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**Characterisation of limb development and locomotion in the
brown kiwi (*Apteryx mantelli*)**

A thesis presented in partial fulfillment of the requirements for the degree of

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Abstract

This thesis covers broad topics concerning limb growth and development and their effects on locomotion in the brown kiwi (*Apteryx mantelli*).

I begin by describing the morphological features of a collection of unknown-age wild kiwi embryos from early development to point of hatch. Using these features, I assign developmental stages to each embryo and compare the progress of development to the same-staged ostrich and chicken embryos. Measurements of the hindlimb, bill and crown-rump length are used to develop an aging scheme based on comparisons with the ostrich and the chicken. The ostrich model and chicken model create age predictions for the unknown aged kiwi embryos. One kiwi embryo was of known age and both models gave identical predictions for this marker embryo, but gave differing predictions for all other kiwi embryos.

Using captive-reared kiwi chicks, I characterise hindlimb, bill and bodyweight growth from the time of hatch to 3 months of age. Growth patterns are very linear within this time period for all measurements but bodyweight. Female kiwi hatch with longer bills than males, but the growth of both sexes converges by the end of the 3-month period. Growth of bodyweight in the males slows earlier than in females. Bodyweight and bill length were then compared to a wild population of kiwi. Captive-reared chicks were found to hatch with shorter bills than the wild birds and to increase in bodyweight at a faster rate than wild birds. Rapid weight gain has been implicated in developmental limb deformities in other precocial and long-legged birds and has the potential to produce similar results in captive kiwi.

I further studied the movement of the hindlimb during locomotion in two adults and one juvenile kiwi by filming them while they were walking on a treadmill. Kinematic parameters were measured from the video recordings and compared to overground parameters from another study. Similarity between the treadmill and overground locomotor parameters validates the use of a treadmill in studying kiwi locomotion. None of the birds achieved the theoretical transition from a walk to a run at a duty factor of 0.5. After normalising for size, the juvenile showed a longer stride length and lower

stride frequency with increasing speed than the adults. Lateral head oscillations were observed during the stride cycle, which I propose having a sensory function as well as a biomechanical one.

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Preface

This thesis has been written and organised as self-contained chapters that will act as submissions to peer-reviewed scientific journals. Therefore, each chapter is written as a fully-referenced paper, causing overlap of some material, but each presents a unique aspect of kiwi hindlimb growth, development or movement.

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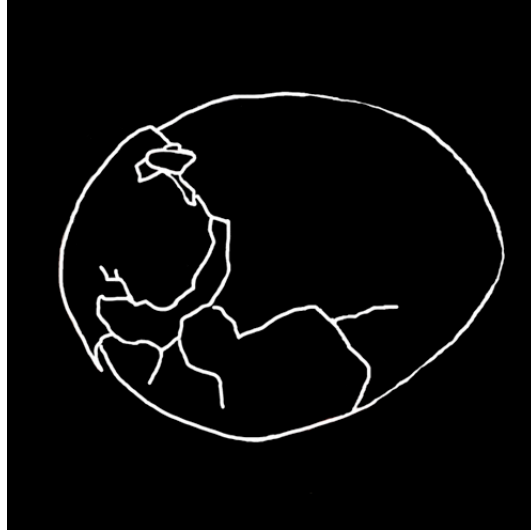
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Chapter One

General Introduction

General Introduction

1.1 Current status and ecology of kiwi

New Zealand is home to a range of unique avian fauna, but the kiwi (*Apteryx* spp.) has emerged as a symbol of concentrated and innovative conservation efforts aimed at ensuring the survival of this iconic species. Under management by the Department of Conservation, community groups and programmes such as BNZ Operation Nest EggTM, kiwi have some amount of protection against the introduced predator threats that have made them a critically threatened group. Mustelid predation is responsible for a 5.8% per annum decline in some populations (McLennan et al., 1996), but other intensively managed populations are no longer in decline and may be experiencing an increase in numbers (Holzapfel et al., 2008). Management practices have established populations in areas that were previously uninhabited by kiwi, leading to a modern day distribution of kiwi throughout the North Island, South Island, Stewart Island and several offshore islands (Holzapfel et al., 2008).

Knowledge of this ‘once abundant’ (Potts, 1872) bird is incomplete. Both the behaviour and appearance of kiwi lend to their cryptic nature - they are nocturnal, excellently camouflaged within their habitat and dwell in hidden burrows. Kiwi probe the forest floor for invertebrates and plant material with their highly specialised, long bill (Davies, 2002, Cunningham et al., 2007). Habitat varies greatly from species to species, and kiwi can be found in native forest, exotic forest, scrub, farmland, tussockland, grassland and wetlands (Heather and Robertson, 1998).

1.1.1 Taxonomy and phylogeny of kiwi

Ratites are loosely united by their lack of flight and by a set of osteological features (Cracraft, 1974, Lee et al., 1997). The ratite group includes the kiwi (*Apteryx* spp.),

ostriches (*Struthio* spp.), rheas (*Rhea* spp.), emus (*Dromaius* spp.), cassowaries (*Casuarius* spp.) and extinct moa (*Dinornis* spp.) and elephant birds (*Aepyornis* spp.). The largest member, the ostrich, weighs approximately 130 kilograms while the smallest member, the kiwi, weighs only 1.1-3.3 kilograms (Reid and Williams, 1975). The ratites can be further grouped with the tinamous to represent one monophyletic branch of modern birds, the Palaeognathae (Cracraft, 1986), which split from the Neognathae around 90 million years ago (Härlid et al., 1997, Haddrath and Baker, 2001).

Kiwi ancestors originated in the southern part of the South Island and subsequently diverged northwards (Baker et al., 1995). Rising water levels during the Pleistocene divided the North and South Islands and allowed the respective populations to evolve in isolation of one another. Conflicting molecular and geological evidence has resulted in disagreement about whether kiwi and the extinct moa colonised New Zealand simultaneously (Cracraft, 1974, Zelenitsky and Modesto, 2003, Bourdon et al., 2009b) or whether kiwi represent a secondary ratite invasion of New Zealand (Houde, 1986, Cooper et al., 1992, Cooper et al., 2001, Haddrath and Baker, 2001). The former is supported by morphology-based phylogenetic trees that have a sister-group relationship of the kiwi (or kiwi and moa) with the rest of the ratites (Cracraft, 1974, Lee et al., 1997, Livezey and Zusi, 2007, Bourdon et al., 2009b), while the latter is supported by molecular-based trees that place kiwi in an Australasian ratite clade that includes kiwi, emus and cassowaries (Cooper et al., 1992, Lee et al., 1997, Cooper et al., 2001, Haddrath and Baker, 2001, Harrison et al., 2004, Slack et al., 2006).

Since their first description in the early 19th century, kiwi have been divided into as many as ten different species and as few as one based primarily on morphological features such as feathers and colouration (Potts, 1872). Nevertheless, more recent genetic analyses of taxonomy have prompted much discussion on how to recognise different species of kiwi (Herbert and Daugherty, 1994, Baker et al., 1995, Burbidge et al., 2003). Five species are currently acknowledged by The Kiwi Recovery Group (Holzapfel et al., 2008): brown kiwi *Apteryx mantelli*, rowi *A. rowi*, tokoeka *A. australis*, great spotted kiwi *A. haastii* and little spotted kiwi *A. owenii*. The extreme population structure of *Apteryx* due to limited gene flow warrants each population being

considered as a unique conservation unit (Baker et al., 1995, Herbert and Daugherty, 1994).

1.2 Avian growth and development

Developmental precociality is a measure of a hatchling's functional maturity, which can vary across a spectrum of possible developmental patterns (Starck and Ricklefs, 1998). On one end of the spectrum are the precocial birds, who hatch with relatively high maturity as indicated by the presence of feathers and little to no dependence upon parental care, while altricial birds are on the opposing end and hatch with low levels of functional maturity as indicated by a lack of feathers and a dependence upon their parents for survival (Nice, 1962). The ratite bird's ability to walk, feed and thermoregulate from a very young age is a defining feature of functionally mature, precocial species.

Limb growth and development in avian species are traditionally characterised by sigmoid growth patterns that are described by the logistic, von Bertalanffy and Gompertz growth models, which present the percentage of growth achieved in relation to the adult size as age increases (Ricklefs, 1967, Ricklefs, 1968). Other models do exist and the appropriate model to fit varies from species to species (Ricklefs, 1967, Brown et al., 2007). The components of these growth equations produce estimations of relative growth rate, overall growth rate and the inflection point where growth begins to slow. By using models that present the underlying principles of growth in a standard way, comparisons can be made between species or across different phylogenetic groups.

1.2.1 Reproductive biology of the kiwi

While the developmental mode of the kiwi and other ratites are similar, the kiwi has markedly different reproductive biology. Kiwi reach sexual maturity at 1.5-2 years, the female has paired ovaries, the male is solely or partially responsible for incubation in most species and egg incubation length is disproportionately long (74-84 days) for a

bird of its size (Reid and Williams, 1975, Calder III, 1979). One to two eggs are laid in a clutch and several clutches can be laid in a year, with each egg being 20-25% of the female's body weight. Eggs average 431 grams in weight in the wild, but are significantly smaller (360 grams) when laid in captivity (Reid, 1981). Large yolk stores, which make up 61% of the fresh egg, make the kiwi egg the most nutritive laid by birds (Reid and Williams, 1975). The yolk is absorbed slowly after hatching and used to maintain the chick for the first 10-15 days of life until it is able to sustain itself by foraging (Prinzinger and Dietz, 2002).

1.2.2 Embryonic development of the kiwi

Observations made on the development of the kiwi embryo by Parker (1892, 1891) remain the authority on the topic, despite being based on 13 specimens of unknown age in what was then known as three species. The availability of embryos for further study, however, is limited due to their low population numbers, status as a protected species and distinction as a taonga species, or one that is culturally relevant to the native Maori people (Holzapfel et al., 2008). Most modern day embryological studies of avian species have utilised Hamburger and Hamilton's (1951) series of developmental stages for domestic chicken embryos as a standard for comparison of developmental events. The series is divided arbitrarily into 46 stages based on the presence or absence of morphological features and provides a classification scheme that is independent of embryo size or incubation length and thus can be applied to any avian species. While there are many published accounts of the embryological development of precocial avian species (Buckner et al., 1950, Daniel, 1957, Fant, 1957, Weller, 1957, Mun and Kosin, 1960, Cooper and Batt, 1972, Caldwell and Snart, 1974, Ryder and Somppi, 1977, Montgomery et al., 1978, Mahoney and Threlfall, 1981, Ancel et al., 1995), the ostrich is the only ratite that has been similarly studied thus far. Gefen and Ar (2001) present a comparison with Hamburger and Hamilton's series in an attempt to establish a tool for age estimation, which is one of the most common uses for embryonic development study. Other benefits of embryonic staging include allowing determination of vulnerable stages of development or establishing a method to calculate a laying or hatch date.

Despite an ostrich's incubation period being exactly twice that of a domestic chicken, some structures in ostrich embryos do not develop in exactly twice the amount of time as would be expected if all structures in an avian embryo followed the same developmental pattern in respect to their incubation time (Gefen and Ar, 2001). Starck's (1998) comparison of several altricial and precocial species found that all birds went through the same normal stages as described by Hamburger and Hamilton (1951), but the timing of developmental events varied by a greater degree in late embryogenesis. From the information available at the time, Starck (1998) found avian embryogenesis to be fairly conservative in its earliest stages and it was only in the later stages that rates of tissue maturation varied. More recent study of species with differing patterns of development, however, suggests late ontogenetic characteristics can affect early embryonic development between and even within a species (Blom and Lilja, 2005, Lilja et al., 2001). Hindlimb length in the precocial ostrich, for example, was significantly larger than forelimb length in early embryogenesis, whereas the opposite was true for the altricial Fieldfare (*Turdus pilaris*), highlighting the early functional demand of the locomotive organ in the ostrich (Blom and Lilja, 2005). Like the ostrich, the hindlimbs of kiwi are crucial in the hatching process where their powerful legs and feet are used to fully crack open their shell (Deeming, 1995).

1.2.3 Postnatal growth of the kiwi

The developmental mode and the postnatal growth rate of a chick are closely linked, in that the level of functional maturity of a hatchling's tissues is inversely related to the ability of those tissues to grow quickly (Ricklefs, 1973). Precocial birds are constrained by tissues that have a limited capability for cell proliferation and thus grow 1/3 to 1/4 as fast as altricial birds (Ricklefs, 1979a, Ricklefs, 1979b). While there is a general recognition of the trade-off between growth and function, multiple tissues and organ systems have been implicated in limiting growth rate, including the development of cartilage in the long bones (Starck, 1994), skeletal ossification (Blom and Lilja, 2004), the legs (Ricklefs, 1979b), skeletal muscle (Ricklefs, 1979a), digestive organs (Lilja et al., 2001) and the nervous system (Ricklefs and Starck, 1998).

The postnatal growth rate of kiwi is rarely quantified due to few long-term population studies that incorporate growth parameters. One brown kiwi population followed for a period of ten years showed growth rates for chicks peaked at 30 days after hatching at 6.3 grams/day and decreased steadily to 0.9 grams/day at 500 days post-hatch (McLennan et al., 2004). While average adult weight occurs at an age of 750-1050 days in this population, histological inspection of kiwi long bones indicate full mature size is actually not attained until 5-6 years of age (Bourdon et al., 2009a). Bourdon identified lines of arrested growth (LAGs), or annual bone growth marks, in kiwi long bones that were previously only found in one modern bird, the moa (Turvey et al., 2005). LAGs are not present in other modern birds because of their rapid bone development, but the kiwi's exceptionally slow postnatal growth allows for the expression of annual rhythms in the cortices of the long bones. The driving force behind such an extended growth period may be a lack of historical predators that would have applied selective pressures for faster growth as well as a shorter incubation period or smaller egg size (Calder III, 1979, McLennan et al., 2004, McLennan, 1988).

1.2.4 Developmental limb deformities in precocial avian species

Commercial broilers whose growth rates have been altered by selection for high body mass or by improved feed efficiency are plagued by skeletal deformities associated with rapid weight gain (Julian, 1998). Other factors, such as nutrient deficiencies, management practices, trauma and infection may also contribute to abnormal body or long bone structure that significantly affects the welfare of afflicted birds (Williams et al., 2000, Oviedo-Rondon et al., 2006, Julian, 1998). Large ratites, such as ostriches, emus and rheas raised for meat production suffer from an array of limb deformities with the same contributing factors (Speer, 1996, Samson, 1997, Squire and More, 1998, Mushi et al., 1999, Cooper et al., 2008). Rotational and angular limb deformities, rolled toes, spraddle legs, tarsometatarsal deformities and other musculoskeletal disorders are also known to occur in wild birds, such as bustards and cranes raised in captivity (Serafin, 1982, Bailey et al., 1996, Naldo et al., 1998, Naldo and Bailey, 2001). One incidence of splayed legs in a kiwi chick is recorded by Doneley (2006), but in general,

limb abnormalities in kiwi have been reported anecdotally and as rare occurrences in young and juvenile birds raised in captive institutions (unpublished reports from New Zealand Wildlife Health Centre).

1.3 Bipedal locomotion

Despite their extreme differences in appearance, both humans and birds use the same fundamental mechanisms for locomotion. In bipeds, the lower limb acts as an inverted pendulum in which the body's centre of mass passes over alternating supporting limbs in a symmetrical gait (Alexander and Jayes, 1978, Cavagna et al., 1977). In such pendular mechanics, kinetic and gravitational potential energy are out of phase with one another, but as speed increases, they become gradually more in phase until there is little to no exchange between the two. At this point, a spring or bouncing mechanism is used to produce a run and is achieved by recovering the elastic energy stored in the tendons (Cavagna et al., 1977). A walk and run are therefore fundamentally different ways of producing an optimum gait for any given speed, that is one that minimises the amount of positive work done (Alexander, 1992). Minimisation of metabolic energy costs or power requirements is primarily important in determining terrestrial gaits (Alexander, 1989, Rubenson et al., 2004).

Gait can be defined by footfall patterns and the relative support time of the limbs, kinematic patterns or the angles of limb joints during locomotion, or by energy fluctuations of the body's centre of mass. There is little agreement as to which method is best (Gatesy and Biewener, 1991, Biknevicius and Reilly, 2006). Footfall patterns are a predominant method in locomotion study, whether used alone or in conjunction with another method, and are based upon the portion of time that a foot remains on the ground during a stride (Hildebrand, 1965). A stride is divided into a stance phase where a given foot is in contact with the ground and a swing phase where it is not. The duty factor is the proportion of the stride where the foot remains in contact with the ground (Alexander and Jayes, 1978), a measure that is traditionally used to distinguish between walking and running gaits. A duty factor above 0.5 indicates a walk, whereas below 0.5 indicates a run. A run of this type is also called aerial running as there is no longer a

period of double limb support. An abrupt change to aerial running can indicate a gait change in humans and horses, but not necessarily in other animals that adopt ‘compliant’ running (Alexander and Jayes, 1983), which is also called ‘groucho’ running (McMahon et al., 1987, McMahon, 1985) or ‘grounded’ running (Rubenson et al., 2004). Grounded running is common in birds and some may never incorporate an aerial phase in their locomotion (Gatesy and Biewener, 1991). Grounded and aerial running may visually appear like different gaits, but switching from one to another does not display energy fluctuations in the centre of mass that would be expected from a change in gait type (Rubenson et al., 2004), nor is there an abrupt change in duty factor. Energetically speaking, grounded and aerial running are both types of bouncing gait and a switch in gait may occur long before incorporating an aerial phase, indicating that duty factor alone cannot identify distinct gaits in a consistent manner (Rubenson et al., 2004, Biknevicius and Reilly, 2006, Hoyt et al., 2006, Hancock et al., 2007).

While all bipedal movement may be a result of similar mechanisms (Cavagna et al., 1977), an animal’s size does have an influence on the components of its gait. In forming the dynamic similarity hypothesis, Alexander and Jayes (1983) predicted that the system of motion in two different bodies can be made identical to one another by multiplying all linear dimensions, time intervals and forces by respective constant factors. After applying this hypothesis to several species of quadrupeds, some kinematic differences became apparent in the groups of cursorial (adapted for running) and non-cursorial animals who had differing postures. Gatesy and Biewener (1991) investigated this departure from dynamic similarity in birds and humans and found that in general, smaller bipeds adopt a more crouched posture, have longer feet and have much higher stride frequencies than larger bipeds. When both small and large animals were compared at dynamically similar speeds, small bipeds actually take longer strides and fewer steps than larger bipeds in relation to their size. As an animal’s size increases, the relative tarsometatarsal length increases, the effective limb length decreases and ultimately the animal is limited in the relative step length it can achieve.

1.3.1 Evolution of avian posture and locomotion

The evolution of characteristic avian posture is primarily a result of the loss of digits, elongation of skeletal elements and reduction in the size and function of the tail and associated musculature (Ostrom, 1975, Gatesy, 1990, Gatesy, 1995, Prum, 2002). The tail underwent a gradual loss of skeletal and muscular elements by reducing the number of caudal vertebrae and the caudo-femoral musculature that ran through the tail of theropods (Gatesy, 1990, Gatesy, 1995). The few remaining caudal vertebrae form a short pygostyle in modern birds and the M. caudofemoralis muscle is weak or absent in ratites (Vanden Berge, 1982). Loss of post-pelvic mass pushed the centre of gravity far in front of the hip joint in modern birds and necessitated a change in the orientation of the femur to a more horizontal position that facilitates placement of the feet under the centre of mass (Gatesy, 1990). Since this femoral position does not allow a great amount of femoral retraction, knee-flexion is the primary method for foot placement (Gatesy, 1990) and its domination can also be linked to the enlargement of the knee flexor muscles over time (Carrano and Hutchinson, 2002). The acquisition of an avian posture also changed the femur:tibiotarsus ratio, showing a relative shortening and thickening of the femur so as to withstand the more perpendicularly oriented forces upon it.

There have been attempts to directly relate the hindlimb structure of modern birds, especially ratites, to their theropod ancestors due to similarities in their footprints (Padian and Olson, 1989); however, reconstructing theropod locomotion as bird-like ignores the many structural changes and adaptations that have occurred throughout evolutionary history. In addition to the theropod body being balanced cantilever by a substantial tail (Gatesy, 1990), the limb length ratio and femoral orientation in modern birds are not features of theropod construction (Gatesy, 1991, Carrano, 1998). Essentially, birds cannot be used as a direct analog for dinosaur locomotion without acknowledging the limitations of this practice (Gatesy and Biewener, 1991, Carrano and Biewener, 1999).

The secondary loss of flight in the ratite birds (Cracraft, 1986, Jones et al., 2000) has lead to another array of avian adaptations for, or because of, ground-based locomotion.

This includes reduced wings, fewer toes, a narrower pelvis, a reduced or absent keel and a high femur to body mass ratio compared to flying birds (Cracraft, 1974, Alexander, 1983a, Davies, 2002). Limb bone lengths of ostriches scale as would be expected for flying birds except for an especially long tarsometatarsus (Alexander, 1983a), a feature of the most cursorial birds (Abourachid et al., 2005). Kiwi, however, scale differently than the larger living ratites, with a proportionally longer femur, longer tibiotarsus and shorter tarsometatarsus (Abourachid and Renous, 2000). The leg bones of the moa scale similarly to the kiwi (Alexander, 1983a), but they appear much more robust than extant ratites of the same approximate size (Alexander, 1983b). The length of the tarsometatarsus can be used to divide birds into two ‘morpho-functional categories’; that is birds with a long tarsometatarsus have a longer swing phase while walking and those with a short tarsometatarsus have a shorter swing phase (Abourachid et al., 2005). Additionally, the swing length of paleognaths present a longer swing duration than neognathous birds, indicating that there might be fundamental differences in the locomotive methods of these two groups of birds.

1.3.2 Applications of gait analysis

Gait analysis is widely used in humans to study and/or quantify numerous aspects of human movement, including osteoarthritis, lower limb amputation, head injury, neurological disorders or diseases, spinal cord injury, age effects and pre- and post-operative results (Whittle, 2007). These same areas of study apply to birds, with investigations into spinal cord injuries in chicks (Muir et al., 1998), lameness in poultry (Kestin et al., 1992, Corr et al., 1998, Corr et al., 2003b, Corr et al., 2003a), and tibiotarsal rotation in ostriches (Cooper, 2007).

The motorised treadmill is a common piece of equipment used in locomotion study of humans and many different animal species (e.g. Taylor et al., 1982, Fredricson et al., 1983, Grubb et al., 1983, Gatesy and Biewener, 1991, Carrano and Biewener, 1999, Gatesy, 1999, Farmer and Carrier, 2000, Reilly, 2000, Rubenson et al., 2004, Abourachid, 2001). Treadmills provide convenience, but do not provide identical conditions to overground locomotion. In comparisons of treadmill and overground

locomotion, treadmills produced conflicting results including no statistical difference in rats (Pereira et al., 2006), a decrease in stance and swing time (Lee and Hidler, 2008) and changes in the hip joint angle and pro/retraction angle of the leg (Savelberg et al., 1998) for humans, an increase in stance time in horses (Barrey et al., 1993, Buchner et al., 1994) and running birds (Gatesy and Biewener, 1991) leading to a higher duty factor at a given speed, and an inability to reach speeds an animal would normally be able to reach in the wild (Gatesy and Biewener, 1991). Most investigators present these differences as warnings for future work that may be hindered by these disparities rather than evidence against the use of treadmills, and in many instances the differences are not considerable enough to discount the treadmill as a useful research tool (Lee and Hidler, 2008).

1.3.3 The function of head movement in locomotion and vision

Head-bobbing, as typified by the pigeon, is generally accepted as an optokinetic movement, rather than a biomechanical one. While bobbing may be linked to locomotion, it is not necessarily a consequence of it (Dagg, 1977, Frost, 1978, Troje and Frost, 2000). The head may lead to some amount of postural stability by contributing to a shift in the centre of gravity, though the contribution would be limited (Dagg, 1977, Fujita, 2002, Fujita, 2003). By thrusting the head forward in time with leg and body movements, bobbing may act as a method of stabilising images for prey detection, landing from flight or obstacle avoidance (Davies and Green, 1988, Jiménez Ortega et al., 2009). Some birds may also choose whether or not to bob their heads in certain situations such as detecting nearby prey or managing difficult terrain (Fujita, 2006). Additionally, the more crouched posture of smaller birds may improve control of head movements (Gatesy and Biewener, 1991).

Lateral head movement may also act as a vision aid, compensating for some inadequacy in the visual field. In owls, side-to-side ‘peering’ movements of the head may aide in gathering monocular depth information, versus that obtained through binocular vision (van der Willigen et al., 2002). Large side-to-side swinging head movements in

chickens may be used to view objects with different parts of the eye or different eyes (Stamp Dawkins, 2002).

The small eyes, limited spatial resolution and small vision centres of kiwi indicate they rely less on vision and more on other sensory cues for navigation and prey detection (Martin et al., 2007). Sensory pits in the bill for olfactory and tactile information have correspondingly large centres in the brain (Martin et al., 2007, Ashwell and Scofield, 2008), a possible consequence of nocturnality (Healy and Guilford, 1990). Ornamental facial feathers and rictal bristles may further aide in obstacle detection (Lederer, 1972, Conover and Don, 1980, Seneviratne and Jones, 2008), distinguishing texture (Carvell and Simons, 1990) and/or detecting depth (Schiffman et al., 1975).

1.4 Thesis aims and organisation

The overall aim of this thesis is to characterise various aspects of limb development from the start of incubation through adulthood. I highlight the great importance of the hindlimbs throughout kiwi life history and use the conclusions made from my investigations to build a better understanding of how the hindlimb of kiwi achieves its form and function. I aim to establish baseline information about the growth and movement of kiwi under normal circumstances so that my investigations can be expanded in future study of kiwi embryology, management practices, locomotor biomechanics and rehabilitation.

Chapter One describes characteristic morphological features of a collection of embryos in various stages of development. These features are used to stage each embryo according to the methods of Hamburger and Hamilton (1951) and to place the embryos in a chronological series. Using these stages, I aim to create an aging scheme based on chicken and ostrich development. The aging scheme is based on external characteristics and allows developmental data to be collected without damaging the few kiwi embryo samples available. Linear measurements of the bill, limbs and crown-rump length are presented with the calculated ages in order to demonstrate an estimated growth pattern.

Chapter Two characterises the growth of the limbs, bill and weight of kiwi chicks from hatching through to approximately 3 months of age. Twenty eight chicks raised from eggs as part of BNZ Operation Nest EggTM at Kiwi Encounter at Rainbow Springs, Rotorua, New Zealand and coming from ten different conservancies located in the central North Island are followed. Rates of growth in the captive-reared chicks are then compared to those of wild birds as gathered by John McLennan (2004, unpublished data) in order to identify any disparities between the rates that might predispose young captive-reared kiwi to developing limb deformities. Growth of the length, width and depth of the femur, tibiotarsus, tarsometatarsus and longest (i.e. middle) toe are presented in order to characterise overall limb development.

Chapter Three compares treadmill locomotion in the kiwi to overground locomotion as described by Abourachid and Renous (2000) in order to verify the treadmill apparatus as a valid method for studying movement in the kiwi. Using video recordings, I analyse the footfall patterns of three locomoting birds in order to identify gait changes with increasing speed, with the specific goal to observe running as defined by an aerial phase. Two adults and one juvenile are used so as to identify kinematic differences between the walk and run in birds of different size and age. Unexpected and unusual patterns of lateral head movement prompted me to track and quantify this behaviour in order to determine whether the movement has a biomechanical explanation or a specific voluntary purpose linked to tactile sensation.

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Chapter Two

**Development of a preliminary method for estimating the age
of brown kiwi (*Apteryx mantelli*) embryos**

Development of a preliminary method for estimating the age of brown kiwi (*Apteryx mantelli*) embryos

Abstract

Morphological features of a collection of unknown-age wild kiwi (*Apteryx mantelli*) embryos from early development to point of hatch are described. Using these features, I assign developmental stages to each embryo and compare the progress of development to the same-staged ostrich (*Struthio camelus*) and chicken (*Gallus* spp.) embryos. Measurements of the hindlimb segments, bill and crown-rump length are used to develop an aging scheme based on the comparisons with the ostrich and chicken. The ostrich model and chicken model create age predictions for the unknown-aged kiwi embryos. One kiwi embryo was of known age and both models gave identical predictions for this marker embryo, but gave differing predictions for all other kiwi embryos. Developmental timing of some features differed between all three species, most markedly in the bill, with growth in the kiwi being relatively faster in order to achieve its larger adult size.

Keywords: kiwi, ratites, precocial birds, embryonic development, hindlimbs

2.1 Introduction

Studies of avian embryo growth and development are not common, but do exist for poultry (Buckner et al., 1950, Hamburger and Hamilton, 1951, Daniel, 1957, Fant, 1957, Mun and Kosin, 1960, Ancel et al., 1995), waterfowl (Weller, 1957, Cooper and Batt, 1972, Caldwell and Snart, 1974, Montgomery et al., 1978) and seabirds (Ryder and Somppi, 1977, Mahoney and Threlfall, 1981). Hamburger and Hamilton's (1951) series of normal stages of development act as a basis for most modern embryological comparisons as the developmental process is divided into 46 stages based on the presence or absence of morphological characteristics independent of incubation length and embryo size. All embryos pass through the same series of developmental events (Ricklefs and Starck, 1998), but the exact chronology of these events can vary even in the earliest stages of development, suggesting that they are not as rigidly conserved as once thought (Richardson, 1999, Lilja et al., 2001, Blom and Lilja, 2005). Study of morphological development of the embryo of the kiwi (*Apteryx* spp.) is limited to the observations of Parker (1891, 1892).

The kiwi's egg is unusually large for a bird its size, weighing up to 25% of the female kiwi's body weight, or approximately 400 g (Reid and Williams, 1975, Calder III, 1979). The incubation period is also exceptionally long, ranging from 74-84 days in the wild (Reid and Williams, 1975). In captivity, however, the incubation period is tightly regulated by more consistent environmental conditions than in the wild. The 78-day incubation period used by captive institutions may be shorter than those found in the wild, where incubation may extend to 91 days (Colbourne, 2002).

The kiwi's large egg size and long incubation period were likely able to evolve because of a historical lack of predators (Calder III, 1979). No selective pressures warranted fast growth and the large egg remained energetically favourable. The relatively recent impact of mustelid predation on kiwi has caused a rapid decline in population size, making all kiwi species threatened and requiring active management (Heather and Robertson, 2005, Holzapfel et al., 2008). Limited access to kiwi and their eggs and their status as taonga species (i.e. one that is culturally significant to the native Maori people (Holzapfel et al., 2008)) both constrains the availability of specimens and precludes

sacrificing healthy embryos for study of normal development. In order to learn more about the kiwi's embryological development with few available samples, a developmental analogue must be sought for initial comparison.

This study aims to examine the growth of the morphological features of kiwi embryos by comparison with two other precocial species: the chicken (*Gallus* spp.), which has a more comparable adult body size; and the ostrich (*Struthio camelus*), which is more closely related phylogenetically (Livezey and Zusi, 2007). By providing detailed descriptions of a collection of kiwi embryos of unknown age, we can directly relate developmental stage-defining features of all three species in order to establish an aging scheme based on morphological measurements. An embryo's age range can be used to estimate the date of laying, date of initial hatch or time of embryonic death, which may help to identify critical or high risk stages of incubation.

2.2 Materials and Methods

Twenty preserved embryos of wild brown kiwi (*Apteryx mantelli*) that had died during incubation were examined. Only one of the embryos (designated embryo I) is of known age at 38 days old (age from date of laying).

Comparable stages of development were determined for the kiwi embryos using Hamburger and Hamilton's (1951) embryo staging methods for domestic chickens and Gefen and Ar's (2001) description of ostrich embryos throughout their development (see Hamburger and Hamilton staging in Appendix). This comparison was based only on external features. Measurements were taken with Vernier calipers (± 0.2 mm) on the right limb while it was flexed as much as possible. Only 15 embryos (those labeled F through T in Figure 2.1) were measured due to a lack of distinguishable limb segments in earlier embryos. Limb segment lengths included digit III, the longest (i.e. middle) toe (from the proximal phalangeal joint to the distal point of the nail), the tarsometatarsus (from the tarsometatarsal to proximal phalangeal joint), the tibiotarsus (from the stifle to tarsometatarsal joint), and the femur (from greater trochanter of the femur to stifle joint). Bill length was measured from the distal tip of the bill to the distal end of the cere at the midpoint of the cere's curve. Bill width was measured at the gape and bill depth

was measured at the cere. Crown-rump length was measured using a string and ruler from the cere of the bill to the pygostyle.

All morphometric parameters except for the bill length were linearly regressed against the estimated age for both the chicken and ostrich models at the minimum (78 days) and maximum (85 days) lengths of kiwi incubation. A second-degree polynomial regression was fit to the bill length measurements. Various incubation times have been reported for kiwi: 74-84 (Reid and Williams, 1975), 77-106 (McLennan, 1988), 75 and 91 (Colbourne, 2002), and 65-75 (Heather and Robertson, 2005). I chose the period of 78-85 days as a mid range of those reported and used these values. The embryos had obtained the majority of their distinguishing features and only grew in size by Hamburger and Hamilton (HH) stage 40, making age comparisons invalid for late stage kiwi embryos. For the last eight embryos (M through T), age was determined by extending the regression line for toe length forward and inserting toe length measurements into the regression line's equation. An estimation of incubation length for this collection of kiwi embryos was determined by inserting the average bill length of wild kiwi at time of hatch (42.9 mm, from Chapter 3, this study) into the regression line equations for bill length.

Each embryo was photographed with a Fujifilm FinePix S200EXR digital camera using a tripod and light table.

2.3 Results

Embryo staging was most effectively determined using limb features, especially the toes. Looking at the presence or degree of joint bending and feathers was not useful in this collection as many embryos were preserved in unnatural positions or had suffered varying degrees of damage.

The photo series in Figure 2.1 displays the embryo collection by increasing incubation age. Table 2.1 presents the Hamburger and Hamilton stage, predicted ages based on the ostrich and chicken models and the key morphological features identified in each kiwi embryo.

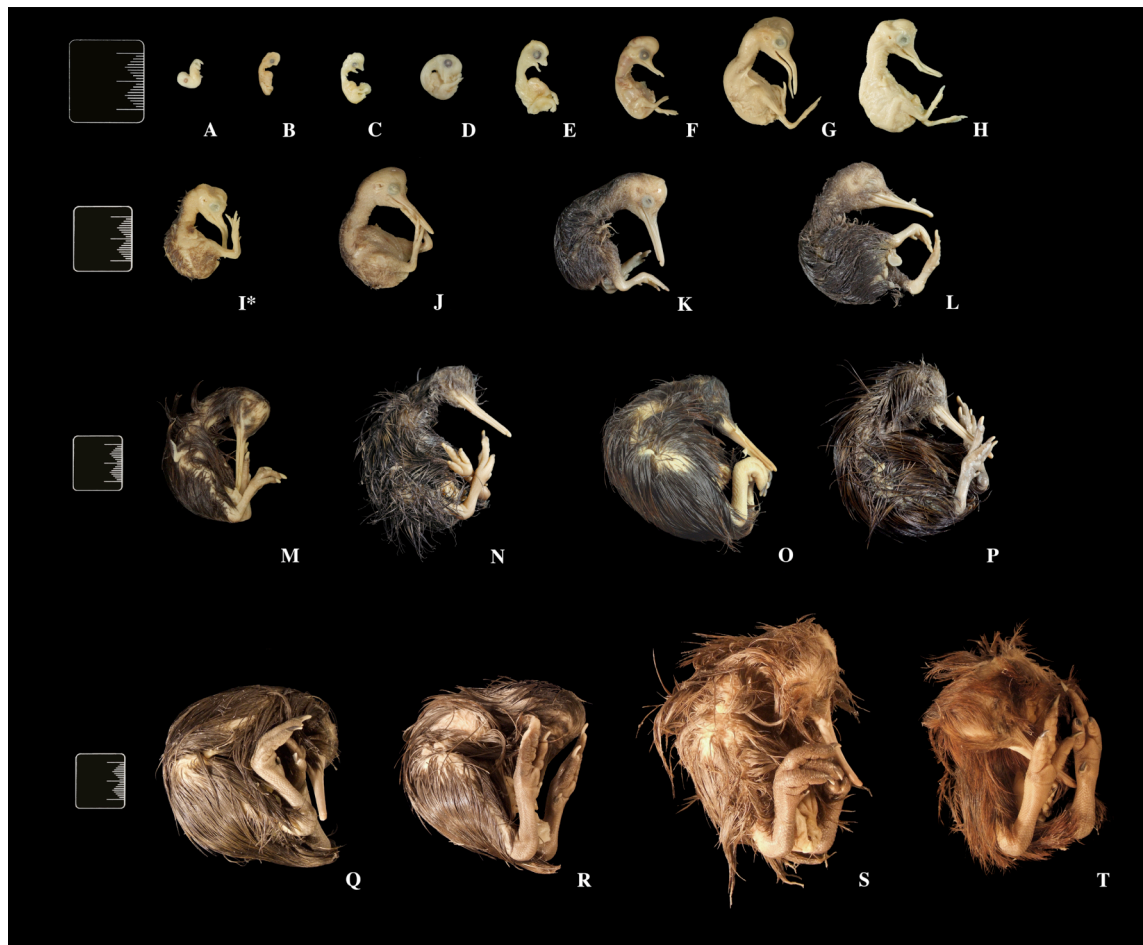


Figure 2.1: Kiwi embryos are arranged in increasing chronological order. Scale bars appear to the left of each row. An asterisk (*) denotes the known-age embryo. See Table 2.1 for details about each embryo.

Differences in timing of head and bill development between the kiwi and the chickens became apparent around Hamburger and Hamilton's stage 28. Embryo C shows a more advanced mandible than the corresponding stage in the chicken. The appearance of feather germs and papillae lagged behind the chicken and did not become apparent until approximately stage 35 in Embryo F. It is possible that feather development occurs earlier, however, since our series is missing stages 32-34. Eyelid development was not apparent to the extent of that in the ostrich and chicken in Embryos E and F.

The best measurements for aging kiwi embryos as determined by the ostrich model were the crown-rump length and tarsometatarsus length (Figure 2.2 *a/b*; Figure 2.3 *c/d*). In contrast, when using the chicken model, the bill length and tibiotarsus length gave

the best predictions (Figure 2.2 *c/d*; Figure 2.3 *a/b*). The least useful measurement was the femoral length (Figure 2.2 *e/f*), which may have been due to the difficulty inherent in measuring this parameter in these specimens. In early through middle stages of embryo development (Embryos A-J), the ostrich and chicken models gave similar age predictions. An estimation of 40.9-44.5 days for the 38-day old, known-age embryo (I*) was produced by both the ostrich and chicken model. In later stages of development (Embryos K-Q), the ostrich model gave somewhat higher age estimations than the chicken model.

Bill growth differed from the other parameters measured in that it was best described by a second-degree polynomial equation rather than a linear one. The curve of the ostrich regression line is more extreme than the chicken and may indicate a slowing of the kiwi bill growth near 48-58 days of incubation. The progression of the chicken regression line is more consistent and does not indicate a slowing in growth. Using 42.9 mm (Chapter 3, this study) as a typical bill length for a wild kiwi at time of hatch, the ostrich model predicts a very long incubation length of 132.0-149.0 days, while the chicken model predicts a more reasonable 89.9-94.8 days.

Table 2.1: Estimation of Hamburger and Hamilton (HH) stage for kiwi embryos, embryo age estimates based on ostrich and chicken models, and feature descriptions are presented for each kiwi embryo. An * indicates the known age embryo (I=38 days).

Embryo ID	HH stage	Predicted age range (days)		Feature descriptions
		Ostrich model	Chicken model	
A	early 24	14.9 - 16.2	14.9 - 16.2	leg bud is approximately twice as long as it is wide, can't completely distinguish toe plate
B	early 25	18.6 - 20.2	16.7 - 18.2	wing straight, legs longer than wings, toe plate present but no demarcation of toes, little embryonic flexure
C	late 28	22.3 - 24.3	22.3 - 24.3	wing bent at elbow, middle toe longest, grooves between toes, neck lengthened, bill clearly recognizable and mandible approximately half the length of bill
D	30	26.0 - 28.3	24.1 - 26.3	grooves visible between digits, second digit slightly longer, webbing between toes evident despite damage to toes, femur obvious, all limb segments are demarcated, mandible approaching end of maxilla, external auditory openings visible
E	late 31	29.7 - 32.4	27.9 - 30.4	wing bent at elbow, toe web contours slightly concave, bill damaged, cannot distinguish feather germs
F	35	33.4 - 36.4	29.7 - 32.4	small amount of webbing at base of the toes, beginning to distinguish phalanges, mandible has nearly reached its full length in relation to maxilla, feather germs on dorsal side
G	36	37.1 - 40.5	37.1 - 40.5	nails resemble cones, plantar pads just visible in profile, feather germs over the embryo's dorsal surface, the thigh and portions of the head
H	late 36	37.1 - 40.5	39.0 - 42.5	nearly identical to embryo G, but claws and plantar pads slightly more conspicuous
I*	late 37	40.9 - 44.5	40.9 - 44.5	claws and plantar pads are obvious, scales visible on all but toes, feather germs visible on entire surface of head and wings and extend down to the middle of the tibiotarsus, dorsal surface feathers from the back of the neck to the tail are pigmented, feather tracks are apparent, small circular opening between the eyelids
J	early 38	44.6 - 48.6	44.6 - 48.6	feathers germs extend over the majority of the tibiotarsus, scales visible on toes
K	late 38	48.3 - 52.6	46.4 - 50.6	nail present on the digit, pigmented feathers cover entire body except top of the head where a portion of skin is missing, nails are semi-transparent, with slight pigmentation at their base, narrow crescent opening between eyelids
L	39	52.0 - 56.7	48.3 - 52.6	scales overlap on all but back of the legs, feathers over head and legs, slightly pigmentation on back of leg and top of toes, eyelids cover entire eye
M	40+	60.3 - 65.7	57.9 - 63.1	pigmented scales on toes, toenails opaque middle toe = 14.8 mm; bill = 29.4 mm
N	40+	63.0 - 68.6	60.4 - 65.8	middle toe = 16.0 mm; bill = 31.8 mm
O	40+	65.3 - 71.1	62.5 - 68.1	middle toe = 17.0 mm; bill = 32.8 mm
P	40+	67.5 - 73.6	64.6 - 70.4	middle toe = 18.0 mm; bill = 32.0 mm
Q	40+	82.6 - 90.0	78.6 - 85.6	claws curve ventrally middle toe = 24.6 mm; bill = 41.4 mm
R	40+	83.9 - 91.4	79.8 - 87.0	middle toe = 25.2 mm; bill = 39.0 mm
S	40+	86.6 - 94.4	82.4 - 89.8	middle toe = 26.4 mm; bill = 39.2 mm
T	40+	86.6 - 94.4	82.4 - 89.8	middle toe = 26.4 mm; bill = 39.8 mm

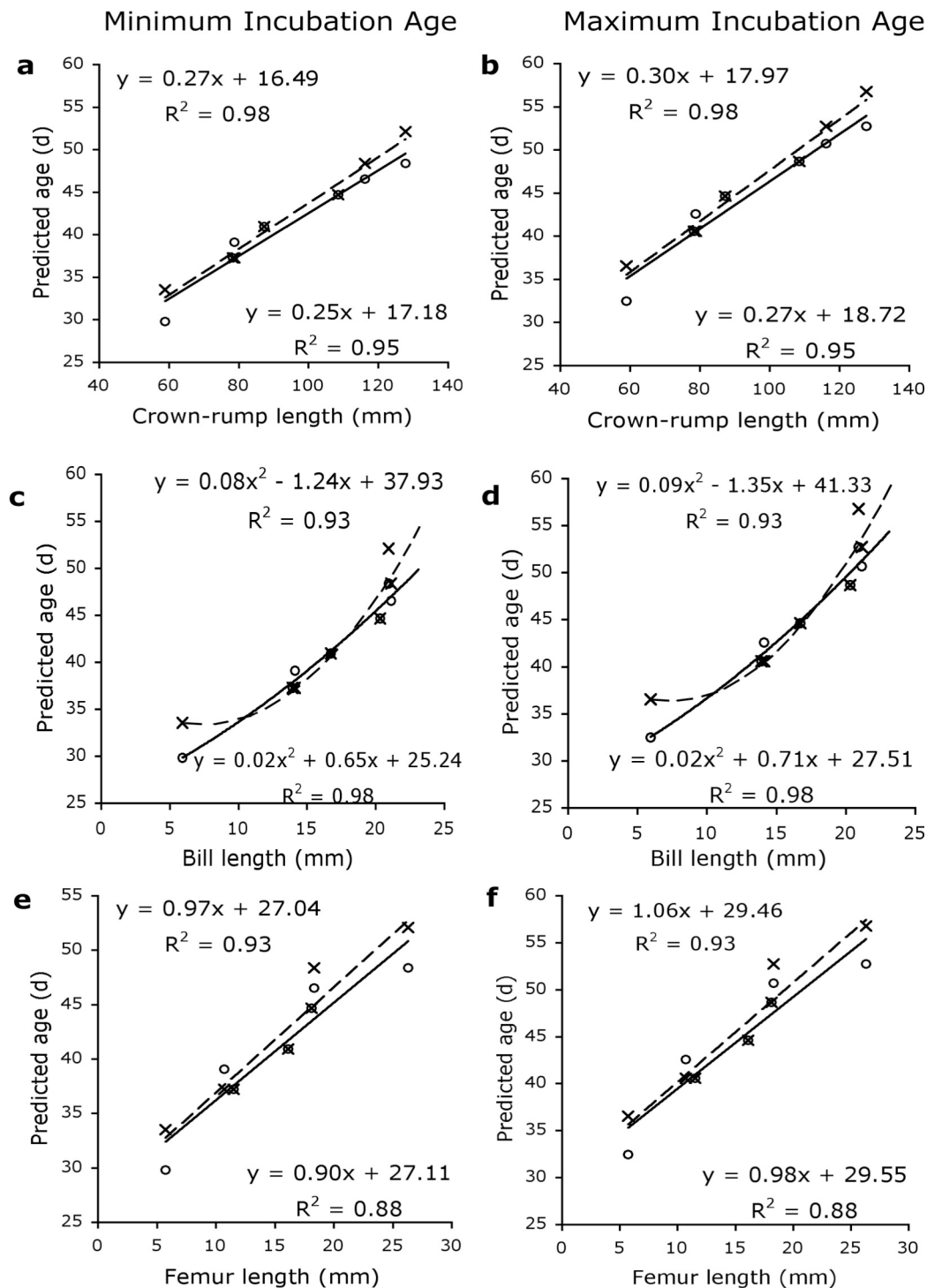


Figure 2.2: Linear regressions of crown-rump (a/b) and femur (e/f) length measurements and polynomial regression of bill length (c/d) with predicted age based on ostrich and chicken models. Predictions for both the minimum (78 days) and maximum (85 days) kiwi incubation period are presented. Equations and R^2 values above the line correspond to the ostrich regression (---) and those below correspond to the chicken regression (—).

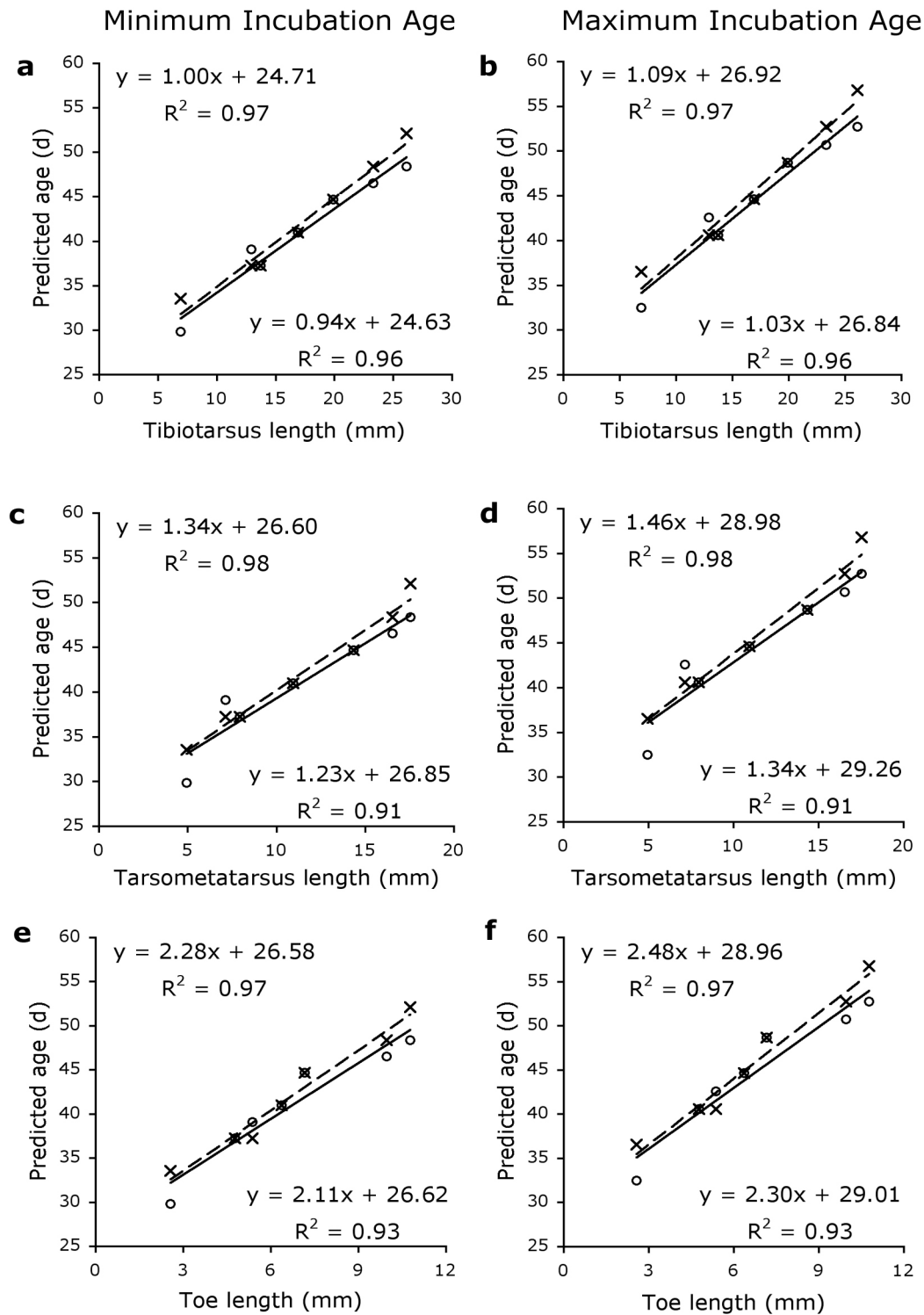


Figure 2.3: Linear regression of tibiotarsus (a/b), tarsometatarsus (c/d) and toe (e/f) length measurements and predicted age based on ostrich and chicken models. Predictions for both the minimum (75 days) and maximum (85 days) kiwi incubation period are presented. Equations and R^2 values above the line correspond to the ostrich regression (---) and those below correspond to the chicken regression (—).

2.4 Discussion

This baseline study of kiwi embryo development in relation to estimated age is a necessary first step towards a better understanding of kiwi embryology. Like Parker (1891), I found that the embryonic kiwi hindlimb increased rapidly and regularly. Parker reported bill length as reaching its full proportional size by approximately the middle of incubation, whereas my models predicted a slowing at a slightly later point (ostrich model) or not at all (chicken model). The ostrich model predicts a higher rate of growth for kiwi bill length than the chicken model and that rate is similar to the rate found in ostrich embryos (Gefen and Ar, 2001). Despite the ostrich being a much larger bird than the kiwi, the kiwi must attain a longer bill length relative to head size than the ostrich. From my estimation of bill growth, this relatively longer bill is achieved by having a similar growth rate to the ostrich throughout the kiwi's much longer incubation period, which has the potential to be twice as long as the period for ostrich egg incubation (42 days).

Neither the chicken model nor the ostrich model gives a better estimation of incubation age for the known-age embryo. Both models over-estimated the age by approximately 2-7 days. Early to middle stages of development had similar age estimations for the kiwi embryos using both models. In later stages, the estimations diverged, with the ostrich model producing greater estimations than the chicken model. This divergence may indicate that early embryo development follows similar patterns in the chicken and ostrich, but much different patterns in late development. For the oldest embryo specimens, which are likely at or close to point of hatch, estimations agree well with the maximum incubation length used in this study. The extreme difference between the estimations of incubation length that was produced by using the bill length regression equations with an average wild hatchling sized bill of 42.9 mm may indicate that the ostrich model does not accurately describe kiwi bill growth throughout the incubation period. At this point, it is unclear whether the chicken, which is more similar to the kiwi in size, or the ostrich, which has a closer phylogenetic relationship, better estimates kiwi embryo age. However, good agreement between ages predicted by ostrich and chicken supports the presented aging scheme as a useful, but not exact, tool and highlights the need for validation of this study with more known-age embryos.

The exact mechanism of an embryo's growth rate is not entirely clear, but may result from an antagonistic relationship between "supply" organs such as the intestines and "demand" organs such as the muscle or bone rather than the chick's postnatal developmental mode (Starck, 1994, Lilja et al., 2001). The developmental mode is traditionally determined by a chick's physical characteristics, behaviour and level of self-sufficiency at hatch, where the most altricial birds hatch completely dependent upon their parents and the most precocial require little to no parental care (Starck and Ricklefs, 1998, Nice, 1962). A bird's precociality is not simply a measure of its postnatal growth rate, which is generally but not exclusively slower in precocial birds (Ricklefs, 1968, Ricklefs, 1973, Ricklefs, 1979b, Ricklefs, 1979a, Starck, 1998), but rather a measure of its functional maturity at the time of hatching. In the case of a precocial species such as the kiwi, ostrich and chicken, the limbs must achieve a great deal of functional capability during the incubation period in order for the chick to walk and feed independently soon after it hatches.

The rate and timing of different embryonic tissues developing functional maturity differs from species to species (Blom and Lilja, 2005) and possibly within species (Lilja et al., 2001) and is a consequence of selection for late ontogenetic characters (Richardson, 1999). Gefen and Ar (2001) observed temporal differences in several structures between ostrich and chicken embryos, much as I found differences between kiwi embryos and both ostrich and chicken embryos. The relatively rapid growth of the kiwi bill became apparent early in the embryo series, at Embryo C, and persisted throughout development to produce the large characteristic bill that allows for feeding and highly specialised prey detection (Cunningham et al., 2007, Martin et al., 2007). The fast embryonic growth leading to advanced function of the kiwi's limbs and bill during incubation may restrict the ability of those tissues to grow quickly during postnatal development (Kirkwood et al., 1989, Ricklefs et al., 1994, Starck, 1994), as is evidenced by kiwi not reaching skeletal maturity until 4-6 years old (Beale, 1991, Bourdon et al., 2009).

The obvious limitations of this study are the use of embryos of non-ideal quality, unknown age and uncertain cause of death, all of which make it possible that these embryos may not accurately represent normal development. The sample population is

also skewed toward older embryos. The abundance of late stage embryos is likely to be a consequence of the BNZ Operation Nest EggTM programme where kiwi eggs are actively sought out and collected from the wild in order to be hatched in captivity. Eggs are less likely to be found until later stages and the more fragile early stage embryos are more likely to be damaged or destroyed during collection or to have decayed beyond use by the time the egg is collected. The conservation status of the kiwi does not allow for the sacrifice of healthy embryos. Validation of current embryo staging models is necessary, and thus as more specimens become available, they should be used for this purpose.

In conclusion, by providing detailed descriptions of a collection of kiwi embryos of unknown age, I have directly related the development stages and defining features of the chicken, the ostrich and the kiwi in order to establish an aging scheme for brown kiwi embryos based on morphological measurements. This model is of use to conservation management in estimating the date of lay, date of initial hatch or the time of embryonic death. My model may also be of use in identifying critical stages of incubation for kiwi embryo growth and development.

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Chapter Three

**Characterisation of hatch-size and growth rates of captive
and wild-reared brown kiwi (*Apteryx mantelli*) chicks**

Characterisation of hatch-size and growth rates of captive and wild-reared brown kiwi (*Apteryx mantelli*) chicks

Abstract

Avian growth rate patterns represent a trade off between a tissue's functional maturity and its capacity for growth. At the time of hatch, the brown kiwi (*Apteryx mantelli*) limb has a high level of maturity in order for the chick to be able to kick its way out of the shell and walk and forage independently from an early age. Growth curves of limb segments, bill length and bodyweight are presented for captive-reared, BNZ Operation Nest EggTM chicks over a period of three months from the point of hatch. Some parameters are slightly larger in the females than in males at time of hatch, including the bill length. Growth in bodyweight began to slow earlier in males than in females. Regressions of limb and bill measurements over time showed linear patterns of growth instead of a sigmoidal curve as seen in other birds, probably due to the short period of observation. Bodyweight and bill length were then compared to these morphometrics in a wild population of kiwi. Captive-reared chicks were found to hatch with shorter bills than the wild birds and to increase in bodyweight at a faster rate than wild birds. Rapid weight gain has been implicated in developmental limb deformities in other precocial and long-legged birds and should be avoided in captive kiwi.

Keywords: kiwi, postnatal growth, development, hindlimbs, limb deformities

3.1 Introduction

Ratites are precocial birds whose slow growth rate is a trade off for the ability to walk and care for themselves from an early age (Ricklefs, 1968, Ricklefs, 1979a). The long bones of precocial birds are highly ossified at hatch so that their limbs can resist the mechanical stress of locomotion early in life (Starck, 1994), leaving smaller cartilaginous centers for cell proliferation and growth than would be seen in fast growing altricial species (Kirkwood et al., 1989a). The cartilaginous cones in the long bones of ostrich, emus and rheas persist until 3-6 weeks of age (Reece and Butler, 1984).

The kiwi is the smallest of the ratites. All five species of kiwi are threatened by introduced mammalian predators and habitat loss, leading to a rapid decline in population numbers (Holzapfel et al., 2008). From hatch to approximately 3-6 months, wild kiwi are highly vulnerable to mustelid predation (McLennan et al., 2004). Once a chick reaches 800 g, or approximately 90-130 days after hatching, predation risk drops dramatically. Programmes such as BNZ Operation Nest EggTM (ONE) proactively counter this susceptibility to predators by raising wild-laid kiwi eggs in captivity and raising young to 800-1000 g in body mass (Holzapfel et al., 2008). The chicks are then released to their parents' home range as juveniles that are largely able to defend themselves against predators.

Growth rates often differ greatly between wild and captive birds (Aubin et al., 1986, Curro et al., 1996). Ostriches, emus and rheas (Sales and Horbanczuk, 1998) are captive-bred for meat production and a number of threatened precocial birds are raised in captivity in order to supplement wild stocks, such as cranes (Ellis et al., 2000), bustards (Bailey et al., 1998, van Heezik and Ostrowski, 2001) and black stilts (Reed, 1994, van Heezik et al., 2005). In all of these species, limb deformities are a common problem that leads to mortality or abnormalities that ultimately lead to them being excluded from the captive breeding stock or being euthanased. Limb deformities in farmed ostriches are a leading cause of death in chicks (Samson, 1997, More, 1996, Mushi et al., 1999). While limb abnormalities in kiwi are not common, they have been reported in Doneley (2006) and anecdotally in young and juvenile birds (unpublished

reports from New Zealand Wildlife Health Centre). The cause of limb deformities is likely to be multifactorial, with rapid growth rates, high protein diets, nutritional deficiencies, excessive quantities of food, poor housing design, lack of exercise, artificial incubation techniques and genetic aberrations being implicated in many different species (Serafin, 1982, Bailey et al., 1996, Speer, 1996, Julian, 1998, Naldo et al., 1998, Squire and More, 1998, Mushi et al., 1999, Williams et al., 2000, Naldo and Bailey, 2001). However, normal patterns of growth must be described before the pathophysiology of growth deformity can be understood.

In light of the reliance of kiwi conservation measures on captive rearing of chicks and the kiwi's shared attributes with species that experience high incidences of leg abnormalities, I wished to examine and compare the growth of captive-reared and wild chicks.

My specific aims were to:

- 1) characterise the growth of the brown kiwi (*Apteryx mantelli*) from time of hatch until approximately 3 months of age by formulating growth curves for selected morphological characteristics important to growth and locomotion; and
- 2) compare the growth of captive-reared kiwi to wild kiwi.

3.2 Materials and Methods

3.2.1 Captive-reared birds

The twenty-eight chicks (16 female, 12 male) monitored for this experiment were from first clutch eggs laid in the wild. All were artificially incubated and hatched at Kiwi Encounter at Rainbow Springs in Rotorua, New Zealand, during the 2007/2008 hatching season as part of the kiwi recovery programme ONE. Only healthy chicks were included in the study. Measurements were taken from 17 October 2007 to 19 January 2008.

3.2.2 Husbandry

All captive-reared chicks were subject to the same captive conditions following hatch according to the recommendations described in Kiwi Best Practice Manual (Robertson and Colbourne, 2007). Briefly, eggs were incubated for different lengths of time, depending on their age on arrival at Kiwi Encounter from their wild source, but all hatched at approximately day 78 of incubation. Chicks spent the first two days post-hatch in a still air incubator (Brisnea Polyhatch, Brisnea Products Ltd, North Somerset, United Kingdom). Incubation temperatures were gradually decreased from a starting temperature of 33.5°C to 28°C over these 48 hours. At day three, the chicks were then moved to a two-compartment brooder. The sleeping compartment was fitted with a heat lamp and matting, whereas the larger compartment was layered with peat moss substrate. A heat lamp maintained a temperature of approximately 28°C in the sleeping compartment and was turned down each day until its removal on day six. Matting in the sleeping area was changed to fern bedding at day six. Water was offered *ad libitum* in the larger compartment and changed daily.

Kiwi chicks possess a large yolk sac at hatching which initially serves as their primary energy source (Prinzinger and Dietz, 2002). Because of this, food (approximately 10 g) was offered on the night of day six. If the chick did not eat on this first night that food was offered, assisted feeding commenced on day seven with approximately 2 g of ox heart being fed. Assisted feeding continued each morning, increasing the amount by 2 g each day until the chick began to eat on its own. Food was offered to chicks each night regardless of whether they were being assist-fed, and those that ate on their own had their amount increased by 10 g each night. The food consisted of a blend of ox heart, rolled oats, cat biscuits, wheatgerm, fruits, vegetables and kiwi vitamin pre-mix (Bomac Laboratories, Auckland, New Zealand).

Chicks were housed in indoor brooders until they reached a weight equal to or above their hatch weight, which occurred at approximately three weeks old. Chick weight dips to about 20% of hatch weight as the yolk sac is absorbed as an energy source for the first few weeks of life until the chick is able to forage for itself. The chicks were then moved to outdoor enclosures, often with one or two other chicks of similar size. All

chicks were identified by a unique subcutaneous passive transponder implant. Birds maintained outside were provided with burrows throughout the enclosure, free access to water, and a dish of food per bird. Food was increased by 20 g each night if all the food was consumed, until chicks ate typically 150-200 g, depending on the individual bird, but a maximum of 250 g was offered so as to avoid the birds becoming overweight. Consumption was monitored by daily checks on the amount of food eaten and birds were weighed weekly.

3.2.3 Morphological measurements

Individual chicks were first measured on day three after hatch and then on every third day until they were moved from their indoor brooders to outdoor enclosures. Measurements were then taken each week. Body weight was obtained to the nearest gram using a digital scale (BS3000L, Shanghai Yousheng Weighing Apparatus Co. Ltd., Shanghai, China).

All measurements of bill and limb segment length were made by the same operator using Vernier calipers (± 0.2 mm) on the right limb while it was flexed. Limb segment lengths included the longest (i.e. middle) toe (from the phalangeal joint to the proximal margin of the claw on the dorsal surface of the toe while it was outstretched), the tarsometatarsus (from the tarsometatarsal to proximal phalangeal joint), the tibiotarsus (from the stifle to tarsometatarsal joint; Figure 3.1), and the femur (from greater trochanter of the femur to stifle joint). Width and depth measurements were taken from the narrowest point just above the tarsal joint on the tibiotarsus and at the midpoint of the tarsometatarsus. Bill length was measured from the distal tip of the bill to the distal end of the cere at the midpoint of the cere's curve. Width was measured at the gape and depth at the point of the cere.



Figure 3.1: Measuring tarsometatarsal length in kiwi chick.

3.2.4 Wild birds

I obtained raw data and unpublished data from John McLennan where radio-tagged birds were monitored and measured from 1992 to 2002 at Lake Waikaremoana in Te Urewera National Park in the North Island of New Zealand. See McLennan et al. (2004) for more information on the study area. Only weight and bill lengths were recorded from these samples.

3.2.5 Statistical Analysis

Regression equations for each variable over time were estimated for both males and females using repeated measures and the MIXED procedure of SAS 9.1 (2003). Contrasts between estimates of the regression lines for both sexes were performed to test if the slopes and intercepts for the male and female regressions differed within and between the captive-reared and wild data sets. The first 10 days post-hatch were excluded from regression analysis and statistical comparisons of growth in mass so as to exclude the time period where chicks use their yolk stores for energy and subsequently lose weight. Some captive-reared birds only stayed at Kiwi Encounter for approximately three weeks and were then released to crèche sites. Weight and bill

length data were analysed both with and without these crèche birds when compared with wild birds to ensure that including their data did not affect significance.

3.3 Results

3.3.1 Captive-reared birds

Mean hatch weight of captive-reared kiwi chicks was 420 g. Thereafter, weight decreased for approximately 10 days as the chicks' yolk stores were used and they learned to eat the artificial diet provided (Figure 3.2). Bodyweight was best described by a second-degree polynomial regression, while all other morphometric parameters were best described by linear regressions. Inclusion or removal of the crèche birds did not affect the statistical outcome of significance between sexes for weight or bill length measurements and are therefore included in the results. The rate of weight gain demonstrated differing patterns for male and female kiwi. Without taking the first 10 days of weight loss into account, the males show a slowing in weight gain toward the end of the 100-day period, while the weight gain in females increased steadily.

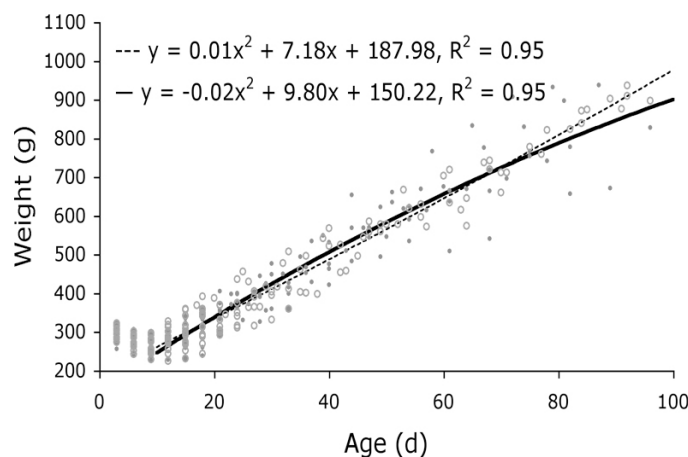


Figure 3.2: Weight gain in captive-reared kiwi (n=28) from hatching through to three months of age. Polynomial regression lines shown for females (---) and males (—) exclude the first 10 days of data.

Bill length showed significant differences in the intercept of the regression lines between the sexes ($p=0.0007$), with females hatching with longer bills than the males

(Figure 3.3). Both sexes continued to grow at a similar rate (females: 0.19 mm/d; males: 0.20 mm/d). Neither bill depth nor width showed any significant differences between the sexes (Figure 3.4).

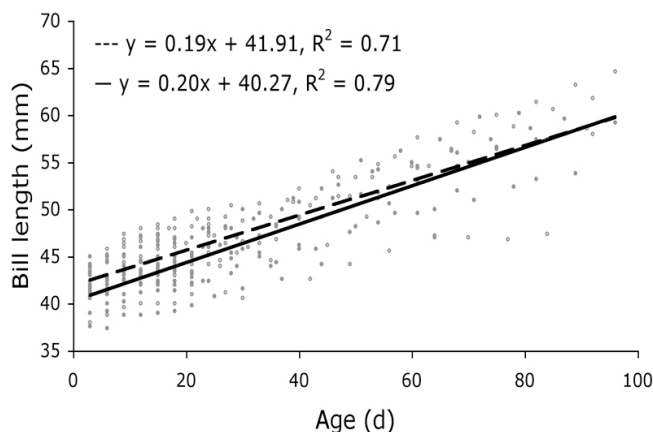


Figure 3.3: Bill length in captive-reared kiwi ($n=28$) from hatching through to three months of age. The growth formulas for females (---) and males (—) are given as linear regressions with associated R^2 values.

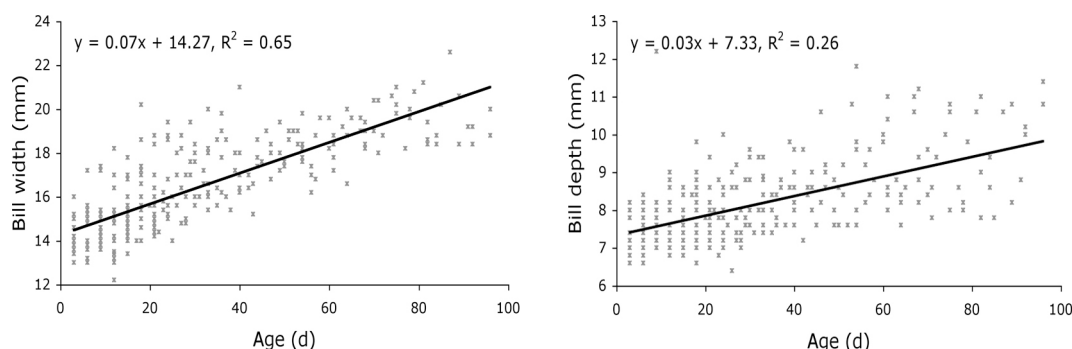


Figure 3.4: Bill width and depth in captive-reared kiwi ($n=28$) from hatching through to three months of age. The growth formulas are given as linear regressions with associated R^2 values.

Longitudinal growth of the long bones was fastest in the tibiotarsus (0.40 mm/d, $SE=0.009$), followed by the femur (0.22 mm/d, $SE=0.014$) and tarsometatarsus (0.22 mm/d, $SE=0.005$). The length ($p=0.005$), width ($p=0.001$) and depth of the tibiotarsus ($p=0.003$) were greater in female hatchlings than in males (Figure 3.5). Females also hatched with a longer tarsometatarsus ($p=0.024$) length than males. No significant differences were found between the sexes in femur length, tarsometatarsus width and

depth, and toe length, width and depth and thus regressions in Figure 3.6 are shown for the whole population.

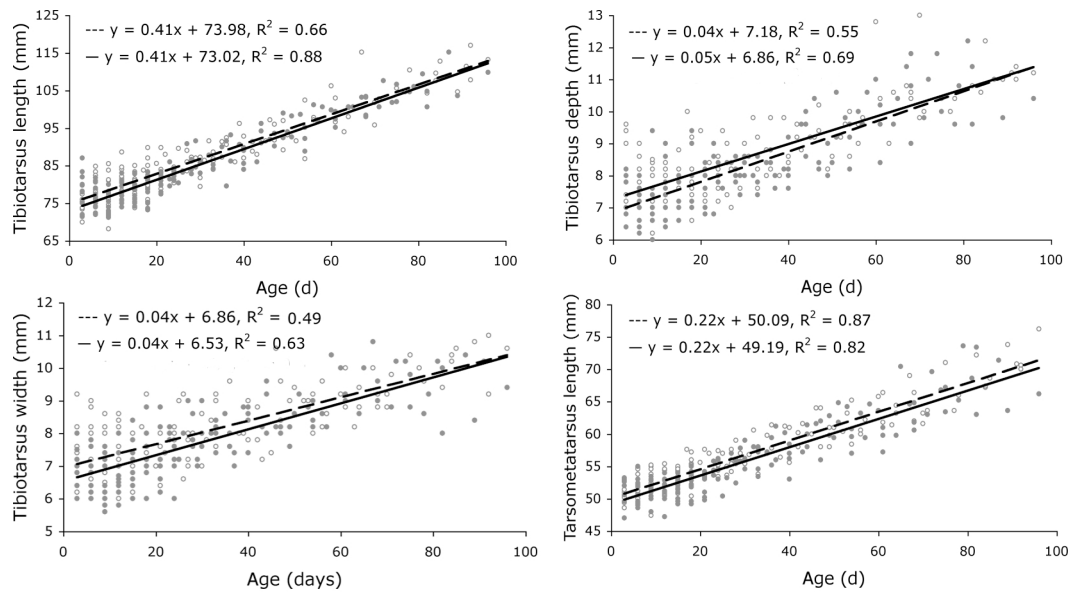


Figure 3.5: Tibiotarsal length, width and depth and tarsometatarsal length in captive-reared kiwi (n=28) from hatching through to three months of age. The growth formulas for females (---) and males (—) are given as linear regressions with associated R^2 values.

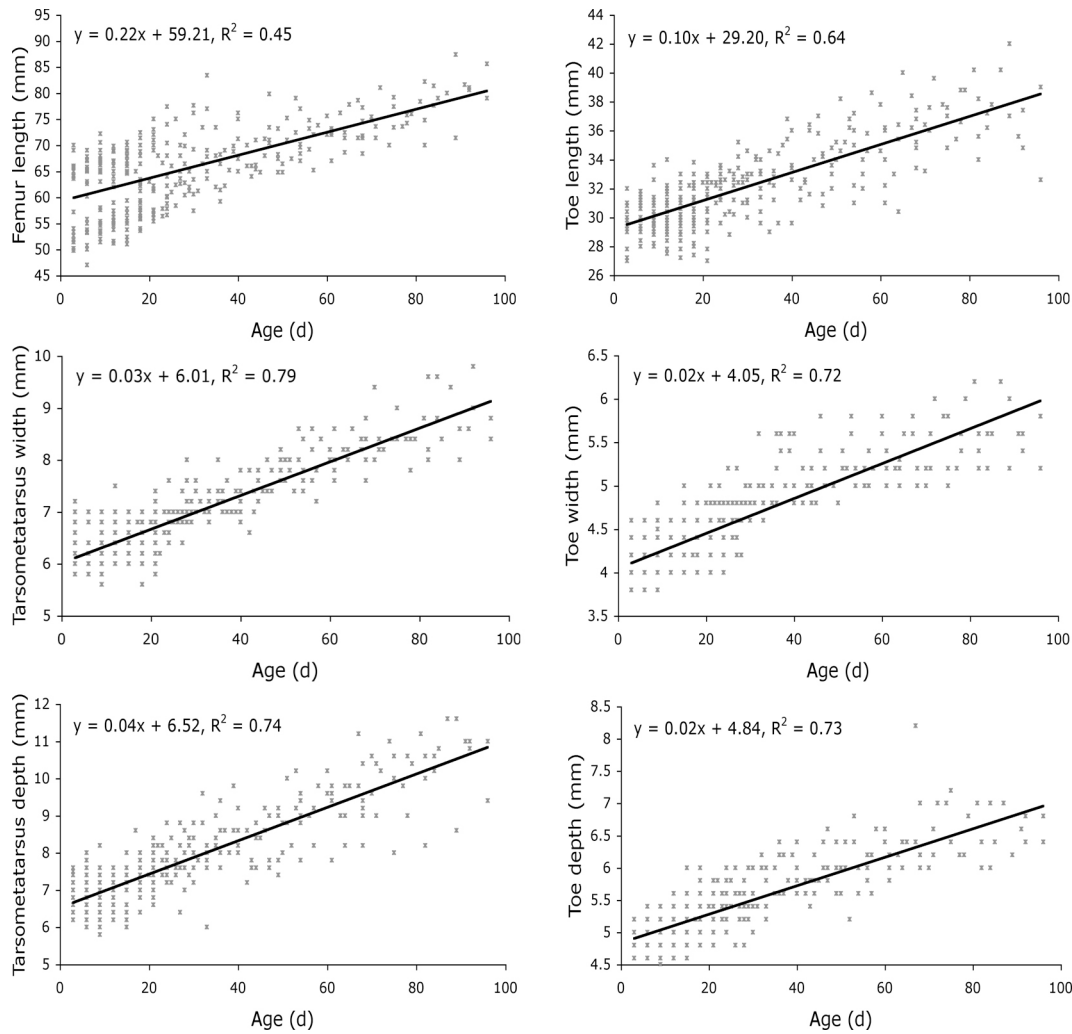


Figure 3.6: Femoral length, tarsometatarsal width and depth, and toe length, width and depth in captive-reared kiwi (n=28) from hatching through to three months of age. The growth formulas are given as linear regressions with associated R^2 values.

3.3.2 Captive-reared versus wild growth

Weight and bill measurements of wild kiwi were not recorded at consistent time intervals as occurred for captive-reared chicks. Hatch weight was not available for the wild chicks and thus the size of wild and captive-reared hatchlings cannot be compared here. The rate of weight increase was significantly faster in captive kiwi than wild kiwi in the time period of 10-100 days of age ($p < 0.0001$).

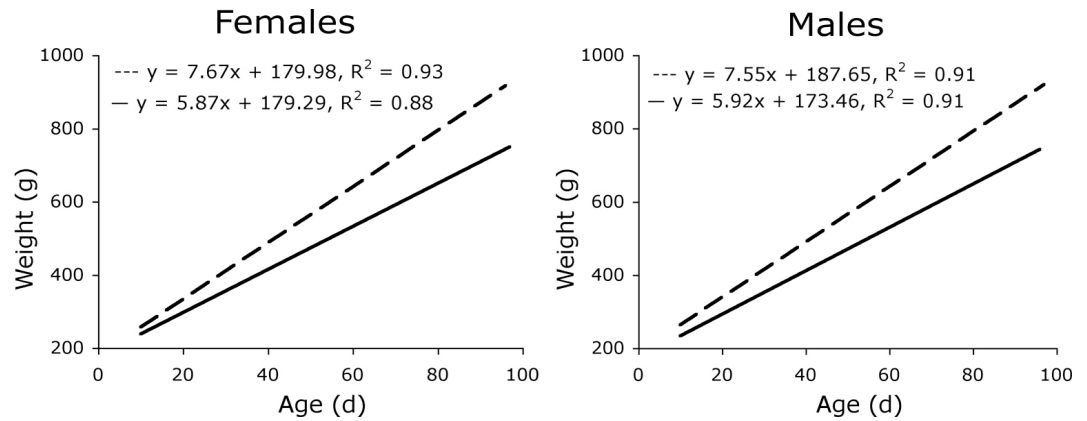


Figure 3.7: Weight gain in female and male captive-reared (---) and wild (—) kiwi from hatch to three months of age. The growth formulas are given as linear regressions with associated R^2 values.

The mean weight gain in captive-reared females was 7.7 g/d (SE=0.14) compared to 5.9 g/d (SE=0.24) in wild females (Figure 3.7). Males showed a similar trend with an average gain of 7.5 g/d (SE=0.15) in captive-reared birds and 5.9 g/d (SE=0.22) in wild birds. There were no significant differences in weight gain between males and females within the groups of captive-reared or wild kiwi.

Captive-reared birds hatched with shorter bills than the wild birds (females: $p=0.025$; males: $p=0.001$) (Figure 3.8). The bills of the captive-reared males grew faster than their wild counterpart ($p=0.004$), but there was no difference in growth rate between captive-reared and wild females. The wild females hatched with significantly longer bills than wild males ($p<0.0001$) and both then proceeded to grow at similar rates. The same was the case with captive-reared females and males ($p<0.001$).

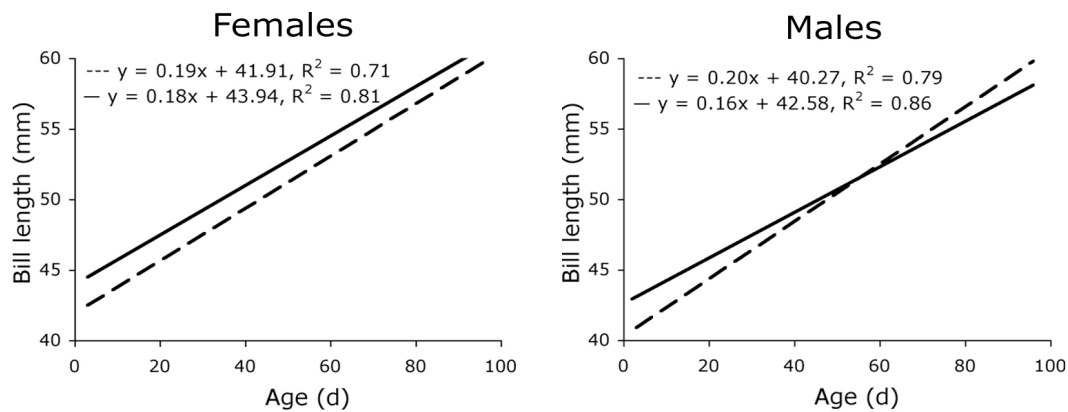


Figure 3.8: Bill length growth in female and male captive-reared (---) and wild (—) kiwi from hatch to three months of age. The growth formulas are given as linear regressions with associated R^2 values.

3.4 Discussion

Physiological limits to growth rates are imposed by adult size and pattern of development (Ricklefs, 1973). Slow growth in precocial birds, such as the ratites and chickens, is a consequence of intrinsic limits to growth rate in tissues such as bone (Ricklefs, 1979b, Starck, 1994, Blom and Lilja, 2004). The number of proliferating cells and the depth of the cartilaginous growth plate may be a major determining factor in the speed of growth in the long bones and thus the overall animal (Kirkwood et al., 1989a, Kirkwood et al., 1989b). At the time of hatching, the kiwi limb has a high level of developmental maturity in order for it to be able to kick its way out of the shell and walk and forage independently from an early age, but high levels of maturity prevents it from growing quickly (Ricklefs, 1973). Hindlimb elements in the day old chick may be up to 58% of their adult length (Reid, 1972), but they can take 4-6 years to reach full maturity (Beale, 1991, Bourdon et al., 2009).

All hindlimb parameters I measured grew in a fairly linear manner, while bodyweight was well described by a second-degree polynomial regression. However, it is unlikely that either of these trends persist in kiwi much older than 3 months; it is more likely that the growth patterns observed in this study are only a small portion of a growth curve that is sigmoid in shape as is seen in wild kiwi (McLennan et al., 2004) and other birds (Ricklefs, 1968). Chicken and ostrich growth are well described by a Gompertz

equation that shows a slowing and eventual plateau of growth when approaching mature size (du Preez et al., 1992, Mellett and Randall, 1994, Aggrey, 2002). Maximum growth rate of ostrich hindlimb elements occurs within the first 3-4 months and begins to slow soon after that (Mellett and Randall, 1994, Mushi et al., 1998). Rate of bone deposition in the hindlimbs of ostrich and emu is initially fast, but decreases as age increases (Castanet et al., 2000). In the kiwi, long bone growth is also fastest in early growth, but it is still slow enough to allow for cyclical growth marks, or lines of arrested growth (LAGs), to appear in the cortices of the long bones, a phenomenon that does not occur in other extant ratites (Bourdon et al., 2009).

The sexual dimorphism in weight that is seen in adult kiwi was not apparent during the period I observed, despite captive-reared females hatching with slightly but significantly larger bill length, tarsometatarsal length, tibiotarsal length, tibiotarsal width and tibiotarsal depth. The weight gain in both male and female captive-reared kiwi during their first three months is significantly faster than that of wild kiwi. These increased rates suggest that captive management regimes may not be accurately imitating the diet and growth conditions of wild kiwi chicks. Increased weight gain due to excess available food, high protein diets and nutrient imbalances during critical stages of limb development, such as the period studied here, have all been implicated in long bone deformities in the ostrich and poultry industries (Samson, 1997, Julian, 1998, Mushi et al., 1999, Oviedo-Rondon et al., 2006). Because the nutrient content of the kiwi's wild diet has only undergone limited investigation (Potter et al., 2009), it is uncertain whether current captive diet formulations are providing chicks with the appropriate nutrition.

For the period of development observed in this study, female kiwi bill length was initially longer than in males at time of hatch. However, regression lines converged by 100 days and showed no difference in bill growth rates between the sexes. Conversely, in wild kiwi that were observed for a longer period of time, females displayed slightly faster bill growth rates and growth continued for a longer period of time than in males (McLennan et al., 2004). The shorter bill length of captive-reared hatchlings versus wild hatchlings in this study suggests that captive-reared kiwi may hatch relatively earlier than wild kiwi. This is supported by the observation that kiwi may have an incubation

period up to 91 days in the wild (Colbourne, 2002), whereas incubation is tightly regulated in captivity so that hatching occurs very close to 78 days. An alternative explanation, if it is assumed that incubation time is equivalent, is that artificial incubation techniques could be responsible for a lower embryo growth rate leading to a smaller bill at hatch. Further study is required to determine the mechanism and significance of the difference in embryonic development between wild and captive incubated kiwi.

Captive rearing of kiwi chicks is a successful method of supplementing wild stock, and there is only limited evidence of limb deformities in captive kiwi. However, my initial investigations into kiwi growth suggest that the weight gain in kiwi chicks in captivity should not be further increased as it already exceeds wild growth rates and may predispose birds to limb growth deformities. Future research should focus on determining the suitability of the kiwi's current captive diet not only for overall health, but also for producing optimum growth rates. Characterisation of kiwi growth patterns would benefit from histological investigation of the growth centres of long bones and observation over a longer period of time than three months as kiwi do not reach their adult size until 5-6 years old.

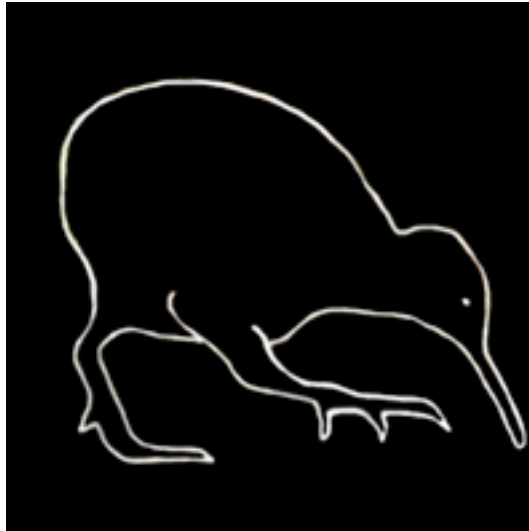
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Chapter Four

**Locomotion parameters and oscillating lateral head motion in
brown kiwi (*Apteryx mantelli*)**

Locomotion parameters and oscillating lateral head motion in brown kiwi (*Apteryx mantelli*)

Abstract

The gaits of two adults and one juvenile brown kiwi (*Apteryx mantelli*) were filmed while walking on a treadmill. Duty factor, frequency, stride length, step length, swing length and foot height were measured from the video recordings and compared to overground parameters from another study. Similarity between the treadmill and overground locomotor parameters validates the use of a treadmill in studying kiwi locomotion. None of the birds achieved the theoretical transition from a walk to a run at a duty factor of 0.5. After normalising for size, the juvenile showed a longer stride length and lower frequency with increasing speed than the adults. Lateral head oscillations were observed during the stride cycle, which I propose having a sensory function as well as a biomechanical one.

Keywords: kiwi, locomotion, gait, treadmill, head movement, sensory behaviour

4.1 Introduction

Kiwi (*Apteryx* spp.) are flightless, nocturnal birds that inhabit New Zealand's native and exotic forests, scrubland and tussockland of both main islands and several offshore islands (Heather and Robertson, 1998, Holzapfel et al., 2008). Kiwi are vulnerable to predation by introduced mammalian predators such as stoats and weasels and are the focus of intense conservation efforts to manage their population decline (McLennan et al., 1996, McLennan et al., 2004, Holzapfel et al., 2008).

The kiwi is the smallest member of the monophyletic group of ratites that also includes ostriches, emus, rheas, cassowaries and the extinct moa and elephant-birds (Cracraft, 1974). Previous study of kiwi locomotion (Abourachid and Renous, 2000) compared kinematic variations in ratites using footfall patterns over sand and this presented descriptive parameters of a single adult kiwi. On the basis of this study and research in other species, it has been suggested that the crouched and compliant posture of kiwi and other small bipeds allows for grounded running, a type of running that does not incorporate an aerial phase. That is, a phase where there is no double limb support (Alexander and Jayes, 1983). Grounded running is common in small bipeds, especially terrestrial birds (Gatesy and Biewener, 1991, Gatesy, 1999) and is a true running gait despite its lack of aerial phase (Rubenson et al., 2004, Hancock et al., 2007). Footfall patterns alone cannot accurately predict a change in gait types in birds, but can provide useful information about kinematic trends over a range of speeds.

Gait analysis is widely used in both humans and birds to study and/or quantify numerous aspects of locomotion, including orthopaedic rehabilitation efforts (Whittle, 2007), spinal cord injuries in chicks (Muir et al., 1998), lameness in poultry (Kestin et al., 1992, Corr et al., 1998, Corr et al., 2003a, Corr et al., 2003b), and tibiotarsal rotation in ostriches (Cooper, 2007). The motorised treadmill is a common piece of equipment in locomotion study of humans and many different animal species (e.g. Fredricson et al., 1983, Gatesy and Biewener, 1991, Farmer and Carrier, 2000, Reilly, 2000) and provides a convenient controlled environment, but does not provide identical conditions to overground locomotion (Gatesy and Biewener, 1991, Barrey et al., 1993, Buchner et al.,

1994, Savelberg et al., 1998, Pereira et al., 2006, Lee et al., 2008). In most instances, kinematic differences are noted but are not considerable enough to discount the treadmill as a useful tool (Lee and Hidler, 2008).

In this chapter, I aim to validate the treadmill as an acceptable technique for the study of locomotion in the kiwi by comparing my findings to those in Abourachid and Renous (2000). I aimed to characterise changes in gait parameters with increasing speed and compare trends between the different birds to identify kinematic differences associated with size and/or age. Finally, I aim to describe the movement of the head and neck of kiwi during locomotion and the possible biomechanical and sensory relevance of these movements

4.2 Materials and Methods

Three brown kiwi were used for my analysis: one juvenile male bird of approximately 10.5 months of age was 974 g and from a captive source; one adult male from a captive institution and approximately 4 years old weighing 1.7 kg; and a third bird that was an adult wild female of unknown age weighing 2.3 kg. All birds were used with permission from their respective Department of Conservation (DOC) conservancy and under the approval of Massey University Animal Ethics Committee.

Each bird was placed on a human treadmill within a 1.2 m x 0.75 m x 0.35 m glass retaining box suspended above the tread (Figure 4.1). The box was fit with a mirror at the front end situated at a 45-degree angle toward the ceiling. One digital video camera was suspended above the treadmill and perpendicular to the tread directly above the mirror so as to capture the front-on reflection of the bird. Another video camera situated at the level of the tread captured the lateral view of the bird.

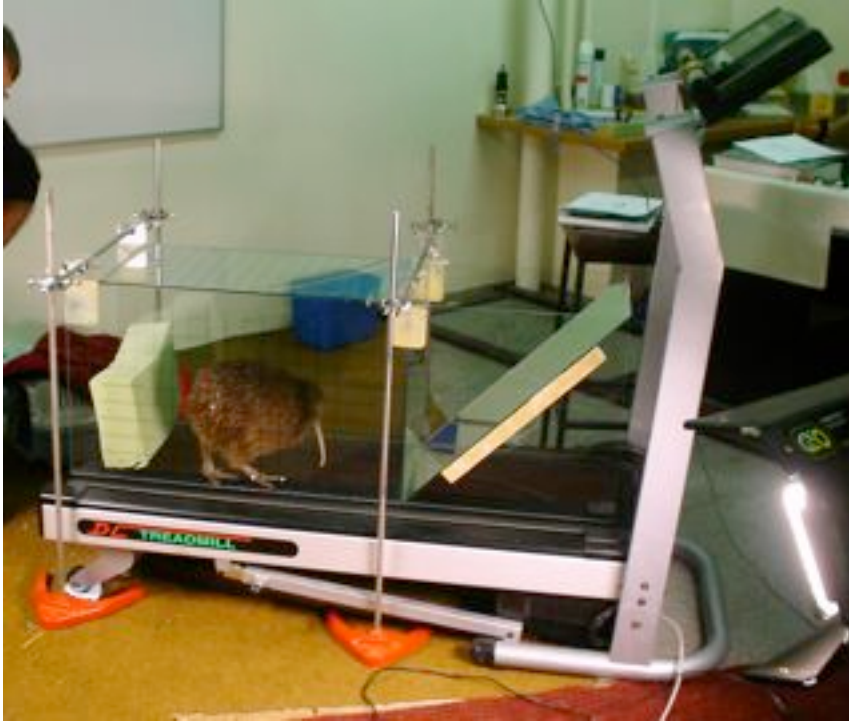


Figure 4.1: Treadmill apparatus for recording of kiwi locomotion. One camera suspended above the mirror captured a front-on reflection of the bird as well as an overhead view. Another camera captured a lateral view.

The speed of the treadmill was slowly increased, allowing the bird to take approximately 20 steps at each speed interval until the bird could no longer match the tread speed, at which point the treadmill was stopped. Each bird was given a 5-minute resting period before another series of speed increases commenced. A large sponge was wedged in the rear of the retaining box to encourage the birds to walk forward and an investigator sat directly at the end of the tread, behind the intended direction of travel of the bird to ensure the safety of the bird throughout the trial.

Video recordings (25 frames/second) were analysed frame by frame in the QuickTime Player program. Lateral views only recorded the right side of the bird and thus the right leg was used in all analyses of each bird. Stride characteristics were gathered from a series of at least four steps at a given speed where the bird was easily matching the tread speed. Data for each series of steps were pooled to create a mean value for the given speed. The birds were prone to slipping on the tread and any steps where there was obvious slipping were not selected for analysis.

4.2.1 Stride characteristics

Speed was determined from the overhead video by dividing the time it took a mark on the treadmill belt to return to the same position by the length of the tread. The stride length, or distance the body moved forward during an entire stride cycle, was calculated as the time for one foot to return to the same position multiplied by speed. Dividing speed by the stride length yielded stride frequency.

Following the method of Pennycuick (1975), the natural log of the stride length and frequency were plotted against each other and the resulting equation yields constants a_5 and a_6 in accordance with the formula:

$$\ln S = \ln a_5 + a_6 \ln V \quad (\text{Eq. 1})$$

The constants were then used in the equations

$$S = a_5 V^{a_6} \quad (\text{Eq. 2})$$

and

$$F = \frac{1}{a_5} V^{(1-a_6)} \quad (\text{Eq. 3})$$

The resulting equations show the relative contribution of stride length and frequency with increasing speed.

The duration of the stance phase, when the foot is in contact with the ground, and the swing phase, when the foot is not in contact, were measured directly from the lateral films. The portion of the cycle where the foot is in contact with the ground is the duty factor (Alexander and Jayes, 1978). A duty factor of 0.5 represents the transition between grounded and aerial locomotion and has classically indicated the shift from walking to running (Hildebrand, 1976). Step length is a measure of the distance the body moves forward while the foot is on the ground and was determined by dividing the stance time by the treadmill speed. Swing length was determined by dividing the swing

time by the treadmill speed.

Foot height was recorded as the maximum height the longest toe reached during the swing phase for each speed. Relative foot height was obtained by dividing absolute foot height by the hip height, h (metres).

ANCOVAs were carried out on all stride characteristics using age as the covariate using SYSTAT (Systat for Windows 7.0; Systat Software, Chicago IL, USA).

4.2.2 Treadmill versus overground locomotion

In order to verify the treadmill as viable method for observing typical gait patterns in kiwi, I compared the parameters obtained from my trials to those recorded for overground locomotion in Abourachid and Renous (2000). My data were converted to relative values in the manner described in Alexander and Jayes (1983) in order to directly compare to the regression line equations reported in their trial with my own. Relative speed was obtained by dividing absolute speed by $(gh)^{0.5}$, relative frequency was the product of absolute frequency and $(h/g)^{0.5}$ and relative stride length was the absolute value divided by hip height. Here g is gravitational acceleration (9.81 m/s^2).

4.2.3 Head movement

The lateral movement of the head during the stride cycle was documented by tracking the position of the base of the bill in relation to the body's midline parallel to the direction of locomotion as seen by the overhead camera. The observations were grouped into slow ($0.3 - 0.44 \text{ m/s}$), medium ($0.45 - 0.59 \text{ m/s}$) and fast ($0.6 - 0.75 \text{ m/s}$) speeds.

4.3 Results

All three kiwi spent a portion of their walking time pressing against the sponge backing of the retaining box. At high speeds, the rubber tread proved to be too slippery for the

birds to maintain traction, resulting in the feet sliding farther forward and backward than what appeared to be normal. In adults, the feet were generally placed along the axis of motion, while the juvenile bird placed its feet parallel to the axis of motion, approximately 2 cm apart.

4.3.1 Stride characteristics

Stride characteristics in absolute and relative terms are illustrated in Figure 4.2. Speed had a significant effect on all absolute and relative parameters except foot height. The duty factor decreased with increasing speed, but did not drop below 0.5 indicating there was a period of double limb support for all speeds I observed. Even at the top speed recorded for the adults, 1.04 m/s, the kiwi did not make the theoretical transition from walk to run by incorporating an unsupported aerial phase in their gait. The absolute values showed that both speed ($p=0.007$) and age ($p=0.042$) had a significant effect on duty factor, but only speed ($p=0.003$) had a significant effect when converted to relative values.

Stride length ($p<0.001$) and frequency ($p<0.001$) increased with increasing speed. Age had an effect on both relative stride length ($p<0.001$) and relative frequency ($p<0.001$). Values obtained from Equation 1 yielded contrasting results for adults and juveniles where stride length had a greater contribution to speed than frequency for the juvenile and the opposite for adults.

$$\text{Juveniles: } S = -0.51V^{0.58} \text{ and } F = -1.98V^{0.42}$$

$$\text{Adults: } S = -0.69V^{0.40} \text{ and } F = -1.46V^{0.60}$$

Step length ($p<0.001$), relative step length ($p<0.001$), swing length ($p<0.001$) and relative swing length ($p<0.001$) increased with increasing speed, but only relative step length was affected by age ($p<0.001$).

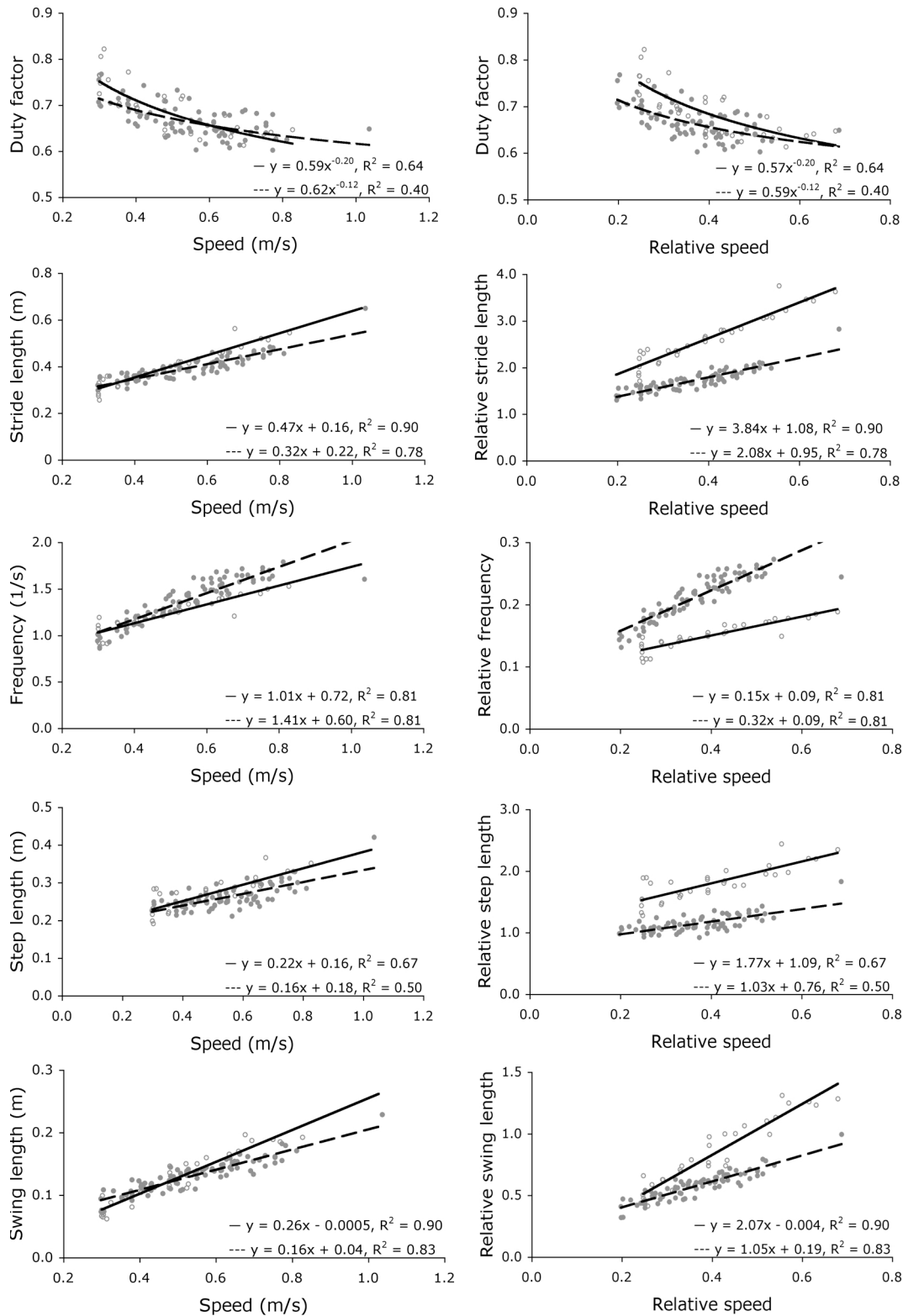


Figure 4.2: Stride characteristics plotted against speed and relative speed in juvenile (1) kiwi (open circles) and adult (2) kiwi (solid circles). Equations of regression lines for juvenile (—) and adult (---) kiwi are provided with associated R^2 values.

The maximum foot height during the swing phase did not vary with speed or with age (Figure 4.3).

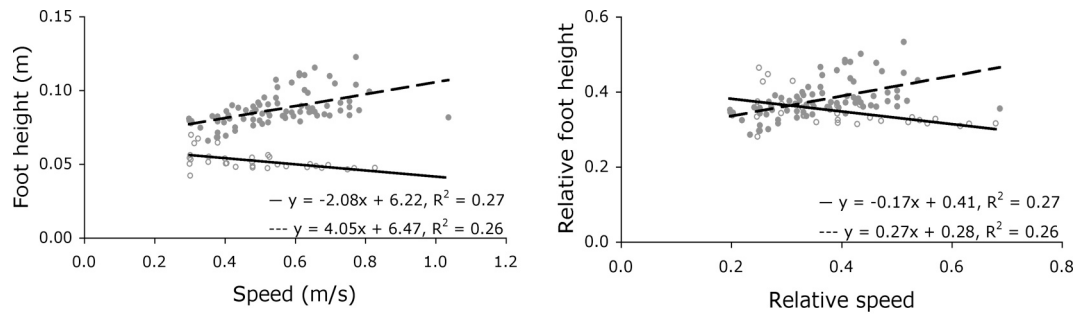


Figure 4.3: Maximum foot height in the swing phase plotted against speed in juvenile (1) kiwi (open circles) and adult (2) kiwi (solid circles). Equations of linear regression lines for juvenile (—) and adult (---) kiwi are provided with associated R^2 values.

4.3.2 Treadmill versus overground locomotion

The regression lines for treadmill and overground data yielded similar results (Table 4.1).

Table 4.1: Regression lines for all relative parameters plotted against relative speed for treadmill locomotion (this study) and overground locomotion (Abourachid and Renous, 2000). The number of animals used to determine these values (n) is in brackets.

	Treadmill – Juvenile (n=1)	Treadmill – Adult (n=2)	Overground (n=1)
Duty factor	$y = 0.571x^{-0.20}$	$y = 0.586x^{-0.12}$	$y = 0.504x^{-0.20}$
Relative stride length	$y = 3.84x + 1.08$	$y = 2.08x + 0.95$	$y = 2.10x + 1.19$
Relative frequency	$y = 0.15x + 0.09$	$y = 0.32x + 0.09$	$y = 0.27x + 0.08$
Relative step length	$y = 1.77 + 1.09$	$y = 1.03 + 0.76$	-
Relative swing length	$y = 2.07x - 0.004$	$y = 1.05x + 0.19$	$y = 1.62x + 1.12$

4.3.3 Head movement

The lateral oscillations of the head showed a definite link with the cycle of foot placement where the head moved contralateral to the raised leg throughout the walking cycle (Figure 4.4). Involuntary biomechanical movement is overlaid with a voluntary movement that was represented by the birds being able to move and/or hold their head on either side of the axis of motion.

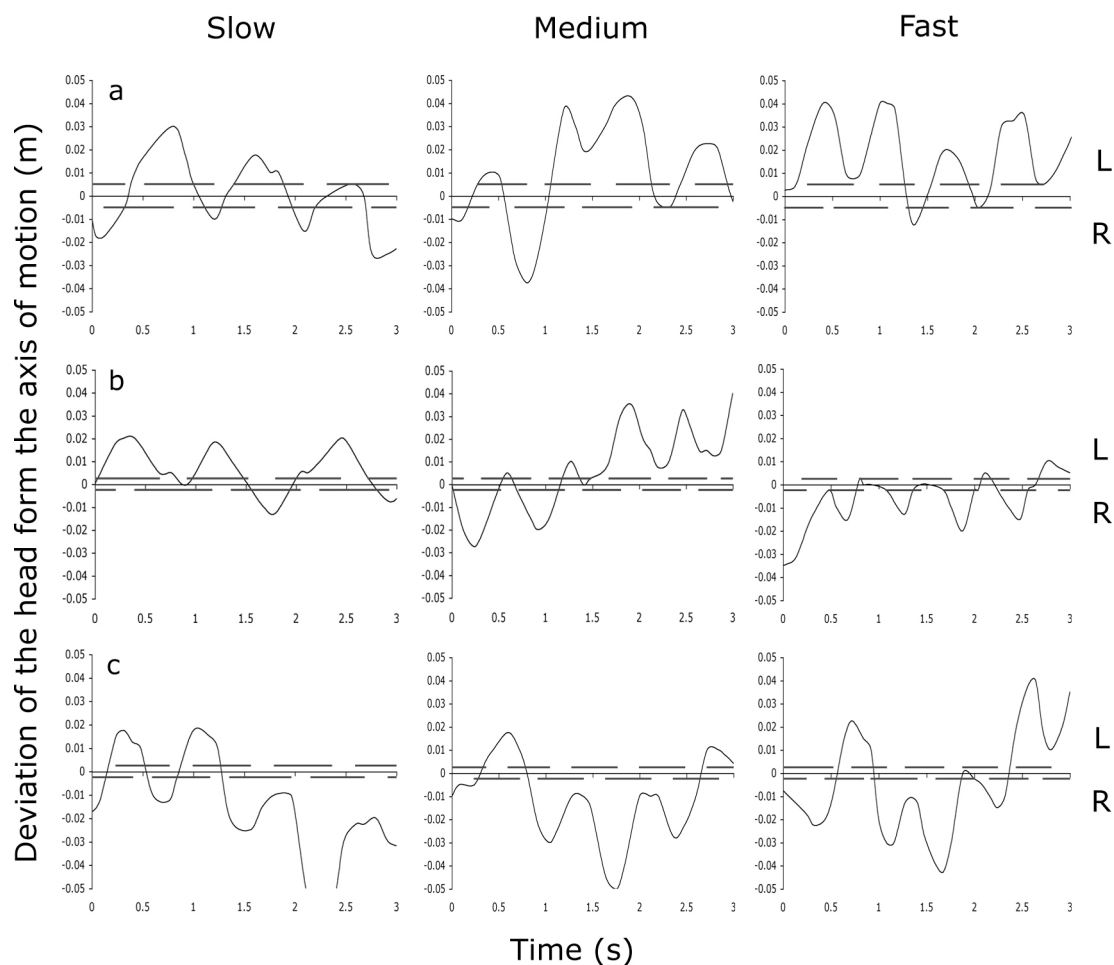


Figure 4.4: Track of head movement superimposed over footfall patterns during locomotion in kiwi. Head movement is measured by the distance of the head from the midline of the body (metres) parallel to the direction of movement and plotted against time (seconds) for one juvenile (a) and two adults (b, c). The dashed lines on each side of the axis of motion represent the length of time each foot (L=left foot; R=right foot) was in contact with the ground.

There was not a large difference between the ‘Slow’, ‘Medium’ and ‘Fast’ speeds in this study and these speeds do not represent the full range of speeds that can be achieved by kiwi. Kiwi *b* demonstrates a small increase in frequency and decrease in magnitude of the head oscillations as speed increases, while the more erratic head movements of *a* and *c* do not make these trends obvious. As speed increased, the duration of foot contact with the ground decreased and step frequency increased, as is represented by the dashed line in Figure 4.4.

The difference in step width between the juvenile and the adults, where the juvenile did not place its foot on the ground at the midline, caused the juvenile’s body to be less stationary throughout the walking cycle than in the adults. However, the step width did not appear to affect the trends in head oscillations between kiwi *a*, *b* and *c*.

4.4 Discussion

Ratites such as the kiwi are cursorial bipeds adapted for running. Small ratites are not considered highly cursorial because they switch to a running gait at lower speeds than larger ratites (Abourachid and Renous, 2000). In this study, kiwi did not display an aerial phase to indicate a transition from walking to running, possibly because they could not reach high enough speeds on the treadmill. The speeds observed in Abourachid and Renous (2000) were slightly higher than those observed here and while an aerial phase was observed in their kiwi, only one kiwi reached 1.04 m/s on one occasion in this study and an aerial phase was not used. Top speeds in kiwi are recorded as being 40-48 kph (11.1-13.3 m/s) (Fowler, 1991), but neither study approached these speeds. In general, duty factors were slightly higher at equivalent speeds on the treadmill as overground. Other birds have demonstrated the same trend when locomoting on treadmills due to a longer stance time (Abourachid and Renous, 2000). An increase in stance time may indicate the importance of a long period of contact with the ground even at high speeds to improve stability or aid in navigation abilities (Gatesy and Biewener, 1991, Abourachid and Renous, 2000). Despite the difference in duty factors, this study found remarkably similar kinematic values and trends using the treadmill to those reported by Abourachid and Renous (2000) about overground kiwi locomotion, confirming treadmill use as a valid method for gait analysis in kiwi.

The locomotion parameters that the juvenile bird exhibited was opposite to what I expected to see in a young bird. The juvenile kiwi increased its stride length more than the adults in order to increase speed, rather than increasing frequency as seen in the young of other species (Muir et al., 1996, Abourachid and Renous, 2000). Differences in walking ability in adult chickens and young chicks may be due to immature muscle reflex pathways (Muir et al., 1996). Instead, the young kiwi displayed kinematic trends that would be expected from a smaller adult bird. When compared at dynamically similar speeds, smaller birds take longer steps than larger birds in relation to their size (Gatesy and Biewener, 1991). This may indicate that juvenile kiwi of 10.5 months of age have reached neurological and/or muscular maturity in areas that control locomotion. In the future, investigations should follow a chick from a very young age through adulthood to determine if kiwi have differing locomotor patterns throughout postnatal development and if so, at what age these patterns arise.

Another noted difference in the gait between the juvenile and adult birds was in the step width. Step width was wider in the juvenile bird than in the adults, a trend that is found in children (Stolze et al., 1997) and *ad libitum*-fed broiler chickens that suffered lameness due to increased bodyweight (Corr et al., 2003a). There were no clinical signs to suggest movement problems due to lameness. Care must be taken in the interpretation of these results as only a single juvenile bird was used. Future study of chick and juvenile locomotion is recommended to confirm my findings and explore possible causes for the observed difference in step width.

Two types of previously undescribed lateral head movement during locomotion were observed. The graphs of head movement show an undeniable link to the pattern of footfalls, but it is uncertain whether a biomechanical explanation is the only one. I suggest that the patterns of head movement are best explained by two mechanisms: voluntary and involuntary. Involuntary movement appears to be the result of counterbalancing the raised leg and is represented by a regular contralateral oscillation to the raised leg. Voluntary movements appear to be superimposed over this pattern and allowed the kiwi to hold its head on one side or another of the axis of movement or move the head in the same direction as the swinging leg. Head movement, though not

strictly lateral movement, is used in many other birds to aid in gathering visual information (Dagg, 1977, Frost, 1978, Davies and Green, 1988, Troje and Frost, 2000, Fujita, 2002, Stamp Dawkins, 2002, van der Willigen et al., 2002, Fujita, 2003, Fujita, 2006, Jiménez Ortega et al., 2009). The poor eyesight of kiwi may benefit from additional head movements in the same way. However, the extent of the eyesight's limitations may be too great to overcome with simple head movements. Instead, the movements may be used for stereoscopic hearing or gathering olfactive or tactile information with the use of the sensitive bill tip (Cunningham et al., 2007, Martin et al., 2007) and rictal bristles. The sensory pits in the tip of the bill are used in prey detection (Cunningham et al., 2007) and could equally be used to detect obstacles. Rictal bristles are thought to act much like mammalian vibrissae (Lederer, 1972), which are used in detecting the physical environment in rats, such as depth and texture (Schiffman et al., 1975, Carvell and Simons, 1990). Specially adapted feathers are also used by auklets to navigate dark and close surroundings of their cave dwellings (Seneviratne and Jones, 2008).

The treadmill model for locomotion is not without its limitations. In general, the birds became more accustomed to the treadmill apparatus even within the short duration of the experiment, indicating more consistent results could be obtained through training. A purpose-built treadmill with a greater range of lower speeds may also improve a kiwi's ability to run, as the slowest speed of the treadmill I used was faster than the slowest walk used by a freely walking kiwi. Conversely, the birds struggled to match the speed of the treadmill at high speeds because of the slippery surface of the tread; the speeds achieved in this study are not likely to be the top speeds kiwi could reach in the wild (Gatesy and Biewener, 1991). The sponge placed at the back of the retaining box encouraged the birds to walk while on the treadmill, but may have inhibited the leg from moving to a fully retracted position; however, kiwi retract their legs to a much lesser degree than protracting them (Abourachid and Renous, 2000), so the sponge may not have significantly restricted leg movement.

In conclusion, this study validates the treadmill as a useful technique for the study of kiwi locomotion. I was successful in describing the locomotion parameters for kiwi at speeds ranging between 0.3 m/s and 1.04 m/s. The change in gait of the juvenile kiwi

with increasing speed differed from that reported previously in juveniles of other species, suggesting a maturation of locomotion, which may mirror the precocial life history of kiwi. There was also a difference in gait width between juvenile and adult kiwi, which requires further study. This study provides the first description of lateral head movements of kiwi during locomotion and the results suggest that this movement may serve dual functions as biomechanical counterweight and also to increase the sensory input of moving kiwi.

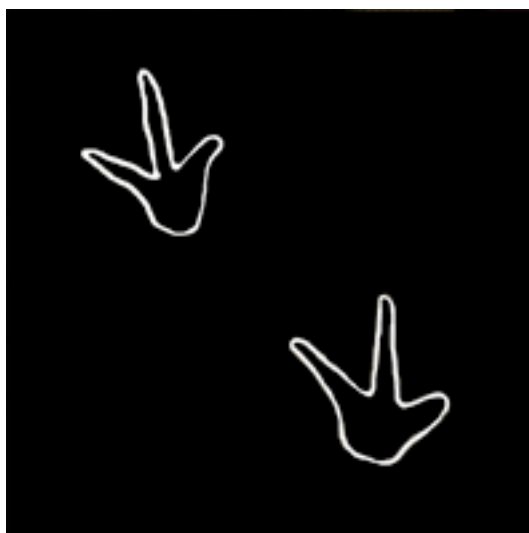
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Chapter Five

General Discussion

General Discussion

The preliminary work done here opens many avenues for future study, primarily as increasing numbers of kiwi move into captivity for captive breeding and rearing programmes and rehabilitation efforts. All three parts of this study highlight the important insights that the investigation of the hindlimb can tell us about overall kiwi growth, development and movement.

A kiwi chick differs greatly from other non-ratite birds in that its legs are of great importance throughout its life. In the absence of an egg tooth, a hatchling's legs are used to kick its way out of the eggshell (Rowe, 1978), an action that would be impossible without sufficient development and growth during the embryonic period. After hatching, a young kiwi chick is not fed by its parents and possesses only about 10 days-worth of nutrient reserves in its yolk sac (Prinzinger and Dietz, 2002). At this point, its limbs must be adequately developed so the chick may begin ranging, foraging, burrowing and using its legs for predator defense. Kiwi chicks hatch with a great deal of functional maturity that allows for immediate independent behaviour, but as a consequence their tissues do not have a great capacity for postnatal growth and therefore grow at a particularly slow rate compared to altricial birds (Ricklefs, 1979a, Ricklefs, 1979b, Ricklefs et al., 1994, Starck, 1994). Growth and maturation of the hindlimb tissues may continue well into adulthood (Beale, 1991, Bourdon et al., 2009). As the young chick grows, limb elements increase at different rates in order to compensate for the changes in distribution of mass and the forces exerted upon them (Main and Biewener, 2007). Smaller birds use different methods of locomotion to achieve changes in speed, but the overall patterns of locomotion do not differ between juvenile and adult kiwi, once again indicating the importance of advanced limb function from an early age in precocial birds (Starck and Ricklefs, 1998).

Due to the relatively large size of the kiwi hindlimb, stages of development could be easily determined using hindlimb features and therefore acted as a strong basis for determining age. That the age of kiwi embryos had differing estimations based on both

the ostrich and chicken models may indicate that even the close phylogenetic relationship of the kiwi and ostrich does not lead to identical developmental patterns. Kiwi embryos were staged using the methods of Hamburger and Hamilton (1951) and then related to ostrich incubation as a proportion of total incubation time. This created a scheme that may be used to determine the age of embryos found in the wild. The aging scheme used linear measurements that do not damage the specimens and could be easily utilised in the field. Studies of embryological development in kiwi would benefit from a greater quantity of fresh or well-preserved samples and specimens of known age to more accurately refine the aging scheme. Conservation managers and those involved in the handling of kiwi embryos should be aware of the great value the embryos have in investigating and preventing future embryonic deaths.

I was unable to compare the hindlimb growth of captive kiwi with wild kiwi because such data were not available for wild kiwi. Therefore, I used the available bodyweight and bill length data to compare captive and wild kiwi growth to give an indication of possible growth rate differences, and I found that growth of both parameters was faster in captive kiwi than in wild kiwi. This is of concern because high growth rates have been linked with limb deformities in other species. The incidence of limb deformities in kiwi is not high, but it does occur in other long-legged and precocial birds whose growth rates are affected by poor diet, lack of exercise and maintenance procedures (Serafin, 1982, Bailey et al., 1996, Speer, 1996, Julian, 1998, Naldo et al., 1998, Squire and More, 1998, Mushi et al., 1999, Williams et al., 2000, Naldo and Bailey, 2001). The mechanisms of long bone growth are not well studied in ratites, despite the importance of the limb throughout their life history, from aiding in hatching to almost immediate locomotion after hatching. Although bill length increased at a slower rate in wild kiwi, captive-reared kiwi hatched with shorter bills than wild kiwi. This finding could indicate that captive birds are hatching relatively earlier than their wild counterparts, or that there is some physiological difference between the two as a result of different incubation methods in captivity and in the wild. The bill length and some hindlimb parameters showed that captive-incubated females were larger at the time of hatch than captive-incubated males. The expected slowing in growth rate to produce a sigmoidal growth curve was not apparent in the 3-month study period I used. In future studies, growth should be investigated over a longer period to determine how growth slows with

age. The low-impact methods used in this growth study were and could easily continue to be incorporated into the daily maintenance routine of the keepers. Continued comparisons with the growth of chicks from wild populations is warranted so that if differences continue to arise, captive managers can adjust their husbandry to slow growth back to optimal levels. Further characterisation of kiwi growth could also allow for the possible use of the kiwi as a developmental analog for moa growth (Turvey et al., 2005) and other k-selected New Zealand species.

Locomotion in kiwi is fundamentally constrained by them being terrestrial and by the nature of the habitat they move within. This study provides descriptive parameters for the bipedal locomotion of kiwi and demonstrates possible mechanisms by which kiwi are able to navigate their dark and dense forest environment. All of the locomotion that I observed was completely grounded, that is, there was no period of time where the animal did not have at least one foot on the ground. Continuous contact with the ground may aid in navigation abilities, as may some of the head movements I observed. In particular, the lateral head movements were unusual and unprecedented in the literature. These movements are compelling because there is little mention of similar side-to-side action of the head in other animals. Those investigations that do mention lateral movements link them to gathering of visual information (Stamp Dawkins, 2002, van der Willigen et al., 2002). It is possible that because of the kiwi's poor eyesight, lateral head movements are needed to gather further visual information. However, kiwi eyesight may be so poor that these small movements would not make any difference, and the head movements may be necessary for extending the sensory range of the tactile facial vibrissae, for improved stereoscopic hearing, or there may be a biomechanical explanation for the head movement. The kiwi's unique body shape and structure, such as a long and flexible neck, might allow easy movement of the head in order to increase balance in a bird that needs excellent navigation skills for activity in dark and dense forests. Further studies of the gait of kiwi should include modelling the role of the head movements as a counter-balancing force in locomotion. A biomechanical explanation does not preclude the use of head movements for gathering better sensory information, and there may be complimentary benefits from it. Recent studies of kiwi perception offer support to the idea that head movements may be related to tactile sensation. Kiwi have a dense concentration of sensory pits in the bill tip that are used in gathering

olfactory and tactile information with correspondingly large centres in the brain, indicating a reliance on sensory cues to guide their activities (Cunningham et al., 2007, Martin et al., 2007, Ashwell and Scofield, 2008). Kiwi also have long rictal bristles, or facial whiskers, located at the base of the bill, which are also present in other birds and thought to function similarly to mammalian vibrissae (Lederer, 1972). In rats, whiskers are used to detect depth and surface texture, and a side-to-side sweeping action with the whiskers has been observed (Schiffman et al., 1975, Carvell and Simons, 1990, Kleinfeld et al., 2006). Specialised facial feathers are also used in the auklet to navigate their dark, narrow cave dwellings (Seneviratne and Jones, 2008). Additionally, I observed that the kiwi bill was kept close to the ground and was swept across the field in front of the bird while it was walking and it was used to investigate the sides and corners of their containing box. It is therefore not unreasonable to suggest that the bill and facial whiskers could be used to gather tactile information with each sweep of the head during movement. The fact that kiwi were able to isolate the head in positions opposite to those expected from biomechanical influence further supports the kiwi's ability to create voluntary, information gathering movements while locomoting. It is likely that voluntary movements for perception are overlaid on a biomechanical movement rhythm.

Many avenues of research are suggested by the fact that treadmill locomotion showed similar gait characteristics to overground locomotion in kiwi as reported in Abourachid and Renous (2000). The treadmill offers a level of experimental control not available when studying an animal in the wild or in an overground setup. Gait analysis in humans utilises various film and treadmill designs in injury rehabilitation that may be possible to adopt for similar purposes in kiwi. The ability to set quantitative goals based on the normal gait presented here will allow medical professionals to evaluate and rehabilitate kiwi with the goal of sending an animal back into the wild. Returning an injured bird to the wild after treatment may well allow it to breed and thus contribute to conservation efforts.

Investigations into energetics may be able to determine whether kiwi utilise the grounded running seen in ostriches and other species (Gatesy and Biewener, 1991, Rubenson et al., 2007). Instead of only characterising footfall patterns, fluctuations in

kinetic and gravitational potential energy with increasing speed should show at what point a kiwi adopts a running gait. The duty factor is unreliable at demonstrating the transition to a true running gait (Rubenson et al., 2004, Biknevicius and Reilly, 2006, Hoyt et al., 2006, Hancock et al., 2007), one where kinetic and potential energy are in phase with one another (Cavagna et al., 1977). Nevertheless, duty factor is still used to separate the more cursorial (adapted to running) birds, those that incorporate an aerial phase at a relatively lower speed, from less cursorial birds, those that incorporate an aerial phase at a relatively higher speed (Abourachid and Renous, 2000). Using only duty factor in the definition of a running bird ignores the possibility that different types of running may be used by different species in order to adapt to their respective environments. Studies of energetics would also allow for better comparison of the modes of locomotion used in kiwi with the extensive work done in ostriches and humans, but would require significantly more complex equipment and more invasive procedures. This study did not indicate any adverse impact of the bird's time spent on the treadmill and suggests that longer sessions may improve a bird's familiarity with the equipment and possibly improve consistency of results. The main limitation I observed with the treadmill model for studying locomotion was the kiwi's need for encouragement to continue walking while the tread was moving, which prompted the use of a sponge wedged into the glass retaining box. By bumping up against the sponge during the walking cycle, some natural patterns of movement may have been altered or obscured. The birds rarely travelled in a straight path for a long period of time, so the retaining box may need to be narrowed to limit the bird's ability to take sideways steps. Additionally, the full range of kiwi's walking and running speed may not have been observed because the treadmill did not reach low enough speeds and at high speeds, the birds were unable to maintain their grip on the rubber tread.

While this study has delivered many key descriptive features of kiwi limb development, growth and locomotion, it also raises a large number of questions. In addition to those discussed above, key questions that have arisen from this study are:

- 1) Can an aging scheme for kiwi embryos be developed based primarily on external limb characteristics?

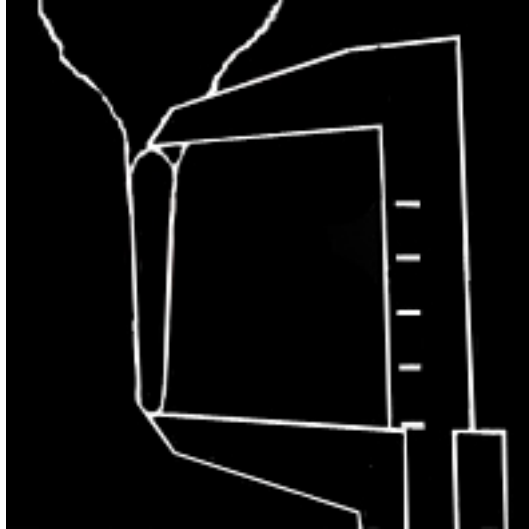
- 2) Should postnatal weight gain in captive-reared kiwi better match those found in the wild in order to prevent developmental limb deformities?
- 3) Does the kiwi use grounded running?
- 4) Does lateral head movement during locomotion in kiwi serve a dual purpose as a biomechanical counterbalance and a mechanism for gathering sensory information about the kiwi's environment?

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Appendix

Appendix

The following information is taken from:

HAMBURGER, V. & HAMILTON, H. L. (1951) A series of normal stages in the development of the chick embryo. *Journal of Morphology*, 88, 49-92.

Refer to original publication for plates. Stages 1-23 not included here.

Stage 24 (*ca.* 4 days)

1. *Limbs*: Wing- and leg-buds distinctly longer than wide. Digital plate in wing not yet demarcated. Toe-plate in leg-bud distinct. Toes not yet demarcated.

2. *Visceral arches* (see plates 7 and 8): First visceral cleft a distinct curved line. Slight indication of two protuberances (“a,” “b”) on mandibular process and of three protuberances (“d,” “e,” “f”) on second arch. Part “c” of mandibular process is receding. Second arch longer ventrally (at “f”) and much wider than mandibular process. Third arch reduced and partly overgrown by second arch; 4th arch flattened. Both are sunk beneath the surface. Third visceral cleft is an elongated groove. Fourth visceral cleft reduced to a small pit.

Stage 25 (*ca.* 4½ days)

1. *Limbs*: Elbow and knee-joints distinct (in dorsal or ventral view). Digital plate in wing distinct, but no demarcation of digits. Indication of faint grooves demarcating the third toe on leg.

2. *Visceral arches* (see plates 7 and 8): Maxillary process lengthened; it meets the wall of the nasal groove (notice the notch at point of fusion). Three protuberances on each side of first visceral cleft (“a” to “f”). In dorsal view, “a,” “b,” and “d” appear as round knobs, and “c” as a flat ridge. Part “f” is conspicuous and projects distinctly over the surface. It will be referred to as the “collar.” Dorsal part of third arch still visible. Third

and 4th visceral clefts reduced to small circular pits.

Stage 26 (ca. 4½-5 days)

1. *Limbs*: Considerably lengthened. Contour of digital plate rounded. Indication of faint groove between second and third digit. Demarcation of the first three toes distinct.
2. *Visceral arches* (see plates 8 and 9): Contour of maxillary process a broken line. Mandibular process lengthened ventrally. Protuberances “a” and “b” project over the surface. The middle protuberance (“b”) is subdivided by a shallow groove. A small knob is distinct at the dorsal edge of “c.” On the second arch, protuberances “d” and “e” are only slightly elevated over the surface. The “collar” (“f”) has broadened and overgrown visceral arches III and IV. A deep groove separates “f” from “c.” The two pits representing the 3rd and 4th visceral clefts are no longer visible.

Stage 27 (ca. 5 days)

1. *Limbs*: Contour of digital plate angular in region of first digit. Grooves between first, second, and third digits indicated. Grooves between toes are distinct on outer and inner surfaces of toe-plate. First toe projects over the tibial part at an obtuse angle. Tip of third toe not yet pointed.
2. *Visceral arches* (see plates 8 and 9): Contour of maxillary process is a curved, broken line. Mandibular process has broadened ventrally (at “c”) and grown forward. Protuberances “a” and “b” project over the surface. Parts “d” and “e” are flat. Protuberances “b” and “e” are close to fusion, but a separating line is still distinct. The “collar” (“f”) has broadened and continued its growth backward. It rises conspicuously above the surface. The groove between “c” and “f” has widened.
3. *Beak*: Barely recognizable.

Stage 28 (ca. 5½ days)

1. *Limbs*: Second digit and third toe longer than others, which gives the digital and toe-plates a pointed contour. Three digits and 4 toes distinct. No indication of 5th toe.

2. *Visceral arches* (see plates 8 and 9): Protuberance “a” still projects over the surface. Mandibular process has lengthened and grown forward. Parts “b” and “e” have fused; a fine suture line is occasionally still visible. Parts “b,” “d,” and “e” no longer project above the surface. External auditory opening is now very distinct between “a,” “b,” and “d.” “Collar” (“f”) projects distinctly over the surface. The neck between “collar” and mandible has lengthened.

3. *Beak*: A distinct outgrowth is visible in profile.

Stage 29 (ca. 6 days)

1. *Limbs*: Wing bent in elbow. Second digit distinctly longer than the others. Shallow grooves between first, second, and third digits. Second to 4th toes stand out as ridges separated by distinct grooves, and with indications of webs between them. Distal contours of webs are straight lines, occasionally with indication of convexity. Rudiment of 5th toe visible.

2. *Visceral arches*: Mandibular process lengthened (compare with stage 28). Mandibular process and second arch are broadly fused. Auditory meatus distinct at dorsal end of fusion. All protuberances have flattened. Neck between “collar” and mandibular process has lengthened. “Collar” stands out conspicuously.

3. *Beak*: More prominent than in stage 28. No egg-tooth visible as yet.

Stage 30 (ca. 6½ days)

1. *Limbs*: The three major segments of wing and leg are clearly demarcated. Wing bent in elbow-joint. Leg bent in knee-joint. Distinct grooves between first and second digits. Contours of webs between first two digits and between all toes are slightly curved concave lines.

2. *Visceral arches*: The mandibular process approaches the beak, but the gap between the two is still conspicuous. Lengthening of neck between “collar” and mandible is very conspicuous. “Collar” begins to flatten.

3. *Feather-germs*: Two dorsal rows to either side of the spinal cord at the brachial level. Three rows at the level of the legs; they are rather indistinct at thoracic level. None on

thigh.

4. *Scleral papillae*: One on either side of choroid fissure; sometimes indistinct but never more than two.

5. *Egg-tooth* distinct, slightly protruding. Beak more pronounced than in previous stage.

Stage 31 (ca. 7 days)

1. *Limbs*: Indication of a web between first and second digits. Rudiment of 5th toe still distinct.

2. *Visceral arches*: The gap between mandible and beak has narrowed to a small notch. “Collar” inconspicuous or absent.

3. *Feather-germs*: On dorsal surface, continuous from brachial to lumbo-sacral level. Approximately 7 rows at lumbo-sacral level. Distinct feather papillae on thigh. One indistinct row on each lateral edge of the tail.

4. *Scleral papillae*: Usually 6; 4 on the dorsal side near the choroid fissure, and two on the opposite side.

Stage 32 (ca. 7½ days)

1. *Limbs*: All digits and 4 toes have lengthened conspicuously. Rudiment of 5th toe has disappeared. Webs between digits and toes are thin and their contours are concave. Differences in size of individual digits and toes become conspicuous.

2. *Visceral arches*: Anterior tip of mandible has reached the beak. “Collar” has disappeared or is faintly recognizable.

3. *Feather-germs*: Eleven rows or more on dorsal surface at level of the legs. One row on tail distinct, second row indistinct. Scapular and flight feather-germs barely perceptible at optimal illumination or absent.

4. *Scleral papillae*: Six to 8, in two groups; one group on dorsal and one on ventral side. Circle not yet closed.

Stage 33 (ca. 7½-8 days)

1. *Limbs*: Web on radial margin of arm and first digit becomes discernible. All digits and toes lengthened.
2. *Visceral arches*: Mandible and neck have lengthened conspicuously. (Compare the ventral contour of body, from heart-region, along neck to tip of mandible, in this and the preceding stages.)
3. *Feather-germs*: Scapular and flight feather-germs not much advanced over stage 32. Tail: three rows distinct, the middle row considerably larger than the others.
4. *Scleral papillae*: Thirteen, forming an almost complete circle, with gap for one missing papilla at a ventral point near the middle of the jaw.

Stage 34 (ca. 8 days)

1. *Limbs*: Differential growth of second digit and third toe conspicuous. Contours of webs between digits and toes are concave and arched.
2. *Visceral arches*: Lengthening of mandible and of neck continues (see previous stage).
3. *Feather-germs*: On scapula, on ventral side of neck, on pro-coracoid, and posterior (flight) edge of wing, feather-germs are visible under good illumination. Feather-germs next to dorsal midline, particularly at limbo-sacral level, extend slightly over surface when viewed in profile. Feather-germs on thigh protrude conspicuously. One row on inner side of each eye. None around umbilical cord.
4. *Scleral papillae*: Thirteen or 14.
5. *Nictitating membrane* extends halfway between outer rim of eye (eyelid) and scleral papillae.

Stage 35 (ca. 8-9 days)

1. *Limbs*: Webs between digits and toes become inconspicuous. A transitory protuberance on the ulnar side of the second digit is probably a remnant of the web. Phalanges in toes are distinct.
2. *Visceral arches*: Lengthening of beak continues. Compare the distance between the eye and the tip of the beak, in this and the preceding stages.

3. *Feather-germs*: All are more conspicuous. Mid-dorsal line stands out distinctly in profile view. At least 4 rows on inner side of each eye. New appearance of feather-germs near mid-ventral line, close to sternum, and extending to both sides of umbilical cord.
4. *Nictitating membrane* has grown conspicuously and approaches the outer scleral papillae. Eyelids (external to nictitating membrane) have extended towards the beak and have begun to overgrow the eye-ball. The circumference of the eyelids has become ellipsoidal.

Stage 36 (ca. 10 days)

1. *Limbs*: Distal segments of both wing and leg are proportionately much longer. Length of third toe, from its tip to the middle of its metatarsal joint = 5.4 ± 0.3 mm. Tapering primordia of claws are just visible on termini of the toes and on digit 1 of the wing. Protuberance on posterior side of digit 2 of wing is missing.
2. *Visceral arches*: Primordium of the comb appears as a prominent ridge with slightly serrated edge along the dorsal midline of the beak. A horizontal groove (the “labial groove”) is clearly visible at the tip of the upper jaw, but is barely indicated on the tip of the mandible. Nostril has narrowed to a slit. Length of beak from anterior angle of nostril to tip of bill = 2.5 mm.
3. *Feather-germs*: Flight-feathers are conspicuous; coverts are just visible in web of wing. Feather-germs now cover the tibio-fibular portion of the leg. At least 9-10 rows of feather-germs between each upper eyelid and the dorsal midline. Sternal tracts prominent, with 3-4 rows on each side of ventral midline when counted in anterior part of sternum, merging into many rows around the umbilicus.
4. *Eyelids*: Nictitating membrane covers anteriormost scleral papillae and approaches cornea. Lower lid has grown upward to level of cornea. Circumference of lids is a narrowing ellipse with its ventral edge flattened.

Stage 37 (ca. 11 days)

1. *Limbs*: Claws of toes are flattened laterally and curved ventrally; dorsal tips are opaque, indicating onset of cornification. Tip of claw on wing is also opaque. Pads on

plantar surface of foot are conspicuous. Transverse ridges along the superior surfaces of the metatarsus and phalanges are first indication of scales. Length of third toe = 7.4 ± 0.3 mm.

2. *Visceral arches*: Labial groove on mandible is now clearly marked off. The comb is more prominent and clearly serrated. Length of beak from anterior angle of nostril to tip of bill = 3.0 mm.

3. *Feather-germs*: Much more numerous, and in most-advanced tracts (e.g., along back and on tail) elongated into long, much-tapered cones. External auditory meatus is nearly surrounded by feather-germs. Circumference of eyelids is bordered by a single row of just-visible primordia; none on remainder of lids. Sternal tracts contain 5-6 prominent rows when counted at anterior end of sternum.

4. *Eyelids*: Nictitating membrane has reached anterior edge of cornea. Upper lid has reached dorsal edge of cornea. Lower lid has covered one-third to one-half of cornea. Circumference of lids now bounds a much-narrowed and ventrally-flattened biconvex area.

Stage 38 (ca. 12 days)

1. *Limbs*: Primordia of scales are marked off over entire surface of leg; ridges have not yet grown out to overlap surface. Tips of toes show a ventral center of cornification as well as the more extensive dorsal one. Main plantar pad is ridged when seen in profile. Length of third toe = 8.4 ± 0.3 mm.

2. *Visceral arches*: Labial groove marked off by a deep furrow at the end of each jaw. Length of beak from anterior angle of nostril to tip of bill = 3.1 mm.

3. *Feather-germs*: Coverts of web of wing are becoming conical. External auditory meatus is surrounded by feather-germs. Sternum is covered with feather-germs except along midline. Upper eyelid is covered with newly-formed feather-germs; lower lid is naked except for 2-3 rows at its edge.

4. *Eyelids*: Lower lid covers two-thirds to three-fourths of cornea. Opening between lids is much reduced.

Stage 39 (ca. 13 days)

1. *Limbs*: Scales overlapping on superior surface of leg. Major pads of phalanges covered with papillae; minor pads are smooth. Length of third toe = 9.8 ± 0.3 mm.
2. *Visceral arches*: Mandible and maxilla cornified (opaque) back as far as level of proximal edge of “egg-tooth.” The channel of the auditory meatus can be seen only at the posterior edge of its shallow external opening. Length of beak from anterior angle of nostril to tip of bill = 3.5 mm.
3. *Feather-germs*: Coverts of web of wing are very long tapering cones. Note great increase in length of feather-germs in major tracts. Four to 5 rows of feather-germs at edge of lower eyelid.
4. *Eyelids*: Opening between lids reduced to a thin crescent.

Stages 40 to 44 are based mainly on the length of the beak and on the length of the third (longest) toe, since other external features have lost their diagnostic value. Of these two criteria, the length of the beak is the better, because it is more easily and accurately measured (with calipers) and shows less variability.

Stage 40 (ca. 14 days)

1. *Visceral arches*: Length of beak from anterior edge of nostril to tip of bill = 4.0 mm. The main channel of the auditory meatus is not visible in strictly lateral view of its external chamber.
2. *Limbs*: Length of third toe = 12.7 ± 0.5 mm. Scales overlapping on inferior as well as superior surfaces of leg. Dorsal and ventral loci of cornification extend to base of exposed portion of toe-nail. Entire plantar surface of phalanges is covered with well-developed papillae.

Stage 41 (ca. 15 days)

1. *Beak*: Length from anterior angle of nostril to tip of upper bill = 4.5 mm.
2. *Third toe*: Length = 14.9 ± 0.8 mm.

Stage 42 (ca. 16 days)

1. *Beak*: Length from anterior angle of nostril to tip of upper bill = 4.8 mm.
2. *Third toe*: Length = 16.7 ± 0.8 mm.

Stage 43 (ca. 17 days)

1. *Beak*: Length from anterior angle of nostril to tip of upper bill = 5.0 mm. “Labial grooves” are reduced to a white granular crust at the edge of each jaw; that of the lower jaw may be partially or completely sloughed off.
2. *Third toe*: Length = 18.6 ± 0.8 mm.

Stage 44 (ca. 18 days)

1. *Beak*: Length from anterior angle of nostril to tip of upper bill = 5.7 mm. The translucent peridermal covering of the beak is starting to peel off proximally.
2. *Third toe*: Length = 20.4 ± 0.8 mm.

Stage 45 (ca. 19-20 days)

1. *Beak*: Length is no longer diagnostic; in fact, the beak is usually shorter than in stage 44, due to a loss (by sloughing off) of its entire peridermal covering. As a consequence, the beak is now shiny all over and more blunt at its tip. Both labial grooves have disappeared with the periderm.
2. *Third toe*: Average length is essentially unchanged from that of stage 44, except in those breeds with a longer period of incubation (21 days) and a heavier build of body. For these latter, length of third toe = ca. 21.4 ± 0.8 mm.
3. *Extra-embryonic membranes*: Yolk-sac is half-enclosed in body-cavity. Chorion-allantoic membrane contains less blood and is “sticky” in the living embryo.

Stage 46 (20-21 days) Newly-hatched chick

