erlacher-Reid 2012, Reisfeld 2013). Studies report that the prevalence of pododermatitis in zoo-housed penguins at some facilities can be as high as 46 – 64% (Erlacher-Reid 2012, Reidarson 1999). While the prevalence of osteoarthritis in penguins is unknown, a gross and histopathological analysis of zoo waterfowl at one facility reported that up to 48% are affected (Degernes 2011). Humboldt penguins (HP; *Spheniscus humboldti*) are a vulnerable species, native to coastal Chile and Peru, that are represented in zoos for conservation and education purposes (Birdlife International 2018 *Spheniscus humboldti*). Like all penguins, HP are susceptible to these lameness-causing diseases and consistent methods to evaluate such lameness are desirable to improve outcomes.

Subjective gait analysis using visual observation and response to analgesic therapy can be affected by observer variation and caregiver bias (Conzemius 2012). Forty percent of caregivers and 44% of veterinarians reported nearly identical subjective improvements in the gait of osteoarthritic dogs given a placebo or treatment despite no improvements in objective force plate analysis (Conzemius 2012). Diagnostic modalities for detection of lameness, including advanced imaging, do not always correlate with severity of discomfort in people or animals (Lascelles 2012, Sarikaya 2015, Kim 2015). In humans, only 9.1% of hips in patients with frequent pain had radiographic changes (Kim 2015). In cats with osteoarthritis, there were no correlations
between behavioral pain reactions and radiographic findings, goniometry, or objective measurement of range of motion (Lascelles 2012).

Several methodologies for objective gait analysis exist, including kinematics, force plates, and pressure sensitive walkways. Kinematic and force plate analysis provide important three-dimensional information but can require non-portable, expensive equipment, and patient tolerance to either the markers placed on the body during kinematic evaluations or specific step timing and placement during force-plate analysis (Torres 2015, Carr 2016, Kim 2011, Caplen 2012). These methods are rarely practical for nondomestic and avian species.

Pressure sensitive walkways (PSWs) are commonly used for gait analysis in people and veterinary application is increasing. With nondomestic species, PSWs allow for measurement of multiple steps in a single trial (compared to a single step with the force plate), easy evaluation of gait symmetry, and flexibility (e.g. patient size and portability). The resulting objective gait analysis from PSW use has been used to assess therapeutic response to analgesics in dogs, pigs, and ducks, as well as gait response to dietary vitamin D supplementation in broiler chickens (Bailey 2019, Horstman 2004, Meijer 2015, Naas 2012).

The goals of this study were to objectively measure gait parameters in zoo-housed HP using a PSW and to compare results between males and females, right and left feet, and those without history of orthopedic disease or pododermatitis (normal) and those with a history of orthopedic disease or pododermatitis (abnormal). The hypothesis is that gait parameters of normal HP will not differ between sex or laterality, but will differ from abnormal HP.
MATERIALS AND METHODS

Twenty-one (13 males, 8 females) adult HP at the Brookfield Zoo were included in this study. A full physical examination was performed on each animal as part of its preventative health care. A medical record review was also performed for each animal. Abnormal HP were identified as those with evidence of active or chronic lameness, pododermatitis, or osteoarthritis based on physical findings during the current examination or in the medical record review (previous examinations and diagnostic imaging).

A 3-meter PSW (Tekscan™ Walkway 7 System; South Boston, Massachusetts, USA) was custom-modified with wooden corral panels to guide the HP in a straight path (Figure 3.1). The PSW was calibrated for an appropriate body weight range for Humboldt penguins. Each HP walked across the PSW until 4 full trials were completed. If a full trial, defined as uninterrupted complete step readings for at least half the length of the PSW, was not obtained, the trial was repeated. A trial was excluded if it included partial footstrikes on the edges of the PSW, changing direction of movement, stopping, or hopping. If the HP did not walk voluntarily, a person walked adjacent to the PSW with a towel draped behind the HP to guide it. The PSW measured and calculated several temporospatial parameters for each HP and limb. Parameters included (1) cadence (steps/min); (2) gait time (sec), distance (cm), and velocity (cm/sec); (3) stance and swing time; (4) step time, distance (length and width), and velocity; (5) stride time, length, and velocity; (6) maximum force (kg and % of body weight); (7) impulse (kg per second and % of body weight per second); and (8) maximum peak pressure (kPa). Gait is defined as one full cycle of the same event (i.e. the left foot heel touching the ground) and is made up of the stance phase (when the foot is in contact with the ground) and the swing phase (when the foot is not in contact with the ground). A stride is defined as the distance that the right and left foot
moves forward during the gait cycle, while a step is the distance only the right or left foot moves forward (i.e. the right step length plus the left step length should equal the stride length). Step width is the lateral distance between the right and left foot strike (Figure 3.2). The PSW software calculates maximum force and impulse generated by each step as percentage of body weight, allowing direct comparison between HP of different weights and comparison between left and right feet to identify symmetry or lack thereof. Calculating the absolute value of the difference between right and left feet provides the expected amount of difference for that parameter between each foot. If an animal has a value that falls outside of this expected range, asymmetry between limbs is likely present.

The average of the four trials for each parameter was calculated. Descriptive statistics and absolute value of the difference between right and left limb (|R-L|) were calculated for each gait parameter. Normality was tested using the Shapiro-Wilk test. Values between R and L feet were compared using a paired t-test or Wilcoxon signed rank test, values between males and females were compared using an independent two-sample t-test, and values between normal and abnormal HP were compared using a Mann-Whitney U test (Microsoft® Excel® for Mac 2011, Microsoft, Redmond, Washington 98052, USA; and SPSS Version 24, IBM Statistics, Chicago, IL 60606, USA).

RESULTS

Physical examinations revealed an overall healthy population. Five of 21 (23.8%; 3 males, 2 females) examined HP were identified as abnormal. Physical examination abnormalities included decreased stifle range of motion, footpad hyperkeratosis, and footpad eschar or callus. Historical abnormalities included chronic lameness and joint swelling of unknown etiology,
chronic pododermatitis and osteoarthritis, and digit subluxation. Three of five abnormal HP had historical right-sided lameness while two had bilateral lameness. Only one HP was on long-term analgesics (oral meloxicam). Information regarding abnormal HP history evaluation is provided in Table 3.1.

Descriptive statistics for body weight, age, and gait parameters are provided in Tables 3.2 – 3.5 for normal and abnormal HP. In normal HP, there were no significant differences between feet or sex for any gait analysis parameters. Left step width was significantly shorter in abnormal (median [range] 9.2 cm [7.2 – 11]) compared to normal HP (mean ± standard deviation 10.7 ± 1.0 cm). No other gait parameters differed between the groups including the absolute value of the difference between right and left step widths. Examples of the footstrikes generated by the PSW software for a normal and abnormal HP are shown in Figure 3.3. Of the 84 analyzed trials, 7 were repeated once, 6 were repeated twice, and 1 was repeated 3 times to obtain a complete set of 4 adequate trials.

DISCUSSION

The PSW is an effective and noninvasive method for objective gait analysis in HPs. Data supported the hypothesis that there would be no differences in the gait parameters of normal HP based on sex or foot laterality. These results provide robust data on normal HP gait parameters that can be used for evaluation of abnormal penguins and temporospatial comparisons.

Left step width differed between normal and abnormal penguins. The clinical significance of this difference is unknown due to variable diagnoses and small sample size of abnormal HP. However, more abnormal penguins (3/5) had historical right-sided disease and none had left-sided disease. Larger step width in Pekin ducks (*Anas platyrhynchos domesticus*) is
associated with imbalance (Duggan 2016) and the waddle of healthy King penguins has a more consistent step width than step length (Kurz 2008). A shortened left step width may be an effect of compensation during right-sided lameness to increase balance, and step width may be a sensitive parameter for detecting lameness due to its narrower variability in penguins. A controlled study with a larger sample size of animals with consistent diagnoses would be needed to confirm that step width is an important indicator of lameness in penguins.

Some penguins required multiple attempts to complete a full trial. While the number of trials requiring a towel to help guide the animal was not recorded, this should be considered in future studies. It is possible that the trial repetitions and presence of extra guidance down the PSW could affect gait parameters by inciting a fight or flight response, masking gait abnormalities. In addition, there was a wide range in gait velocity in the current study, the significance of which is unknown in this species. Thus, it is recommended to develop a method to assure a uniform velocity and decreased necessity for additional repetitions during the trials in future studies. This might include a steady standardized panel moving behind the animal, or conditioning the animals to walk down the PSW at a targeted pace.

Because this group had multiple causes of historical lameness, it is challenging to directly compare the percentage of HP identified as abnormal in this study (23.8%) to studies of penguin pododermatitis prevalence, which trend higher toward 46 – 64% (Ehrlacher 2012, Reidarson 1999). There are currently are no other published reports of general lameness prevalence in penguins under professional care.

The importance of species-specific objective gait analysis is highlighted with the unique gait of the penguin. Avian gaits vary tremendously by species ranging from hopping and landing stances employed by flighted birds, to purely bipedal walking seen in ratites and roadrunners, to
waddling gaits seen in aquatic bird species. As a result, the animal’s strategy to compensate for lameness would depend on its type of gait (Caplen 2012). Studies of normal gait for each species, or type of gait, are needed to bridge the gap between subjective and objective gait analysis (Campbell 2014, Caplen 2012).

The gaits of Adelie (Pygoscelis adeliae), emperor (Aptenodytes forsteri), and white-flippered penguins (Eudyptula albosignata) cost more metabolic energy than gaits of other bird species of similar body weights that walk or run such as rheas and turkeys (Pinshow 1977). Force plate analysis in emperor penguins concluded that penguins generate the same amount of force as a rhea, just twice as fast due to their relatively short legs, and that waddling actually helps to conserve mechanical energy compared to what would be required for the penguin to walk (Griffin 2000). Furthermore, penguins have more frontal than lateral plane motion variability in the waddle, which is the opposite of bipedal animals such as humans (Kurz 2008). These studies emphasize foundational differences in gait between taxa and gait types due to natural history and anatomical adaptations. Thus, it would be inappropriate to compare the gaits of such different bird species directly.

In conclusion, the PSW objectively identified symmetrical gait in normal HP and shorter left step width in abnormal HP. While the abnormal penguin data set was not controlled or of adequate sample size in this study to stand alone, these preliminary findings indicate that contralateral step width may be a marker of lameness. The lack of other differences may suggest that lesions were not significant enough to cause significant gait changes at the time of the study or that the novelty of walking across the PSW could cause a stress response that may mask gait abnormalities. Baseline values from this study will be used to objectively monitor progression of disease and response to therapy in this population of HP if they develop clinical lameness in the
future. This research will also set the framework for investigating efficacy of various therapeutics in lame birds and will allow comparison of gait parameters to other related penguin species.
Table 3.1.

Information for Humboldt penguins (*Spheniscus humboldti*) classified as abnormal due to chronic intermittent lameness (n=5).

<table>
<thead>
<tr>
<th>Penguin</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Historical lameness</th>
<th>Historical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>28.3</td>
<td>Right</td>
<td>Stifle osteoarthritis (right marked, left mild)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>26.7</td>
<td>Bilateral</td>
<td>Spondylosis</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>5.7</td>
<td>Right</td>
<td>Right digit 3 subluxation and sclerosis</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>20.9</td>
<td>Bilateral</td>
<td>Decreased stifle range of motion</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>7.6</td>
<td>Right</td>
<td>Right pododermatitis</td>
</tr>
</tbody>
</table>

Table 3.2.

Body weight, age, and objective gait analysis results of 16 normal Humboldt penguins (*Spheniscus humboldti*) using a pressure sensitive walkway.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>4.4 ± 0.36 (4.3 - 4.6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.5 ± 6.6 (8.3 – 14.7)</td>
</tr>
<tr>
<td>Cadence (steps/min)</td>
<td>268 ± 53.2 (242 – 294)</td>
</tr>
<tr>
<td>Gait distance (cm)</td>
<td>250 ± 23.5 (239 – 262)</td>
</tr>
<tr>
<td>Gait time (sec)</td>
<td>5.2 ± 1.8 (4.3 – 6.1)</td>
</tr>
<tr>
<td>Gait velocity (cm/sec)</td>
<td>54.0 [35.4 – 102]</td>
</tr>
</tbody>
</table>

Values reported as mean ± standard deviation (95% confidence interval) if normally distributed or median [range] if not normally distributed (Shapiro-Wilk Test p < 0.05).
Table 3.3.

Objective gait analysis results of 16 normal Humboldt penguins (*Spheniscus humboldti*) using a pressure sensitive walkway

| Parameter                  | Right                   | Left                     | |Right – Left| |
|----------------------------|-------------------------|--------------------------|------------------------|-----------------|------------------------|------------------------|------------------------|
| Stride length (cm)         | 25.5 ± 4.9 (23.1 – 27.9) | 25.9 ± 5.5 (23.2 – 28.6) | 0.4 [0 – 5.5]          |
| Stride time (sec)          | 0.5 ± 0.1 (0.4 – 0.5)    | 0.5 ± 0.9 (0.4 – 0.5)    | 0 [0 – 0.1]            |
| Stride velocity (cm/sec)   | 54.5 [36.3 – 102]       | 54.0 [35.4 – 101]       | 0.5 [0 – 2.6]          |
| Step length (cm)           | 13.1 ± 3.0 (11.7 – 14.6) | 13.0 ± 2.8 (11.6 – 14.4) | 0.6 [0 – 3.5]          |
| Step time (sec)            | 0.2 [0.2 – 0.3]         | 0.2 ± 0.4 (0.2 – 0.3)    | 0.03 [0 – 0.1]         |
| Step velocity (cm/sec)     | 60.7 ± 19.6 (51.1 – 70.3)| 52.0 [38.6 – 112]       | 4.7 [0.5 – 18.7]       |
| Step width (cm)            | 10.3 ± 0.8 (9.9 – 10.7)  | 10.7 ± 1.0 (10.2 – 11.2) | 0.8 ± 0.7 (0.5 – 1.2) |
| Stance time (sec)          | 0.3 ± 0.1 (0.3 – 0.4)    | 0.3 ± 0.1 (0.3 – 0.4)    | 0 [0 – 0.1]            |
| Swing time (sec)           | 0.2 ± 0                 | 0.2 [0.1 – 0.3]         | 0 [0 – 0.1]            |
| Max force (% BW)           | 105 ± 10.1 (100 – 110)  | 101 ± 9.3 (96 – 106)     | 5.4 ± 4.2 (3.3 – 7.5)  |
| Impulse (%BW*sec)          | 21.2 ± 4.4 (19.1 – 23.3) | 20.5 ± 4.8 (18.2 – 22.9) | 2.5 ± 1.9 (1.6 – 3.4)  |
| Max Peak Pressure (kPa)    | 86.1 ± 12.1 (80.2 – 92.0)| 84.5 ± 13.8 (77.7 – 91.3)| 9 [1 – 37]            |

Values reported as mean ± standard deviation (95% confidence interval) if normally distributed or median [range] if data not normally distributed (Shapiro-Wilk Test p < 0.05).

|Right – left| = absolute value of the difference between right and left foot parameters.

Table 3.4.

Body weight, age, and objective gait analysis results of 5 abnormal Humboldt penguins (*Spheniscus humboldti*) using a pressure sensitive walkway

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>4.06 [3.15 – 4.26]</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.9 [5.67 – 28.3]</td>
</tr>
<tr>
<td>Cadence (steps/min)</td>
<td>279.3 [183 – 339.2]</td>
</tr>
<tr>
<td>Gait distance (cm)</td>
<td>246.2 [168.1 – 253.2]</td>
</tr>
<tr>
<td>Gait time (sec)</td>
<td>3.9 [2.99 – 8.68]</td>
</tr>
<tr>
<td>Gait velocity (cm/sec)</td>
<td>70.9 [19.7 – 85.3]</td>
</tr>
</tbody>
</table>

Values reported as median [range].
Table 3.5.

Objective gait analysis results of 5 abnormal Humboldt penguins (*Spheniscus humboldti*) using a pressure sensitive walkway

| Parameter                  | Right            | Left             | |Right – Left| |
|----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Stride length (cm)         | 28.4 [12.8 – 35.2] | 28.3 [12.9 – 35.4] | 0.1 [0 – 0.3] | | | |
| Stride time (sec)          | 0.43 [0.36 – 0.66] | 0.43 [0.37 – 0.66] | 0 [0 – 0.1] | | | |
| Stride velocity (cm/sec)   | 71.8 [19.6 – 92.1] | 71.3 [19.9 – 92.8] | 0.1 [0.1 – 0.7] | | | |
| Step length (cm)           | 14.1 [5.5 – 15.8] | 13.8 [7.3 – 19.3] | 1.2 [0.6 – 3.5] | | | |
| Step time (sec)            | 0.23 [0.18 – 0.3] | 0.23 [0.17 – 0.35] | 0.03 [0.02 – 0.05] | | | |
| Step velocity (cm/sec)     | 75.3 [18.6 – 100] | 68.9 [21.1 – 92.5] | 6.8 [0.1 – 17.2] | | | |
| Step width (cm)            | 10.1 [8.5 – 10.5] | 9.2 [7.2 – 11] | 0.7 [0.3 – 2.9] | | | |
| Stance time (sec)          | 0.25 [0.21 – 0.53] | 0.25 [0.22 – 0.5] | 0.01 [0.01 – 0.03] | | | |
| Swing time (sec)           | 0.16 [0.13 – 0.19] | 0.17 [0.15 – 0.18] | 0.01 [0 – 0.03] | | | |
| Max force (% BW)           | 108.1 [87.9 – 115.2] | 109.8 [83.1 – 127.7] | 5.6 [1.8 – 12.5] | | | |
| Impulse (%BW*sec)          | 18.1 [15.9 – 29.3] | 18.7 [16.5 – 25] | 1.1 [0.5 – 4.3] | | | |
| Max Peak Pressure (kPa)    | 76 [47 – 104] | 83 [48 – 107] | 3 [1 – 10] | | | |

Values reported as median [range].
**Fig. 3.1.** Adult Humboldt penguin (*Spheniscus humboldti*) on a pressure sensitive walkway.

**Fig. 3.2.** Definitions of gait parameters overlaid on foot-strike data from a Humboldt penguin (*Spheniscus humboldti*) walking on the Tekscan™ Walkway 7 System.
Fig. 3.3. Foot-strikes generated by the Tekscan™ Walkway 7 System software for a Humboldt penguin (Spheniscus humboldti) without (top) and with (bottom) history of lameness-causing disease. The normal penguin had less strikes, longer steps, and less force as percentage of body weight than the abnormal penguin.
CHAPTER 4: EVALUATION OF A PRESSURE SENSITIVE WALKWAY FOR OBJECTIVE GAIT ANALYSIS IN NORMAL AND ARTHRITIC DOMESTIC DUCKS (*CAIRINA MOSCHATA DOMESTICA*)

**ABSTRACT:** The objective of this study was to evaluate the use of a pressure sensitive walkway (PSW) for objective gait analysis in normal domestic ducks (*Cairina moschata domestica*) and those with experimentally induced arthritis. Eighteen healthy adult ducks walked across the PSW four times in each experiment at each time point. For experiment 1, gait parameters (step and stride distances and velocities, maximum force, impulse, and peak pressure) were calculated for each foot in each duck (time 0). For experiment 2, six of these ducks were randomly selected, anesthetized, and administered a unilateral intra-tarsal injection of monosodium urate solution to induce arthritis. Serial PSW trials were repeated at 1, 2, 3, 4, 8, and 24 hours post-injection. Gait parameters were calculated and compared at each time point, including baseline at time 0. Among the normal ducks, there were no significant differences between right and left feet for any gait parameter. Maximum force and impulse were significantly lower for the affected limb at the 3- and 4-hour time points in ducks with unilateral induced arthritis. This asymmetry was resolved by 8 hours post injection. This PSW transient arthritis model allows for objective assessment of lameness in domestic ducks with maximum force and impulse serving as the most sensitive gait parameters for lameness detection. This method has potential as a model to assess analgesic efficacy for zoo-housed waterfowl and other avian species.
INTRODUCTION

Lameness, due to pododermatitis, osteoarthritis, and nutritional diseases, is a common health condition in birds with significant impacts on welfare in human care settings. Objective gait evaluation with a pressure sensitive walkway (PSW) has been used as a tool for welfare assessment in poultry, as well as assessment of lameness and response to therapy in domestic mammals (Duggan 2016, Hortsman 2004, Naas 2012, Verdugo 2013, Wyneken 2015). Objective gait analysis of birds with lameness-causing diseases could provide non-biased assessment and therapeutic monitoring for zoo clinicians.

Avian analgesic efficacy studies use validated nociception models to establish evidence-based clinical standards for appropriate medications and dosing regimens, improving health and welfare of avian species (Cole 2009, Ceulemans 2014, Guzman 2013, Guzman 2014a, Guzman 2014b, Paul-Murphy 2009a, Paul-Murphy 2009b). The thermal threshold model conditions a bird to stand on a perch that differentially increases in temperature under one foot causing the bird to lift its foot to escape the stimulus. With effective analgesic treatment, the bird will require a stronger stimulus to lift its foot (increased thermal threshold) (Ceulemans 2014, Guzman 2013, Guzman 2014a, Guzman 2014b). Monosodium urate (MSU) induced arthritis transiently mimics articular gout. An incapacitance meter perch measures the amount of weight placed on a limb that has had a MSU solution injected into the tarsal joint. With effective analgesia, the bird will bear more weight on the affected limb than without treatment (Cole 2009, Paul-Murphy 2009a, Paul-Murphy 2009b). The majority of efficacy studies have been performed in psittaciformes and falconiformes, measuring weight-bearing at a standstill. There has yet to be a dynamic avian nociception model developed to address lameness for aquatic birds, which are commonly diagnosed with lameness from diseases such as pododermatitis and osteoarthritis.
Traditional gait assessment in veterinary medicine is based on skilled subjective evaluation, but remains flawed due to “caregiver” placebo bias, variability between observers, and differences in evaluation based on variation in gait dynamics between species (e.g. waddling penguin, walking ratite, hopping passerine) (Carr 2016, Conzemius 2012, Evans 2005). Objective gait analysis methods commonly used in veterinary medicine, such as force plate and kinematic analysis, have been validated primarily for canine and equine patients. These methods, while useful, can be challenging to adapt to nondomestic species due to portability, cost, inflexibility related to patient size and gait type, and behavioral compliance with evaluation methods (Carr 2016).

The pressure sensitive walkway (PSW) objectively provides temporospatial information using a pressure-sensing mat that communicates with a computer software system to calculate several gait parameters. The mat contains a matrix of resistive thin force sensors that gather information on the vertical ground reaction forces and contact area of the weight-bearing foot as the person or animal walks across the mat (Tekscan™ Load cell vs. force sensor). Advantages of the PSW include portability, ability to measure multiple steps in a single trial (as opposed to static step analysis with the force plate), ability to easily evaluate gait symmetry, and flexibility in patient size. Objective gait assessment technology has been used in human, domestic animal, production animal (poultry, bovine, and porcine) and has identified some differences in gait parameters that subjective evaluation has missed (Agostinho 2012, Besancon 2003, Duggan 2016, Horstman 2004, Kim 2015, Lascelle 2006, McDonough 2001, Meijer 2014a, Naas 2010, Pickel 2011, Verdugo 2013). The PSW has yet to be utilized for zoological species in human care or as an avian analgesia efficacy tool using MSU-induced arthritis.
The purpose of this study is to evaluate the PSW as an objective gait analysis tool for normal ducks and those with experimentally induced arthritis as an avian model with the goal of using this tool for future analgesia efficacy studies. Authors hypothesize that the PSW will successfully measure gait parameters of ducks and that objective gait parameters will not differ significantly between right and left feet. In addition, authors predict that a unilateral tarsal MSU injection will cause transient lameness in domestic ducks that will be detectable and quantifiable by the PSW.

MATERIALS AND METHODS

This study was approved by the University of Illinois Institutional Animal Care and Use Committee (protocol # 17226). Twenty adult domestic Muscovy ducks (Cairina moschata domestica) of unknown sex were acquired from a university-approved vendor. Ten ducks were housed in two rooms (9m x 4m) each containing a longitudinal pool (7.6m long x 1.4m wide). Floors were sealed, smooth, painted concrete. One side of the room was filled with continuously flowing water and a deep end for swimming. The other side had varied substrate of rubber coated platforms, outdoor carpet, and pine shavings. Water and poultry feed were provided ad libitum. (Purina Premium Poultry Feed, Flock Raiser Crumbles, Purina Animal Nutrition LLC, Shoreview, Minnesota 55126, USA). The rooms were pressure-washed and cleaned at least once daily.

Five days after arrival, each duck was weighed and had a complete physical examination performed by a veterinarian (JDS). Animals were individually identified using 1-2 plastic colored cable ties placed around either the right or left tarsometatarsus. Eighteen ducks met the inclusion criteria of lacking foot lesions or apparent lameness. Ducks were allowed to acclimate
to their environment for three weeks. Health was assessed through the duration of this study via daily visuals by animal care staff and physical examination including body weight at intake and before each experiment.

For experiment 1, baseline objective gait analysis was performed. A 3-meter long pressure sensitive walkway (PSW; Tekscan™ Walkway 7 System; Tekscan™, South Boston, Massachusetts 02127, USA) covered with a 2mm thickness smooth rubber mat (Multy Home LP, Concord, Ontario, Canada) was set up in an adjacent room. Custom-designed panels were added in order to help guide the ducks down the walkway (Figure 4.1). One side was wooden and the other was clear acrylic for observational purposes. The PSW was calibrated for an appropriate body weight range for ducks. Each duck was encouraged to walk down the PSW at least 4 times (4 trials). If a full trial, defined as uninterrupted complete step readings for at least half the length of the PSW, was not obtained, the trial was repeated until 4 full trials were collected for each animal. Exclusions included partial footstrikes on the edges of the PSW, changing direction of movement, stopping, or partially taking flight. If the duck did not walk along the PSW voluntarily, a person walked adjacent to the PSW with a towel draped behind the duck to guide it along the PSW. The walkway software measured and calculated several temporospatial parameters for each animal and each limb. Parameters included number of strikes; cadence (steps/min); gait time, distance and velocity; stance and swing time; step time, length, width, and velocity; stride time, length and velocity; maximum force in kilograms and percent of body weight; impulse in kilogram meter per second and percent of body weight meter per second; and maximum peak pressure.

For experiment 2, six ducks were randomly selected for the MSU lameness induction model using a computer based random number generator. MSU crystal solution (3%) was
prepared in accordance with methods described previously (Paul-Murphy 2009a, Paul-Murphy 2009b). Specifically, 5mL 1N NaOH was added to 195mL sterile water and pH was adjusted to 12. The alkaline solution was boiled and stirred, 1g of uric acid was added, boiled for another 5 minutes, and then was removed from heat. An additional 1N NaOH was added until the solution cleared. This sat at 23°C for 48 hours, pH was adjusted to 8 using NaOH, and sat at 23°C for 48 more hours. It was centrifuged at 693xg for 15 min, the supernatant was decanted, and the crystals were washed in sterile saline (250mL). The solution was centrifuged again at 693g for 20 min, and the supernatant decanted. Crystals were re-suspended in 25mL sterile saline in a sterile glass flask which was then capped and autoclaved at 93.3°C for 2 hrs. The suspension was pipetted with continuous stirring into sterile 30ml glass vials, centrifuged at 390xg for 15 min, and supernatant decanted. Then 33mL of sterile saline was used to suspend the crystal and make a 3% MSU solution. The presence of crystals in solution was confirmed by microscopic examination.

Baseline objective gait analysis (time 0) was performed using the same methodology as experiment 1 immediately before the lameness induction procedure. For each of the six ducks, general anesthesia was induced using 5% isoflurane and 100% oxygen at 1L/min via facemask under manual restraint. Delivered isoflurane percentage was adjusted to maintain a moderate to light plane of anesthesia. Heart rate was monitored using a stethoscope and respiratory rate by visual observation. Once at a working plane of anesthesia, the tarsus was aseptically prepared, the MSU solution was thoroughly mixed, and 0.2mL was injected into the cranial aspect of the right or left tarsal joint (side determined by a coin-toss) using a 1-cc syringe and 22-ga, 1-in hypodermic needle (Figure 4.2). Correct placement was confirmed via aspiration of synovial fluid prior to injection and palpation of joint capsule expansion following injection. Following
injection isoflurane was immediately discontinued and ducks were recovered from anesthesia under manual restraint, followed by undisturbed time in a holding kennel for 30 – 60 min. Following recovery, serial PSW trials using the same methodology as experiment 1 were performed at 1, 2, 3, 4, 8, and 24 hours post MSU injection. Ducks were held in individual plastic or metal kennel between the 1, 2, 3, and 4 hour trials. They were placed in a larger kennel with food and water between the 4 and 8 hour trials and then returned to their normal housing until the 24 hour trial the following day. After the 24 hour PSW trial, each duck was anesthetized with alfaxalone (5-10 mg/kg intramuscular or intravenous; Alfaxan®; alfaxalone, 10mg/ml, Jurox Pty Limited, Rutherford, NSW 2320, Australia) and euthanized with an overdose of intravenous pentobarbital (Euthasol®; pentobarbital sodium 400mg/ml, Le Vet. Pharma BV, Wilgenweg 7, 3421 TV, Oudewater, The Netherlands) based on protocol requirements. Carcasses were incised along the coelom, sex was determined, and then were appropriately disposed of. Six of the remaining ducks from experiment 1 were used in a pharmacokinetic study prior to the current study, and the remaining ducks were used in a pharmacodynamics study after the current study (Bailey 2018, Bailey 2019).

For statistical analysis of experiment 1, the average of the four trials for each parameter and each foot were calculated. Descriptive statistics and absolute value of the difference between right and left limb (|R-L|) were calculated for each gait parameter. Normality was tested using the Shapiro-Wilk test. Values between R and L feet were compared using a paired t-test or Wilcoxon signed rank test for normally and non-normally distributed data, respectively. All statistical analyses were performed using commercial software (Microsoft Excel for Mac 2011; Microsoft, Redmond, Washington 98052, USA; SPSS Version 24; IBM Statistics, Chicago, IL 60606, USA). For experiment 2 statistical analysis, the average of the four trials for each
parameter was calculated. A repeated measures analysis of variance was performed for each
parameter and absolute value of the difference between right and left limb (|R-L|) at time points
0-24 hours for all six ducks combined. Statistical significance was determined at a level of p <
0.05.

RESULTS

All eighteen ducks were in good body condition and had no significant abnormalities
upon initial physical examination. The ducks remained healthy throughout the study period (all
maintained or gained weight and none had abnormalities on physical examination prior to each
experiment) and successfully completed the PSW trials. Of the 72 trials analyzed in experiment
1, 10 were repeated once and 5 were repeated twice in order to obtain a complete set of 4
adequate trials. Descriptive statistics for body weight and gait parameters for experiment 1 are
provided (Tables 4.1 and 4.2). Among the ducks in experiment 1, there were no significant
differences between right and left feet for any gait parameter.

The six ducks included in experiment 2 tolerated anesthesia, MSU injection, recovery,
and PSW trials well with no complications. Of the 144 trials analyzed in experiment 2, 12 were
repeated once and 4 were repeated twice in order to obtain a complete set of 4 adequate trials.
Ducks were anesthetized using isoflurane for an average of 7.2 min (range 5 – 13 min). Based on
gross post-mortem examination, there were four males and two females. The repeated measures
analysis of variance revealed that the |R-L| of maximum force in kilograms and in percentage of
body weight ($F_{2.536,12.678} = 23.619; \ p < 0.0001$), in addition to impulse in kilograms meter per second
and in percentage of body weight meter per second ($F_{2.347,11,735} = 14.845, \ p < 0.0001$), significantly
differed at only the 3 and 4 hour time points in ducks with unilateral induced arthritis (Figures
4.3 and 4.4). No other parameters significantly differed between baseline and post-MSU injection at any time point. Examples of foots-trikes generated by the PSW in a duck before and after lameness induction are shown in Figure 4.5.

**DISCUSSION**

The PSW provided repeatable objective gait analysis in domestic ducks. All ducks successfully participated in the PSW protocol and no differences were identified in gait parameters between feet in normal ducks. Most ducks voluntarily walked along the PSW when placed at one end with a person standing behind them. In addition, the unilateral MSU arthritis induction method induced transient lameness in these ducks and is an avian nociception model for analgesic efficacy studies in ducks.

Lameness was present during 3-4 hours post-MSU injection. However, there were resting periods between hours 4 and 8 and 8 and 24, and it is unknown at what point between hours 4 and 8, the lameness resolved. While a transient method for lameness induction provides a more humane method than a permanent one, this result suggests that for an analgesic efficacy study, the lameness duration may not be sufficient to detect effects of medications for a clinically applicable time period. For example, tramadol plasma concentrations in this species maintained levels therapeutic for humans for at least 6 hours and the M1 metabolite maintained presumed therapeutic concentrations for at least 24 hours (Bailey 2019). Future studies investigating a higher dose or concentration of MSU may yield a more effective lameness duration that would mimic true disease and allow for effects lasting as long as target medications. In addition, the type of mat covering the PSW should be consistent, as in this study, because different cover mats can significantly affect the magnitude of gait parameters (Kieves 2019).
The gait parameters following MSU injection significantly reduced the maximum force (kg and % of body weight) and impulse (kg meter per second and % of body weight meter per second) put on the injected limb. There were no significant differences in step length between feet, indicating that the ducks were able to compensate and not change their stride length or shape. These findings differ from that found in other species of poultry with different lameness-causing diseases. For example, high growth-rate breeding lines of broiler chickens (Gallus gallus domesticus) and Pekin ducks (Anas platyrhynchos domesticus), and birds fed an ad libitum diet, had a lower velocity, larger step width, and increased double support time (spent more time with two feet supporting the body) compared to low growth-rate lines and birds fed a restricted diet, respectively (Duggan 2016). Additionally, turkeys kept on wet litter substrate developed pododermatitis, resulting in decreased velocity, increased double support time, decreased stride length, and increased stance time compared to those kept on dry litter (Mayne 2007, Wyneken 2015). Larger body size in chickens and ducks, and pododermatitis in turkeys affected different gait parameters compared with the MSU injections of the current study (maximum force and impulse). These findings indicate that different diseases (e.g. obesity, pododermatitis, osteoarthritis) cause different gait abnormalities.

Future studies warrant 1) testing a higher MSU dose to aim for longer lameness duration, 2) exploring objective gait analysis in other aquatic avian species using the PSW and 3) investigating efficacy studies for commonly used analgesics in waterfowl such as tramadol and meloxicam. Investigating the presence of inflammation in the joint after MSU injection via thermography, joint fluid analysis, and histopathology, and confirming the presence of MSU crystals as the cause of lameness by comparing to saline control injections were beyond the scope of this study but would be beneficial to evaluate in the future.
Species-specific gait assessment modalities are important due to the vast variability between gaits even among avian species. In addition, not only species-specific, but also disease-specific pain models are needed to determine the most effective analgesic therapies for different avian species. In conclusion, objective gait analysis using the PSW offers a flexible technique to detect gait abnormalities in a variety of species with the potential to assess, monitor, and promote improvements in the welfare of birds with lameness-causing diseases.
Table 4.1.

Experiment 1: Body weight and objective gait analysis results of normal ducks (*Cairina moschata domestica*; n=18) as measured by a pressure sensitive walkway

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>2.25 (1.74 – 3.65)</td>
</tr>
<tr>
<td>Cadence (steps/min)</td>
<td>342.0 ± 62.7 (314.0 – 370.0)</td>
</tr>
<tr>
<td>Gait distance (cm)</td>
<td>222.0 ± 42.0 (203.0 – 241.0)</td>
</tr>
<tr>
<td>Gait time (sec)</td>
<td>1.56 (1.13 – 3.72)</td>
</tr>
<tr>
<td>Gait velocity (cm/sec)</td>
<td>147 ± 39.1 (129 – 165)</td>
</tr>
</tbody>
</table>

Values reported as mean ± standard deviation (95% confidence interval) or median (range) if data not normally distributed (Shapiro-Wilk Test *p* < 0.05).

Table 4.2.

Experiment 1: Objective gait analysis results of normal ducks (*Cairina moschata domestica*; n=18) as measured by a pressure sensitive walkway

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Left</th>
<th>Right</th>
<th>[Right – Left]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stride length (cm)</td>
<td>51.3 ± 7.1 (48.1 – 54.5)</td>
<td>50.5 ± 7.5 (47.2 – 53.9)</td>
<td>1 (0.1 – 9.3)</td>
</tr>
<tr>
<td>Stride time (sec)</td>
<td>0.36 (0.25 – 0.67)</td>
<td>0.37 ± 0.08 (0.34 – 0.4)</td>
<td>0.01 (0 – 0.08)</td>
</tr>
<tr>
<td>Stride velocity (cm/sec)</td>
<td>148.0 ± 37.7 (131.0 – 165.0)</td>
<td>147.0 ± 37.6 (130.0 – 164.0)</td>
<td>2.7 (0.4 – 10.4)</td>
</tr>
<tr>
<td>Step length (cm)</td>
<td>26.2 ± 5.0 (23.9 – 28.5)</td>
<td>26.1 ± 4.7 (24.0 – 28.2)</td>
<td>1.4 (0.2 – 15.0)</td>
</tr>
<tr>
<td>Step time (sec)</td>
<td>0.19 ± 0.04 (0.17 – 0.21)</td>
<td>0.19 (0.12 – 0.34)</td>
<td>0.02 (0 – 0.09)</td>
</tr>
<tr>
<td>Step velocity (cm/sec)</td>
<td>146.0 ± 41.0 (128.0 – 164.0)</td>
<td>148.0 ± 37.6 (131.0 – 165.0)</td>
<td>9.2 ± 5.8 (6.6 – 11.8)</td>
</tr>
<tr>
<td>Step width (cm)</td>
<td>8.5 ± 1.4 (7.8 – 9.1)</td>
<td>8.5 ± 1.4 (7.7 – 9.2)</td>
<td>0.5 (0 – 1.2)</td>
</tr>
<tr>
<td>Stance time (sec)</td>
<td>0.17 (0.11 – 0.84)</td>
<td>0.19 ± 0.06 (0.16 – 0.22)</td>
<td>0.01 (0 – 0.62)</td>
</tr>
<tr>
<td>Swing time (sec)</td>
<td>0.19 (0.13 – 0.33)</td>
<td>0.19 ± 0.03 (0.18 – 0.20)</td>
<td>0.01 (0 – 0.24)</td>
</tr>
<tr>
<td>Max force (% BW)</td>
<td>120.3 ± 15.0 (113.6 – 127.1)</td>
<td>122.3 ± 12.5 (116.7 – 127.9)</td>
<td>4.0 (0.1 – 19.1)</td>
</tr>
<tr>
<td>Impulse (%BW*sec)</td>
<td>13.5 (9.4 – 36.7)</td>
<td>13.2 (9.1 – 25.1)</td>
<td>0.5 (0 – 20.9)</td>
</tr>
<tr>
<td>Max Peak Pressure (kPa)</td>
<td>73 ±15 (66 – 80)</td>
<td>76 ± 14 (70 – 82)</td>
<td>8± 5 (6 – 11)</td>
</tr>
</tbody>
</table>

Values reported as mean ± standard deviation (95% confidence interval) or median (range) if data not normally distributed (Shapiro-Wilk Test *p* < 0.05).
Fig. 4.1. Muscovy duck (*Cairina moschata domestica*) on pressure sensitive walkway (PSW) within a custom-designed chute composed of wooden and acrylic panels.

Fig. 4.2. Intratarsal injection of monosodium urate solution in an anesthetized Muscovy duck (*Cairina moschata domestica*).
**Fig. 4.3.** Boxplot depicting the absolute value of the difference between maximum force (kg) of right and left feet of Muscovy ducks (*Cairina moschata domestica*) as measured by a pressure sensitive walkway at serial time points following induction of lameness with intratarsal injection of monosodium urate solution. Asterisks represent a significant increase at hours 3 and 4.
**Fig. 4.4.** Boxplot depicting the absolute value of the difference between impulse (kg*m/sec) of right and left feet of Muscovy ducks (*Cairina moschata domestica*) as measured by a pressure sensitive walkway at serial time points following induction of lameness with intratarsal injection of monosodium urate solution. Asterisks represent a significant increase at hours 3 and 4.
Fig. 4.5. Footstrikes generated by the Tekscan™ Walkway 7 System software for a domestic Muscovy duck (*Cairina moschata domestica*) before (top) and after (bottom) induction of arthritis using a unilateral intratarsal injection of monosodium urate solution.
CHAPTER 5:
CONCLUSIONS AND FUTURE DIRECTIONS

Lameness in birds under professional care in zoos, aquariums, and rehabilitation facilities is a historical and frequently encountered problem for caretakers and veterinarians. While the significance of this problem in free-ranging avian populations is unknown, challenges arise due to degenerative, infectious and environmental processes while experiencing longer life expectancies in human-care settings. Causes of lameness such as osteoarthritis and pododermatitis have been investigated, and methods of treatment have been explored. However, objective monitoring of lameness in zoo animals is lacking. This study is the first to explore the use of a PSW for objective gait analysis in zoo-housed penguins and to develop a lameness model in domestic ducks.

The PSW identified symmetrical gait in normal penguins and ducks, and asymmetrical gait in penguins with historical lameness-causing disease (shorter left step width) and in ducks with transient induced unilateral tarsal arthritis using MSU injections under anesthesia (decreased maximum force and impulse on the affected limb 3 and 4 hours post injection and resolved by 8 hours post injection). While the sample size of abnormal penguins was small (n=5) and the causes of lameness were varied, the PSW has potential to serve as a method of case-by-case monitoring of lameness progression and/or objective assessment of individual therapy efficacy. A case series evaluating gait parameters of individual lame penguins over time before and after instituting therapeutic regimens is indicated. This also can be expanded to evaluating gait in other avian species, especially anseriformes, since the PSW objectively and quantitatively identified gait changes in domestic ducks.
The MSU lameness induction model investigated in this project caused gait changes in ducks at only the 3- and 4-hour time points post injection. Future studies warranted include testing a higher MSU dose to aim for longer lameness duration, sufficient enough to evaluate analgesic effects of commonly used medications in avian species including opioids, such as tramadol, and non-steroidal anti-inflammatory, such as meloxicam.

Eliminating the overarching problem of lameness in birds under human care is daunting and likely not possible. However, identifying and minimizing the causes, and learning how to effectively treat and objectively monitor are reachable goals and are essential in providing exceptional animal welfare, maintaining healthy zoo populations, and fulfilling conservation and educational goals for these charismatic avian species.


Erlacher-Reid C, Dunn JL, Camp T, Macha L, Mazzaro L, Tuttle AD. Evaluation of potential variables contributing to the development and duration of plantar lesions in a population of


