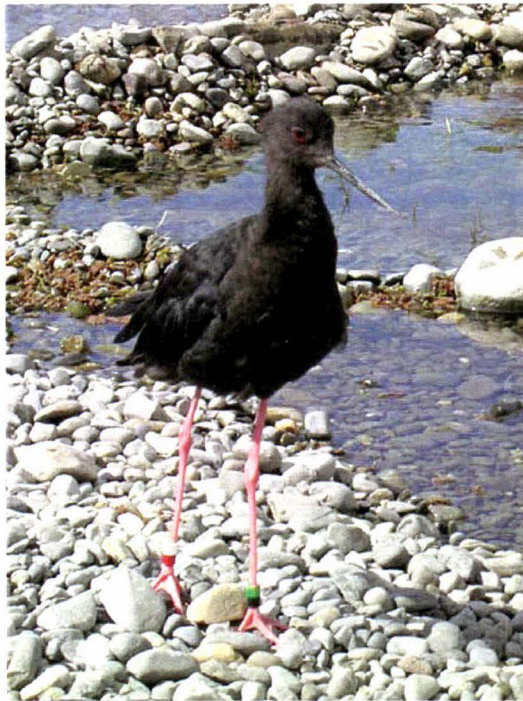


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**Limb growth and development in the  
endangered, captive reared,  
black stilt (*Himantopus novaezelandiae*)**



A captive adult black stilt, Twizel. Photo: Bridget Wrenn.

A thesis presented in partial fulfilment of the requirements for the degree of  
Master of Science in Conservation Biology  
at Massey University, Turitea, Palmerston North, New Zealand

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## Abstract

Captive management is a crucial part of the conservation of one of New Zealand's endemic and critically endangered wading birds, the black stilt (*Himantopus novaezelandiae*). Acquired limb deformities have become apparent in captive reared black stilt, highlighting a lack of knowledge of normal limb growth and maturation. Body weight, and tarsus, carpal and remex length were measured on stilts on *ad libitum* and restricted fed diets. It was found that body weight, and carpal and remex length was significantly slowed by food restriction. Male stilts did not fully compensate in subsequent growth, whereas female stilts compensated in subsequent growth to greater than that of females previously on *ad libitum* diets. Food restriction also significantly increased the percentage of time stilts spent foraging and interacting with each other. Bone histology was undertaken on the tarsus and first phalanx of the wing. This data, combined with the morphological measurements enabled the identification of the critical growth periods, in which black stilt are most likely to develop limb abnormalities. These periods include: the first three weeks of age for leg abnormalities and between 13-25 days of age for wing abnormalities. The manganese (Mn) concentration in black stilt bone and feathers was also investigated. Key findings were that stilts with acquired limb deformities were not deficient in Mn, and feather Mn did not correlate with bone Mn concentration, and therefore can not be used as a non-invasive technique to monitor Mn bioavailability in the black stilt. The cause of angel wing and slipped tendon in captive reared black stilt was not resolved by the research. A Mn deficiency was eliminated but it is still uncertain whether rapid growth rates may have contributed to the abnormalities. This research has shown that dietary restriction in the early growth phase can be used to limit growth rates of black stilt, although the effects of this restriction on long term growth show sex-dependent differences. Further research into the incidence of limb abnormalities in the black stilt is encouraged, with the aim of contributing to the recovery of the critically endangered, wild population.

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## Contents

	Page
<b>Abstract</b>	i
<b>Acknowledgements</b>	ii
<b>Contents</b>	iv
<b>List of figures</b>	v
<b>List of tables</b>	viii
<b>Thesis organisation</b>	x
<b>Chapter 1:</b> Literature review	1
<b>Chapter 2:</b> Captive reared black stilt ( <i>Himantopus novaezelandiae</i> ): The influence of dietary restriction on growth and behaviour of the endangered species	15
<b>Chapter 3:</b> A histological description of bone maturation in the black stilt ( <i>Himantopus novaezelandiae</i> )	70
<b>Chapter 4:</b> Manganese concentration in bone and feathers of the black stilt	94
<b>Chapter 5:</b> General discussion, implications for conservation and future research options	121
<b>Appendix 1:</b> Composition of the Wombaroo Insectivore Rearing Mix	135
<b>Appendix 2:</b> Images of morphological measurements of the tarsus, carpal and remiges	136
<b>Appendix 3:</b> Images of brooder setup	138
<b>Appendix 4:</b> Composition of Kiwi Premix	139



## List of Figures

### Chapter 2: Captive reared black stilt (*Himantopus novaezelandiae*): The influence of dietary restriction on growth and behaviour of the endangered species

	Page
Figure 1. Observed, mean and predicted body weights of birds on i) an <i>ad libitum</i> diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction.	33
Figure 2. Mean body weight.	34
Figure 3. Observed, mean and predicted carpal length of birds on i) an <i>ad libitum</i> diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction.	37
Figure 4. Mean carpal length.	38
Figure 5. Observed, mean and predicted remex length of birds on i) an <i>ad libitum</i> diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction.	40
Figure 6. Mean remex length.	41
Figure 7. Observed, mean and predicted tarsus length of birds on i) an <i>ad libitum</i> diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction.	43
Figure 8. Mean tarsus length.	44
Figure 9. Mean length of tarsus, carpal and remiges of black stilt on an <i>ad libitum</i> diet.	45
Figure 10. Mean growth variables of male sub-adult black stilts on <i>ad libitum</i> diet or 80% dietary restriction as juveniles.	47
Figure 11. Mean growth variables of female sub-adult black stilts on an <i>ad libitum</i> diet or 80% dietary restriction as juveniles.	47
Figure 12. Mean carpal and remex growth and the growth of the chick which developed the initial signs of angel wing.	49
Figure 13. The interaction of behaviour of chicks on <i>ad libitum</i> and restricted diets.	51

### **Chapter 3: Bone maturation and skeletal deformities in the black stilt**

	Page
Figure 1. Longitudinal section through the growth plate of the distal tip of the tibio-tarsus of a Rhode Island red cockerel aged four weeks of age.	75
Figure 2. Longitudinal section through the proximal growth plate of the tarso-metatarsus of a black stilt aged 40 days of age.	80
Figure 3. Estimation of lengths of the zones of proliferation and hypertrophy in the proximal growth plate of the tarsus of black stilts aged one day to five months of age.	81
Figure 4. Estimation of lengths of the zones of proliferation and hypertrophy in the distal growth plate of the tarsus of black stilts aged one day to five months of age.	81
Figure 5. Longitudinal section through the distal growth plate of the tarso-metatarsus of a black stilt aged 21 days of age.	82
Figure 6. Longitudinal section through the proximal growth plate of the first phalanx of a black stilt aged one day of age.	83
Figure 7. Estimation of lengths of the zones of proliferation and hypertrophy of the proximal growth plate in the first phalanx of black stilt aged one day to three months of age.	84

#### **Chapter 4: The role of manganese in the incidence of limb abnormalities in the black stilt**

	Page
Figure 1. Bone and feather Mn in captive reared black stilt with and without limb deformities.	104
Figure 2. Mean Mn concentrations in captive reared black stilt in comparison to wild reared hybrid stilts.	105
Figure 3. Correlation between feather Mn and age of captive reared black stilts over the first 16 months of age.	106
Figure 4. Correlation between bone Mn and age of captive reared black stilts over the first 16 months of age.	106
Figure 5. Correlation between bone Mn and age of captive reared black stilts over the first 33 days of age.	107
Figure 6. Mean Mn concentrations in neonatal feathers of captive raised black stilt with wild and captive parents.	108
Figure 7. Correlation between bone and feather Mn (mg/kg) in hybrid stilts.	109
Figure 8. Correlation between bone and feather Mn (mg/kg) in captive black stilts.	109



## List of Tables

### Chapter 2: Captive reared black stilt (*Himantopus novaezelandiae*): The influence of dietary restriction on growth and behaviour of the endangered species

	Page
Table 1. Diet fed to juvenile black stilt captive reared at the Kaki Captive Breeding Centre.	23
Table 2. Components of the captive mix fed to captive reared black stilt.	23
Table 3. Components of the adult captive mix fed to captive reared black stilt.	26
Table 4. Description of stilt behaviour.	27
Table 5. Best models and parameter estimates for predicting growth variables for stilts on an <i>ad libitum</i> diet, 80% dietary restriction or 70% dietary restriction.	31
Table 6. Growth models of body weight.	32
Table 7. Growth models of carpal length.	36
Table 8. Growth models of remiges length.	39
Table 9. Growth models of tarsal length.	42
Table 10. Mean growth variables of sub-adults on an <i>ad libitum</i> diet or 80% dietary restriction as juveniles.	46
Table 11. Mean increase in growth variables between age 31 days and sub-adults.	48
Table 12. Wing measurements of chick with initial signs of angel wing.	49
Table 13. Behaviour significantly affected by dietary restriction.	50

### Chapter 3: Bone maturation and skeletal deformities in the black stilt

	Page
Table 1. Description of the epiphyseal growth zones in bone.	74

**Chapter 4: The role of manganese in the incidence of limb abnormalities in the black stilt**

	Page
Table 1. Components of the captive mix fed to captive reared black stilt.	99
Table 2. Approximate composition of the Wombaroo Insectivore Rearing Mix.	100
Table 3. Nutritional analyses of the artificial captive black stilt diet and mayfly larvae.	100

## **Thesis organisation**

This thesis has a chapter reviewing the literature on the background of the black stilt, possible causes of limb deformities in captive reared avian species and bone maturation in avian species. It has three research chapters which have been written in the format of journal papers, where co-authors recognise supervision. These are titled: Captive reared black stilt (*Himantopus novaezelandiae*): The influence of dietary restriction on growth and behaviour of the endangered species; A histological description of bone maturation in the black stilt (*Himantopus novaezelandiae*); and Manganese concentration in bone and feathers of the black stilt. It has a final chapter discussing overall findings, the implication the findings have for conservation of the black stilt and future research options. A reference list is at the conclusion of each chapter. The final section is of appendices.



## **Chapter 1**

### **Literature review**



A 13 week old captive reared black stilt. Photo: Bridget Wrenn.



## Literature Review

Human colonisation of New Zealand, by both Maori and European's, has modified New Zealand landscapes and biodiversity. Humans established a vast range of foreign plant and animal species, causing habitat modification, predation and competition, accompanied by human hunting and ecosystem destruction, these factors have contributed to the extinction and overwhelming decline of New Zealand's endemic and native biodiversity (Atkinson and Cameron 1993, Craig, et al. 2000).

The black stilt, also known as kaki (*Himantopus novaezelandiae*), is a critically endangered wading bird endemic to New Zealand (Maloney and Murray 2001). It has been suggested as being one of the world's rarest wading birds (Reed, et al. 1993). The species previously inhabited wetlands throughout New Zealand, but like many of New Zealand's endemic species, introduced mammalian predators and habitat degradation have led to a severe reduction in their natural distribution (Atkinson and Cameron 1993, Reed, et al. 1993, Craig, et al. 2000). The black stilt is now restricted to the upper reaches of the Waitaki River Basin, where it had previously been distributed throughout New Zealand (Reed, et al. 1993, Maloney and Murray 2001).

The black stilt, as with all species endemic to New Zealand, evolved with relatively no predation pressures (Craig, et al. 2000). Bird of prey such as the Australasian harrier (*Circus approximans*), the New Zealand falcon (*Falco novaeseelandiae*), and the now extinct Haast eagle (*Harpagornis moorei*), weka (*Gallirallus australis*) and other now extinct rails, pukeko (*Porphyrio porphyrio melanotus*) and gulls (*Larus* spp.) were the only natural predators the black stilt evolved with (Pierce 1986, Reed, et al. 1993). The introduced predators, ferrets (*Mustela furo*), hedgehogs (*Erinaceus europaeus*) and cats (*Felis catus*) have been recorded as having the greatest effect on ground nesting birds, such as the black stilt, in the Upper Waitaki River Basin (Sanders and Maloney 2002). Ecological characteristics of the black stilt such as nest timing, nest location, solitary nesting behaviour, anti-predator behaviour, fledgling period and plumage, have been suggested as making them more vulnerable to introduced predators than other wading birds in New Zealand (Pierce 1986, Reed, et al. 1993).

Habitat degradation has also contributed to the decline of the black stilt. Wetland drainage and riverbed modification for hydro-electric schemes have depleted feeding and nesting grounds of the stilt (Reed, et al. 1993). The introduction of invasive plants such as crack willow (*Salix fragilis*) and Russell lupin (*Lupinus polyphyllus*) to braided river beds has also reduced feeding and nesting sites of the black stilt, as vegetation has stabilised riverbeds, reducing the creation of side streams and ponds. Vegetation also provides increased cover for predators (Reed, et al. 1993).

A third factor that has had a detrimental effect on black stilt survival is the occurrence of interbreeding with its close relative, the pied stilt (*H. himantopus leucocephalus*). Hybridisation between these two species is now considered a major problem to the survival of the black stilt due to the critically low population status of the black stilt (Reed, et al. 1993). Hybridisation has been encouraged in areas where there is a sexual imbalance in a sub-population, and as black stilt tend to mate for life and do not spread very far from birth rearing areas, hybrid pair formation occurs. Hybrid stilts are a threat to the survival of black stilt as their gene pool is different. Hybrids may inherit behavioural traits from pied stilt parentage, such as migrating out of the Waitaki River Basin during winter, which results in black stilt hybrids missing out on essential pair bonding as black stilt remain in the Waitaki River Basin all year round (Reed, et al. 1993).

Predation pressures, habitat loss and modification, and hybridisation are all key factors which have and will continue to push the decline of the black stilt. For this reason captive breeding and management, combined with other management strategies, are essential for the survival of the critically endangered stilt in the wild. Captive management is a component of the Kaki Recovery Plan which aims to increase the wild population to at least 250 breeding individuals by captive rearing, and releasing large quantities of juveniles and sub-adults (Maloney and Murray 2001). The establishment of both Kaki Recovery and Kaki Captive Management Plans have so far lead to a recovery of the wild population from the record estimated low of c. 28 individuals in 1980 (Reed, et al. 1993), to the most recent wild population count (28<sup>th</sup> August 2006) residing at a total of 182 black stilt, 100 of these individuals being adults (E. Sancha, Department of Conservation, unpublished data). Captive management has been a part of the black stilt recovery program since 1981 when



the first eggs were collected from the wild for artificial incubation and captive rearing (Maloney and Murray 2001).

The formulation of an artificial diet for captive held and reared black stilt has posed problems to managers as a number of nutritionally related problems have arisen resulting in adverse affects on stilt recruitment. Goitre, caused by an iodine (I) deficiency (Sancha, et al. 2004b), and fibrous osteodystrophy caused by a calcium (Ca), phosphorus (P) imbalance (Sancha, et al. 2005), have previously been overcome. Iodine deficiency in captive held black stilt pairs resulted in a significant number of peri-hatching deaths of their offspring, it also resulted in enlarged thyroid glands of captive reared stilts, both factors resulting in reduced stilt recruitment (Sancha, et al. 2004b). This deficiency has since been corrected by I supplementation to the captive diet (Sancha, et al. 2004b). The metabolic bone disease, fibrous osteodystrophy, affected two captive reared chicks in the 2003/2004 chick rearing season, these chicks became lame and euthanasia was necessary (Sancha, et al. 2004a). The lesions observed upon necropsy indicated a diet with a poor Ca to P ratio. At the time both chicks sustained abnormalities, they were eating mainly mealworms as they were in the transition phase between eating aquatic invertebrates and the captive mix. As typical in most insects, mealworms are high in P and low in Ca (McDonald 2006). The Ca:P imbalance has since been increased by calcium carbonate supplementation to the captive diet (Sancha, et al. 2005). Ultra violet lights were also installed in chick brooders (Sancha, et al. 2005) as vitamin D<sub>3</sub> deficiency is known to contribute to osteodystrophy in captive reared species including young African grey parrots (Stanford 2006).

Further nutritionally related abnormalities have been recognized in captive black stilt. Pododermatitis has been recorded as an ongoing problem (E. Sancha, Department of Conservation, personal communication) and has been related to nutritional factors such as lack of exercise and obesity in captive poultry (Macwhirter 1994), penguins (Reidarson, et al. 1999) and raptors (Rodriguez-Lainz, et al. 1997, Muller, et al. 2000), and vitamin A deficiency in unspecified avian species (Rodriguez-Lainz, et al. 1997).

The most recent nutritionally related abnormalities observed in captive reared black stilt



(2003/2004 and 2004/2005 chick rearing seasons) were further deformities of the long bones. These manifested as either an outward rotation of the carpo-metacarpus (angel wing) or luxation of the gastrocnemius tendon from the condyles of the tibio-tarsus (slipped tendon or perosis) (B. Gartrell, Massey University, personal communication). Skeletal deformities have been highlighted as issues of concern in a range of avian species raised in captivity, most commonly reported as occurring in domesticated meat-type fowl and have been related to rapid growth rates and mineral deficiencies.

A possible cause of the limb deformities seen in captive reared black stilt are rapid growth rates. R. Maloney & E. Sancha, (Department of Conservation, personal communication) reported that captive reared black stilt chick growth over the first 25 days after hatching is relatively rapid compared to their wild reared counterparts. The initial rapid growth rate causes them to be heavier than wild reared chicks over the same time period, but after about 25 days, growth rates decrease and captive reared black stilt maintain similar growth rates to individuals who have been raised by their parents in the wild. It is a possibility that the rapid growth rates of captive reared black stilt have contributed to both angel wing and slipped tendon, as they were fed on an *ad libitum* diet.

A precocial chick hatches with downy feathers and soon after hatching it has the ability to leave the nest and forage for itself (Skutch 1976). In comparison to altricial chicks, whom hatch helpless and are completely dependent on their parents for weeks to months, a precocial chick's brain, muscles and limbs account for a large proportion of their body weight, but their digestive tract accounts for a relatively smaller portion (Skutch 1976). These factors aid chicks with the ability to be mobile soon after hatching and having the ability to keep up with their parents (Skutch 1976). Mobility soon after hatching requires musculoskeletal tissues to grow and mature at a suitable rate. Growth is dependent on the allocation of tissue production to either growth or function (Ricklefs, et al. 1998). Tissue allocation may act as a constraint to growth as it may be more prominently allocated to function rather than growth. This idea was proposed as, more mature, precocial chicks grow at a slower rate than lesser developed, altricial chicks, thus tissues are allocated more dominantly to function and growth to respective developmental modes. Further support for

this growth constraint is that all species growth slows with age (Ricklefs 1973, Ricklefs, et al. 1998).

Stilts are precocial birds (Skutch 1976, Pierce 1982). The black stilt, in particular, remains in the nest for 13-38 hours after hatching, and after this time, forages within a couple of meters of the nest with no guidance from their parents (Pierce 1982). Tissue development is therefore mainly devoted to function, but as a growing juvenile, is still required for growth.

Commercial diets encouraging rapid weight gains in meat-type fowl have been related to a range of skeletal abnormalities. Rapid growth rates have been related to tibial dyschondroplasia in broiler chickens, turkeys and ducks (Cherel, et al. 1991, Hester 1994, Lilburn 1994, Orth and Cook 1994, Riddell 1997, Bradshaw, et al. 2002). Tibial dyschondroplasia is characterized by a bird having abnormal masses of cartilage below the growth plate, usually at the tibio-tarsus joint. It commonly causes anterior bowing of the tibio-tarsus and lameness in broiler chickens (Riddell 1997). Rapid growth rates have also been suggested as being associated with tibio-tarsal rotation in ostrich chicks (Reece and Butler 1984, Hahulski, et al. 1999, Mushi, et al. 1999) and broilers (Bradshaw, et al. 2002). Tibial-tarsal rotation can lead to slipped tendon, thus resulting in birds that are unable walk (Reece and Butler 1984). Leterrier and Nys (1992) found fast growing chickens had a lower tibial bone density than slower growing chickens. Fast growth rates result in weaker bones, as during rapid growth, large cartilaginous growth plates occur, resulting in reduced mechanical strength (Kirkwood, et al. 1989). This may have resulted in greater difficulty for the faster growing chickens to carry their heavier than normal body weight, thus leading to the development of slipped tendon and lateral and medial angulation on the tarsus joint (Leterrier and Nys 1992). Rapid growth rates at a young age have also been suggested as contributing to angel wing in waterfowl (Kear 1973, Olsen 1994, Smith 1997), bustard species (Naldo and Bailey 2001), a northern goshawk (Zsivanovits, et al. 2006) and greater sandhill cranes (Serafin 1982). Rapid growth rates can commonly be related to high concentrations of protein in the diet (Kear 1973, Serafin 1982, Mushi, et al. 1999, Zsivanovits, et al. 2006) but can also be related to excess food consumption (Flinchum



2006) and lack of exercise (Smith 1997, Klasing 1998a).

Mineral deficiencies have also been linked to limb abnormalities observed in captive reared avian species. Speer (1996) *in* (Naldo and Bailey 2001) reported manganese (Mn), copper, biotin and choline deficiencies in chickens and turkeys to result in chondrodystrophy of the distal tibio-tarsus and proximal tarso-metatarsus, subsequently resulting in slipped tendon due to the deformed hock joint. Chondrodystrophy is a disorder of long bone growth plates effecting linear growth resulting in enlarged inter-tarsal joints, varus and valgus abnormalities and luxation of the gastrocnemius tendon (Naldo and Bailey 2001). Chondrodystrophy has been observed in the great and Australian bustards, it has been associated with Mn and zinc deficiencies and excess protein in some species (Naldo and Bailey 2001). Deformed and weak joints, such as the hock joint, may result in slipped tendon. Mn deficiency has also been highlighted in Klasing (1998b) to be responsible for shortened and thickened long bones, deformed tibio-metatarsus joints and weakened cartilage. The tendon is known to detach from weakened tibio-metatarsus joints leading to rotation of the distal tibio-tarsus and proximal tarso-metatarsus bones (Klasing 1998b). Klasing (1998b) stated that these bone abnormalities are likely in captive raised precocial birds that are raised on an artificial diet high in energy, protein and Ca, but deficient in Mn. Wallach (1970) in Mushi et al. (1999) also associated abnormal bone development with mineral deficiencies such as Mn. Mn deficiency was suspected to be the cause of limb deformities in captive reared takahe (*Porphyrio [Notornis] mantelli*). From 1985 to 1994 a low number of takahe per year suffered medial dislocation of patellas between the ages of two to seven weeks; a number of chicks also developed constricted tendons in their toes. Since potassium permanganate solution was added to their artificial diet in 1994, the occurrence of both these problems has been eliminated in captive reared takahe (Eason and Willans 2001). Klasing (1998b) also mentioned that Mn is first used to develop new skeletal muscle and other tissues over development of connective tissues. This causes joint cartilage and tendons to be too weak to handle the strength of the skeletal muscles that pull on them. He then went on to state that if growth is slowed, muscle growth and joint strength are likely to be more appropriate for each other.

The limb abnormalities observed in the greater sandhill crane have similarities to the abnormalities observed in captive reared black stilt. The endangered species of greater and Florida sandhill cranes were artificially incubated and reared in captivity at the Patuxent Wildlife Research Center. Both species were fed on the same experimental diets varying in protein, metabolizable energy and sulphur amino acid contents. Florida sandhill cranes grew at the slowest rates on all diet types and did not develop any limb abnormalities, whereas, the faster growing greater sandhill cranes exhibited limb abnormalities (Serafin 1982). The diets encouraging reduced growth rates resulted in a lower incidence of abnormalities. Serafin (1982) concluded that the wing abnormalities observed in these birds may have resulted from weight stress put on tendons and ligaments associated with the carpal joint due to rapid feather growth. Wing abnormalities consisted of unilateral or bilateral twisting of the wing feathers. Leg deformities in the greater sandhill cranes were displayed as relatively weaker areas of the bone. This included the proximal ends of the tibio-tarsus and tarso-metatarsus and the distal end of the tibio-tarsus, resulting in the bones rotating or bending, potentially reaching a point where the individuals could no longer stand. Serafin (1982) concludes that the leg abnormalities observed in these birds most likely resulted from the birds not being able to support their rapid weight gain.

Another threatened avian species that has exhibited similar limb abnormalities to the black stilt is the houbara bustard. This species is raised in captivity for release at the National Avian Research Center, United Arab Emirates. Naldo & Bailey (2001) reported limb abnormalities in three captive reared houbara bustards. The first bustard developed angel wing at 21 days of age. The second bustard also developed angel wing but at as early as 10 days of age. At three weeks of age this bustard exhibited bilateral varus abnormality of the distal tarso-metatarsus and inward rotation of the phalanges. By eight months of age this bird exhibited moderate lameness and continued to do so into adulthood. The third bustard hatched with some varus deformity in one leg, and by four days of age both legs were affected. By 47 days of age this bird was reported as having slipped tendon over the left inter-tarsal joint. This was corrected through surgery but two days later the gastrocnemius tendons had luxated in both legs. All three bustards reported on had shorter tarso-metatarsal bones than normal bustards (Naldo and Bailey 2001). Naldo & Bailey (2001)



went on to suggest that the primary cause for the leg abnormalities exhibited in these bustards was chondrodystrophy. Naldo & Bailey (2001) did not diagnose the cause of angel wing exhibited in the bustards but referred to the following factors that can influence the development of angel wing: added weight from blood filled quills of the remiges, interruption during incubation, excessive heat during the early growth phase, excessive energy, high levels of protein in the diet, a vitamin E or Mn deficiency, hypovitaminosis D<sub>3</sub>, management practices, and genetic or environmental factors.

Earlier work carried out by Naldo et al. (1998) on four species of bustard chicks at the National Avian Research Center, United Arab Emirates, found that all cases of angel wing from 1993-1995 occurred in chicks of less than one month of age. The study looked at chicks aged 0-180 days old. The same study also reported the frequency of angular and rotational limb deformities. These included bustards with chondrodystrophy, slipped tendon and deviation of the radius and ulna. Their report did not differentiate between these abnormalities but 92% of the abnormalities observed occurred between the ages of 0-90 days of age, the phase of which resources would most likely be allocated to growth rather than function. Naldo et al. (1998) reported that the occurrences of these abnormalities were reduced after 1993 due to changes in management practices.

These studies highlight the multi-factorial causes of limb abnormalities in avian species and the need for detailed studies on limb maturation. An important part of recognising limb abnormalities is the ability to distinguish between normal and abnormal bone maturation. The limited population size of an endangered species, such as the black stilt, and limited incidences of abnormalities, can often restrict diagnostic ability due to the limited information on normal growth plate morphology and maturation rates (Kirkwood, et al. 1989). Due to the population status of the black stilt, and the limited number of archived stilts, it was only appropriate to investigate two possible causes of the acquired limb deformities in captive reared black stilt: 1. rapid growth rates and 2. a Mn deficiency.

The aims of this research were to investigate:

a) the effect of an *ad libitum* and restricted diet on the growth rates of juvenile black stilts, and their ability of restricted diet fed stilts to compensate in growth once changed to an *ad libitum* diet. We hypothesised that the more food stilt chicks ate, the faster they would grow and that compensatory growth would be observed when stilts that had early feed restriction were changed to an *ad libitum* diet. The second aim of this study was to use the growth rate data to attempt to identify the critical growth period in which captive reared black stilt would most likely be prone to developing further limb deformities. Behavioural observations were also used to examine the effect of the restricted diet on chick behaviour.

b) to document the normal bone maturation of the tarso-metatarsus and first phalanx of the wing of the black stilt and to correlate this to morphological measurements of limb growth. This will identify the critical phase in which the black stilt grows most rapidly and will be most likely to develop further acquired limb abnormalities.

c) the manganese concentration in bone and feathers of black stilt by determining: 1. if Mn concentrations vary between stilts with and without limb deformities; 2. if captive reared black stilt are deficient in Mn in comparison wild parent reared hybrid stilts; 3. to better understand the variables involved with Mn body stores in bone and feathers including age and parental heritage. We also aimed to determine whether feather Mn reflected skeletal Mn, as a positive correlation would support assays of feather Mn as a non-invasive technique to monitor body Mn concentration in individuals.

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## Chapter 2

### Captive reared black stilt

*(Himantopus novaezelandiae):*

### The influence of dietary restriction on growth and behaviour of the endangered species



A one day old captive reared black stilt. Photo: Bridget Wrenn.

## **Captive reared black stilt (*Himantopus novaezelandiae*): The influence of dietary restriction on growth and behaviour of the endangered species**

Bridget J. Wrenn, Brett D. Gartrell, Doug P. Armstrong, Nick Cave and Emily Sancha

### **Abstract**

Captive management is essential for the survival of the endangered wading bird, the black stilt (*Himantopus novaezelandiae*). Long bone limb abnormalities were seen in captive reared chicks over the 2003/2004 and 2004/2005 chick rearing seasons. Captive reared black stilt grow faster than their wild reared counterparts and, as rapid growth rates have attributed to limb abnormalities in other avian species, dietary restriction was implemented with the aim of reducing growth rates of captive reared black stilt. Chicks were allocated to one of three treatment groups. Group A (control) fed *ad libitum* and groups B and C were fed 80% and 70%, respectively, of what group A consumed from approximately 12-34 days of age. The primary aim of this study was to investigate the effect of an *ad libitum* and restricted diet on the growth rates of juvenile black stilts and to find out whether this early restriction had any effect on subsequent growth once changed to an *ad libitum* diet. We hypothesised that the more food stilt chicks ate, the faster they would grow as juveniles, and compensatory growth would be observed when restricted fed stilts were changed to an *ad libitum* diet. The second aim of this study was to use the growth rate data to attempt to identify the critical growth period in which captive reared black stilt would most likely be prone to developing further limb deformities. Behavioural observations were also used to examine the effect of the restricted diet on chick behaviour. Dietary restriction significantly reduced black stilt body weight and carpo-metacarpus (carpal) and remex length over their first 34 days of age. Dietary restriction also significantly increased the percentage of time black stilts spent foraging and interacting with each other. We have successfully shown that the growth rates and final body weight and final lengths of the carpal and remiges of captive reared black stilt can be reduced significantly by food



restriction. Male black stilt did not fully compensate in subsequent growth, whereas females compensated in subsequent growth to greater than that of females previously on an *ad libitum* diet. Food restriction will be recommended to the Kaki Captive Breeding Centre as an option to consider if limb deformities become more frequent in captive reared black stilt in the future.

## Introduction

The black stilt, or kaki (*Himantopus novaezelandiae*), is a critically endangered wading bird endemic to New Zealand (Maloney and Murray 2001). The species was once distributed throughout New Zealand but like the habitat requirements of many of New Zealand's endemic species, introduced predators and habitat degradation have led to a severe reduction in its natural distribution (Atkinson and Cameron 1993, Reed, et al. 1993, Craig, et al. 2000). The black stilt is now restricted to the braided rivers of the Upper Waitaki Basin, where its population reached a record low of c.28 individuals in 1980 (Reed, et al. 1993). The instigation of the black stilt recovery and captive management plans have improved the status of the black stilt, leading to a gradual recovery of the wild population. The most recent wild population count (28<sup>th</sup> August 2006) identified 182 black stilt, 100 being adults (E. Sancha, Department of Conservation, unpublished data). A key goal of captive management is to aid in the recovery of the wild population through supplementation with captive reared juveniles (Maloney and Murray 2001). Early captive management practices had mixed success (Reed, et al. 1993) but advancements in the development of the captive diet and husbandry techniques saw 115 stilts fledge in captivity during the 2005/2006 chick rearing season (Sancha, et al. 2006).

The health of non-domesticated individuals in captivity has become an issue of concern for a number of species, especially endangered species whose survival relies on captive management. Avian species whose health concerns in captivity have been primarily dietary related include black stilt (Sancha, et al. 2004), kiwi (Boardman 1994), takahe (Eason and Willans 2001), a range of Psittaciformes (Torregrossa, et al. 2005) and hummingbirds (Elliston and Perlman 2002). It can be difficult to formulate an artificial captive diet for wild species as it is not usually feasible to replicate their natural diet. One of the main problems with formulating a captive diet is the key issue of unknown nutrient requirements of the species of concern. Captive avian diets are commonly formulated based on poultry requirements (Harper and Turner 2000, Liukkonen-Anttila, et al. 2002, Miao, et al. 2003, Lemm, et al. 2004), as most is known about these species. This may be a good base for formulating captive avian diets but many species will have species specific nutritional



requirements, and health problems are likely to arise. Ongoing evaluation and development of the diets is usually necessary to make them appropriate for individual species.

This has been the case in the black stilt as a number of nutritional related abnormalities have been identified in captive stilts resulting in adverse affects on health of the captive flock. Iodine deficiency, causing goitre (Sancha, et al. 2004), and fibrous osteodystrophy (Sancha, et al. 2005) have previously been overcome but further abnormalities have been recognised. Pododermatitis has been recorded as a common problem in captive black stilt (E. Sancha, Department of Conservation, unpublished report). It has been related to nutritional factors such as lack of exercise and obesity in captive poultry (Macwhirter 1994), penguins (Reidarson, et al. 1999) and raptors (Rodriguez-Lainz, et al. 1997, Muller, et al. 2000) and vitamin A deficiency in unidentified avian species (Rodriguez-Lainz, et al. 1997). Long bone deformity was noted in some captive reared black stilt chicks during the 2003/2004 and 2004/2005 chick rearing seasons and was also suspected as being nutritionally related. This manifested as either an outward rotation of the carpo-metacarpus (angel wing) or luxation of the gastrocnemius tendon from the condyles of the tibio-tarsus (slipped tendon or perosis) (B. Gartrell, Massey University, unpublished report).

Common causes of angel wing in other avian species have included high protein intake (Serafin 1982, Macwhirter 1994, Smith 1997, Naldo and Bailey 2001, Flinchum 2006, Zsivanovits, et al. 2006), lack of exercise (Smith 1997) or over feeding (Flinchum 2006) resulting in rapid growth rates. Other suggested causes of angel wing include mineral deficiencies (Macwhirter 1994, Naldo and Bailey 2001, Flinchum 2006), vitamin deficiencies (Naldo and Bailey 2001, Flinchum 2006), interruption during incubation, high temperature during early growing stages or an individual may be genetically predisposed to the condition (Kear 1973, Naldo and Bailey 2001).

A number of factors were also considered for the causation of slipped tendon in captive reared black stilt. As with angel wing, high protein diets (Serafin 1982, Mushi, et al. 1999, Bradshaw, et al. 2002), rapid weight gain (Haye and Simons 1978, Naldo, et al. 1998, Mushi, et al. 1999), limited exercise (Klasing 1998a, Mushi, et al. 1999), genetic factors

(Macwhirter 1994, Naldo, et al. 1998, Mushi, et al. 1999), mineral deficiencies (Serafin 1982, Klasing 1998c, Naldo, et al. 1998, Mushi, et al. 1999) and incubation factors (Serafin 1982, Macwhirter 1994) have been suggested as having a role in causing slipped tendon in avian species. Further factors expressed as a possible cause of slipped tendon include high fat diets (Macwhirter 1994) and amino acid deficiencies (Serafin 1982, Macwhirter 1994, Naldo and Bailey 2001). Naldo & Bailey (2001) suggest that slipped tendon is also likely to be associated with chondrodystrophy as the deformation of the hock joint encourages tendon luxation. The development of chondrodystrophy has been shown to be caused by deficiencies such as manganese (Naldo and Bailey 2001, Bradshaw, et al. 2002), copper (Naldo and Bailey 2001), biotin (Naldo and Bailey 2001), choline (Naldo and Bailey 2001, Bradshaw, et al. 2002), niacin, vitamin E, folic acid, pyridoxine and zinc (Bradshaw, et al. 2002).

The Department of Conservation (DoC) has recognized that black stilt raised in captivity grow at faster rates than those raised in the wild (R. Maloney & E. Sancha, DoC, unpublished report). The initial rapid growth rate causes them to be heavier than wild reared chicks over the same time period, but after about 25 days, growth rates in captive stilts decrease to maintain similar growth rates to individuals who have been raised by their parents in the wild (R. Maloney & E. Sancha, DoC, unpublished report). Rapid growth rates were therefore suggested as a possible cause for the limb malformations observed in captive reared black stilt. Many studies have reported rapid growth rates in juvenile, captive reared, avian species resulting in skeletal abnormalities, including meat-type fowl (Cherel, et al. 1991, Hester 1994, Lilburn 1994, Orth and Cook 1994, Bradshaw, et al. 2002, Waldenstedt 2006) and endangered species such as the greater sandhill crane (Serafin 1982) and a range of bustard species (Naldo, et al. 1998).

High protein diets have been suggested as being related to rapid growth rates, which attribute to limb deformities, in captive avian stock (Serafin 1982, Macwhirter 1994, Smith 1997, Naldo and Bailey 2001, Flinchum 2006, Waldenstedt 2006). A high concentration of protein in the captive black stilt diet was suggested as being an unlikely cause of the observed limb abnormalities because they are an insectivorous species, feeding on aquatic



invertebrates (Sanders 1997) and thus should be adapted to a high protein diet (Klasing 1998a). Adult insects are generally 50-75% protein and larvae are generally 40-70% protein (Klasing 1998a). Rapid growth rates have also been related to limited exercise (Klasing 1998a) and excess feed intake in poultry (Lilburn 1994, Bradshaw, et al. 2002, Flinchum 2006, Waldenstedt 2006). Although these are possible causes for the rapid growth rates observed in the black stilt, the most probable cause is excessive food intake, as captive reared chicks are offered a diet fed *ad libitum*.

The primary aim of this study was to investigate the effect of an *ad libitum* and restricted diet on the growth rates of juvenile black stilts and to find out whether a restriction had any effect on subsequent growth once changed to an *ad libitum* diet. We hypothesised that the more food stilt chicks ate, the faster they would grow. The second aim of this study was to use the growth rate data to attempt to identify the critical growth period in which captive reared black stilt would most likely be prone to developing further limb deformities. Behavioural observations were also used to examine the effect of the restricted diet on chick behaviour.

## Methods

### Chick growth

During the 2005/2006 black stilt chick rearing season the body weight, the tarso-metatarsal and carpo-metacarpal length (from here on referred to as tarsus and carpus respectively) and the length of the first remex (primary feather on the wings) was measured on black stilt chicks raised at the Kaki Captive Breeding Centre, Twizel. As a part of the Kaki Recovery Programme, the field staff attempt to locate all wild nests and collect all clutches within one day of the total clutch being laid. This encourages the natural multiple clutching behaviour exhibited by black stilt, thus increasing productivity for the species. All eggs were collected from a total of seven captive black stilt pairs and 13 wild black stilt pairs. The collected eggs were artificially incubated and stilts were reared in captivity (Maloney and Murray 2001). Chicks were measured between November 2<sup>nd</sup> 2005 and February 6<sup>th</sup> 2006. Individual chicks were measured on every third day from the ages of 1-34 days old. The Massey University Animal Ethics Committee gave approval for the research described in this paper (MUAEC 05/101).

#### *1. Experimental design and dietary manipulations*

All chicks were randomly allocated into one of three treatment groups. Group A (control) fed *ad libitum* and groups B and C were fed 80% and 70%, respectively, of what group A consumed from approximately 12 days of age (table 1). Captive reared black stilt food is normally replenished twice a day, but is offered *ad libitum*, so available food lasts until the next feed. The captive mix was made up daily (table 2) and was thoroughly mixed until the meat was well broken down and it was dry and crumbly, it was stored at 4°C and was used the same day.



Table 1. Diet fed to juvenile black stilt captive reared at the Kaki Captive Breeding Centre.

Age (days)	Diet Received
0-3	Aquatic invertebrates (mainly mayfly larvae) every 2-3 hours, collected from river's near Twizel.
3-7	Aquatic invertebrates, captive mix with small mealworms and an extra water tray.
7-12	Captive mix with small mealworms and an extra water tray.
~12 (when DoC staff had confirmed that all chicks within a brooder were eating captive mix).	Fed captive mix twice daily, once at approximately 9:30am and once at approximately 4:30pm.

Table 2. Components of the captive mix fed to captive reared black stilt.

Ingredient	Rate
Ox-heart	500 g
Wombaroo Insectivore Rearing Mix (appendix 1)	250 g
Iodised salt (dissolved in a little water)	¼ teaspoon (level)
Calcium carbonate	11.4 g

Dietary restricted groups received the same diet as control groups until management confirmed that the chicks were feeding on the captive mix at approximately 12 days of age. Once chicks in restricted feed groups were eating solely the captive mix they received 70% or 80% of what control chicks had consumed on corresponding days of age according to the following:

1. Calculations were based on the age of the youngest chick in the brooder.
2. The amount (g) of food consumed per day in control brooders was measured. The estimated consumption per feed was calculated by subtracting the amount remaining from the amount provided when the plates were collected at the end of each feed. This was repeated at both the morning and afternoon feeds, giving a total amount of food consumed per brooder per day.
3. The mean total food consumed per chick per day was calculated.
4. The mean amount of food consumed per chick per day in control groups was used to calculate the amount of food the restricted groups received for each corresponding day of age. The appropriate restriction (70% or 80%) of the mean of all control groups preceding

a restricted group was calculated for the consumption of food per chick per day in the corresponding restricted brooder for each specific day of age.

5. Chicks received half of the total restricted amount at the morning feed and half at the afternoon feed.

In total 92 chicks were measured from day one through to day 34. The control group consisted of 56 chicks, which were reared in 12 sets. The 80% dietary restriction group consisted of 20 chicks, which were reared in six sets and the 70% dietary restriction group consisted of 16 chicks reared in five sets.

As part of the management of black stilt at the Kaki Captive Breeding Centre, chicks are allocated to brooders so all chicks within a brooder are within three days of age, and there is a maximum of six chicks and a minimum of two chicks per brooder. The first four brooders were designated to be control groups to establish *ad libitum* intakes to calculate restricted feed amounts. From then brooders were allocated to control, 80% or 70% dietary restricted treatment groups.

## 2. Chick measurements taken

Descriptions of the measurements follow:

Body weight (g, 2 decimal places) was measured using a bucket and digital scales (MM-2000, Universal Weighing Enterprise Co.) from day one to approximately day 28. The remaining body weight measurements taken were carried out using Pesola scales. Individual behaviour would determine the upper age limit to be able to measure a stilt using digital scales as movement within the bucket led to inaccurate readings. All length measurements were made using calipers, and left limbs and remiges were measured. Tarsus length (tarso-metatarsus) (mm) was measured from the distal end of the bone with the foot contracted to the cotyles of the tarso-metatarsus when this joint was in a flexed position (appendix 2 fig. 1). Carpal length (carpo-metacarpus) (mm) was measured from the tip of the carpo-metacarpus to the ridge of the carpo-metacarpal – radio-ulnar joint, which was also exposed when the wing was in a flexed position (appendix 2 fig. 2). Remex



length (primary feather length) (mm) was measured from the point in which the feather shaft emerged from the skin to the tip of the feather. The feather measured was the first primary feather on each wing (appendix 2 fig. 3).

### *3. Husbandry*

All management practices were the same for both control and dietary restricted black stilts. From days 0-7 chicks were kept indoors at all times and checked two hourly from 8:00am-9:00pm. The brooder had a 250W bulb, the entire floor was covered with grass matting, there was a heat-pad underneath the brooder dummy, and the dummy was lowered. Chicks were put into a night box at 9:15pm each night and were weighed daily upon removal from the night box the following morning by DoC staff. From days 7-10 the chicks were allowed outside into the small aviary and returned to inside the brooder by 5pm. On cold or wet days, as judged by management, the chicks were kept inside. On day 10 the 250W bulb inside the brooder was replaced with a 150W bulb. Grass matting was used on the heat-pad only and newspaper covered the floor of the remainder of the brooder. The chicks were allowed into the large aviary during the day and brought inside by 5pm. A hose was left running into the brooder pond during the day. On day 15 a 75W bulb replaced the 150W bulb in the brooders. On day 21 the chicks were fed outside on warm days and the door was left open at night if the weather was fine. Also between days 0-21 ultra violet lights were used in the brooders between 8:00am and 8:00pm. At approximately day 30 the chicks were weighed, examined, permanently banded, and feather samples were taken. From day 30 they were locked outside both day and night until they were transferred to the main aviaries after measurements were taken on day 34.

The husbandry was modified in two brooders in which individuals in restricted dietary groups developed aggressive behaviour towards other chicks within their brooder. Behavioural problems were minimised in both these cases by setting up a partition inside the brooder and providing food on two plates, one on each side of the partition (appendix 3).



**Sub-adult growth**

Thirty three black stilts that had been hand reared at the Kaki Captive Breeding Centre, Twizel, New Zealand, during the 2005/2006 chick rearing season were further raised in captivity until released as sub-adults in September 2006. These individuals had been sexed for the DoC using DNA analysis. Twenty-one of the stilts had been raised on an *ad libitum* diet, and 12 stilts had been raised on an 80% dietary restriction over their first 12-34 days of age. At approximately 35 days old stilts were transferred, to large aviaries at either the Kaki Captive Breeding Centre, Twizel, or Peacock Springs, Christchurch, New Zealand. From 35 days approximately six weeks of age, all stilts were fed on an *ad libitum* diet of the captive mix outlined in (table 2). After approximately six weeks of age, all stilts were fed an *ad libitum* diet of the adult captive mix (table 3). All stilts were recaptured two weeks prior to their release at approximately 8-9 months of age and the growth variables body weight (g), left tarsus length (mm), left carpal length (mm) and first remiges length (mm) on the left wing were re-measured as previously described.

Table 3. Components of the adult captive mix fed to captive reared black stilt.

<b>Ingredient</b>	<b>Rate</b>
Ox-heart	1 kg
Friskies Go-Cat (dry cat food). Chicken, beef, calcium and vegetable flavour	72 g
Kiwi Premix (appendix 4)	Approx. 5.5 g
Iodised salt	Approx. 3.2 g
Calcium carbonate	13.9 g
Water	56 g

**Behavioural observations**

Behavioural observations took place from 9<sup>th</sup> December 2005 to 1<sup>st</sup> February 2006. The behavioural observations were carried out on groups that were in four of the eight brooders at the Kaki Captive Breeding Centre, as visual access to the remaining brooders was limited. Observation sessions commenced when a brooder group of birds was solely eating the captive mix (at approximately day 12), and concluding observations were made at approximately day 34. All observations took place three times a week per brooder, approximately every second day between 10:25am and 12:25pm due to management constraints. Each observation session lasted for 21 minutes. The behaviour of a chick was noted each minute, for 20 minutes. As there were up to six chicks per brooder, the minute for which individual chick behaviour was recorded had a 10-30 second interval between individual chicks. Individual chicks within a brooder were easily identified by different coloured temporary leg bands. From approximately 30 days old chicks were identified by the permanent leg bands that were applied as apart of DoC management practices. In total we observed three control groups with a total of 17 individual chicks, and three food restricted groups with a total of 10 chicks at an 80% and three chicks at a 70% dietary restriction.

Stilt behaviour was classified according to the following categories (table 4):

Table 4. Description of stilt behaviour recorded.

<b>Categorised behaviour</b>	<b>Description</b>
Stationary behaviour	Sleeping, sitting, standing, hock sitting and roosting
Foraging behaviour	Foraging
Drinking Behaviour	Drinking
Interactive behaviour	Chasing or pecking x and chased by or pecked by x (where x is another stilt)
Other active behaviour	Scratching, jumping, running, walking, bathing, preening, stretching and defecating

**Statistical analysis**

All chicks in which their measurements were interrupted part way through our research due to management reasons beyond our control were removed from the data set before statistical analysis.

The statistical analysis on the growth data were carried out by fitting nonlinear mixed models with the PROC NLMIXED procedure (Wolfinger 2006). The growth model used was the logistic growth function as follows (Starck and Ricklefs 1998):

$$\text{Growth variable}_t = a/(1+\exp^{-k(t-i)})$$

Growth variable<sub>t</sub> = growth variable at time t (days)

a = asymptote of the growth curve

k = growth rate constant

i = inflection point of the growth curve

t = age (days)

Models were run to account for the effect of random variation between individuals (random effect) on the parameters a, k and i. They were also run to account for the effect of dietary restriction (treatment effect), individually (80% or 70%) and combined (80% and 70%), on the parameters a, k and i. The lowest Akaike Information Criterion (AIC<sub>c</sub>) value indicated the most parsimonious model to the original data set for each growth variable and in the case where there was more than one most parsimonious model of equal fit to the original data set, the simplest model was chosen to predict growth. One-way ANOVA's and Tukey's post-hoc Pairwise Comparisons were carried out to determine the significance of dietary restriction on the endpoints of the growth variables at day 31.

A t-test was used to assess the significance of the growth variables: body weight, tarsus length, carpal length and remiges length, as sub-adults, between stilts that had been raised on either an *ad libitum* diet or on an 80% dietary restriction as juveniles (*ad libitum* diet  $n = 21$ ; 80% dietary restriction  $n = 12$ ). T-tests were also used to assess the significance of the growth variables of both males and females, between sub-adult age and at 31 days of age,



that had been on either an *ad libitum* diet or an 80% dietary restriction as juveniles (males: *ad libitum* diet  $n = 14$ , 80% dietary restriction  $n = 6$ ; females: *ad libitum* diet  $n = 7$ , 80% dietary restriction  $n = 6$ ). A t-test was also used to assess the significance of the difference of the increase of each growth variable between 31 days of age and sub-adult age between stilts that had been on either an *ad libitum* diet or an 80% dietary restriction as juveniles.

The behavioural data was analysed in the categories of interest (table 4), where active behaviour not of analysis interest was included in 'other active behaviour'. The behaviour data for stilts on 80% and 70% dietary restriction were combined due to the small sample size. One-way ANOVA's were used to assess significance between behaviour of chicks fed at an *ad libitum* and restricted rate. All statistical analysis was carried out using SAS version 9.1 (2003).

## Results

Food restriction had a significant negative effect on body weight ( $P = 0.0005$ ), carpal length ( $P < 0.0001$ ) and remex length ( $P < 0.0001$ ), but not tarsus length ( $P = 0.0687$ ) from days 13-34. The parameter values and growth equations which should be used to predict growth are displayed for the best fitting models (table 5).

Chick body weight, carpal length and remex length were also significantly negatively effected at day 31 by dietary restriction ( $P = 0.0032, 0.0001, 0.0002$  respectively). Pairwise comparisons indicated that there was a significant difference between chicks in both restricted dietary treatments and the chicks fed *ad libitum*, but there was no significant difference between chicks on a 70% and 80% dietary restriction. Chicks on a dietary restriction did not have significantly shorter tarsi than chicks fed *ad libitum* on day 31 ( $P = 0.1079$ ). End point significance was tested at day 31, as there was a limited sample size of 70% dietary restricted chicks at day 34.

Table 5. Best models and parameter estimates for predicting growth variables for stilts on an *ad libitum* diet, 80% dietary restriction or 70% dietary restriction.

Growth variable	Dietary treatment	a	k	i	Random effect	Treatment effect	Growth variable <sub>t</sub> = $a/(1+\exp^{-k(t-i)})$
Body Weight (g)	<i>Ad libitum</i>	187.12	0.16	17.50	-	-	$187.12/[1+\exp^{-0.16(t-17.50)}]$
	80% and 70% restriction	187.12	0.16	17.50	On a	-9.46 (on a)	$(187.12-9.46)/[1+\exp^{-0.16(t-17.50)}]$
Carpal Length (mm)	<i>Ad libitum</i>	78.13	0.18	16.58	-	-	$78.13/[1+\exp^{-0.18(t-16.58)}]$
	80% and 70% restriction	78.13	0.18	16.58	On i	-0.02 (on k)	$78.13/[1+\exp^{-(0.18-0.02)(t-16.58)}]$
Remex Length (mm)	<i>Ad libitum</i>	100.58	0.20	25.22	-	-	$100.58/[1+\exp^{-0.20(t-25.22)}]$
	80% and 70% restriction	100.58	0.20	25.22	On i	-5.16 (on a)	$(100.58-5.16)/[1+\exp^{-0.20(t-25.22)}]$
Tarsus Length (mm)	<i>Ad libitum</i>	85.73	0.09	8.83	-	-	$85.73/[1+\exp^{-0.09(t-8.83)}]$
	80% restriction	85.73	0.09	8.83	On a	-0.01 (on k)	$85.73/[1+\exp^{-(0.09-0.01)(t-8.83)}]$
	70% restriction	85.73	0.09	8.83	On a	-0.01+0.01 (on k)	$85.73/[1+\exp^{-(0.09-0.01+0.01)(t-8.83)}]$



### Body Weight

The best fitting model for predicting body weight gain accounted for random variation between individuals at the asymptote of the growth curve with the combined dietary restriction (treatment effect) also at the asymptote of the growth curve ( $\Delta_i = 0$ ) (table 6). This model is the most parsimonious for the data and is the best for predicting future body weights as it indicates the most significant reduction in variance about the data. The best fitting growth model displayed an excellent fit to the mean body weight of black stilt (figure 1). The predicted growth model for the 70% dietary restriction at 34 days of age has a large standard error (SE) (10.5) as there were only two individuals included in the data set at this age.

Table 6. Growth models of body weight. Smallest AIC<sub>c</sub> indicating the best model.

<sup>a</sup> Random effect accounting for random variation between individuals; treatment effect accounting for the combined effect of the 80% and 70% dietary restriction; 80% and 70% treatment effect accounting for the individual effect of the 80% and 70% dietary restriction separately. <sup>a</sup>, <sup>k</sup> and <sup>i</sup> referring to the growth parameters in the growth model. <sup>b</sup> Number of parameters in model. <sup>c</sup> Corrected Akaike's Information Criterion adjusted for small sample size. <sup>d</sup> Delta AIC<sub>c</sub> indicating difference in AIC<sub>c</sub> value from that of the best model. <sup>e</sup> Akaike weight indicating relative support for the model ( $w_i = e^{-\Delta_i/2} / \sum e^{-\Delta_i/2}$ ).

Model <sup>a</sup>	K <sup>b</sup>	AIC <sub>c</sub> <sup>c</sup>	$\Delta_i$ <sup>d</sup>	$w_i$ <sup>e</sup>
No random effect	4	5163.9	503.5	0.0000
Random effect on k	5	4981.6	321.2	0.0000
Random effect on i	5	4759.8	99.4	0.0000
Random effect on a, treatment effect on a & i	6	4672.4	12	0.0010
Random effect on a, treatment effect on i	6	4672.2	11.8	0.0012
Random effect on a	5	4670.7	10.3	0.0023
Random effect on a, treatment effect on a, k & i	6	4662.7	2.3	0.1241
Random effect on a, treatment effect on k	6	4662.3	1.9	0.1516
Random effect on a, treatment effect on a & k	6	4662.3	1.9	0.1516
Random effect on a, 80% and 70% treatment effect on a	7	4662.0	1.6	0.1762
Random effect on a, treatment effect on a	6	4660.4	0	0.3921

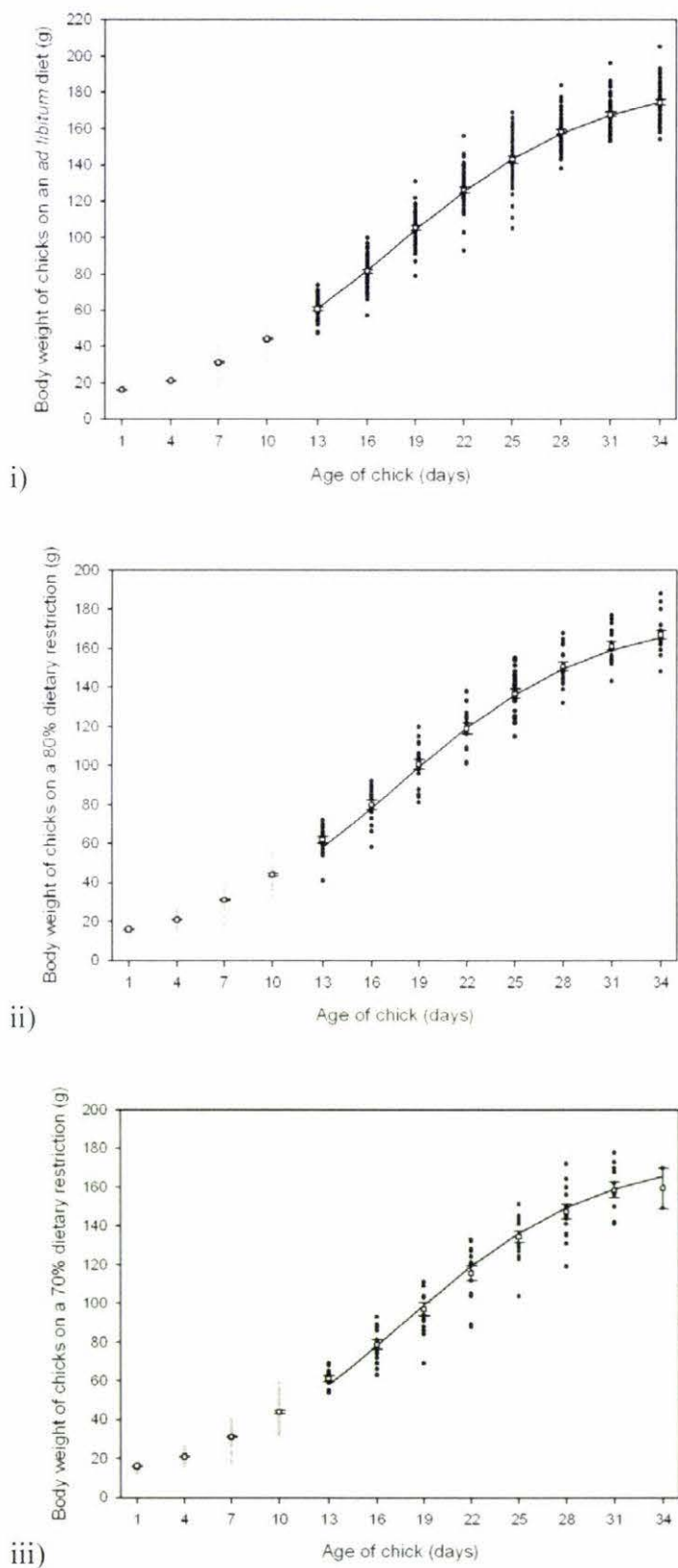


Figure 1 legend see over page.

Fig. 1. Observed, mean and predicted body weights of birds on i) an *ad libitum* diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction. The  $\bullet$  represent the observed body weights between days 1-10. They are the same for each graphed dietary treatment as these measurements were taken before the chicks were on the artificial diet and therefore before the age at which their diets were fed at a restricted rate or *ad libitum* for this study. The  $\bullet$  circles represent observed measurements of chicks once eating the captive mix (food restricted where appropriate), the  $\square$  represent mean body weight  $\pm$  1 SE and the solid black line represents the following predicted growth curve: Best model = Random effect on a, treatment effect on a;  $AIC_c = 4660.4$ . *Ad libitum* body weight (g) =  $187.12/[1+\exp^{-0.16(t-17.50)}]$ , 80% and 70% dietary restriction body weight (g) =  $(187.12-9.46)/[1+\exp^{-0.16(t-17.50)}]$

A comparison of the mean body weights from the three dietary treatments are displayed (figure 2). Body weight over the first 34 days of age follows the same general trend for all dietary treatments with the expected result that stilts fed *ad libitum* were heavier than stilts fed a restricted diet.

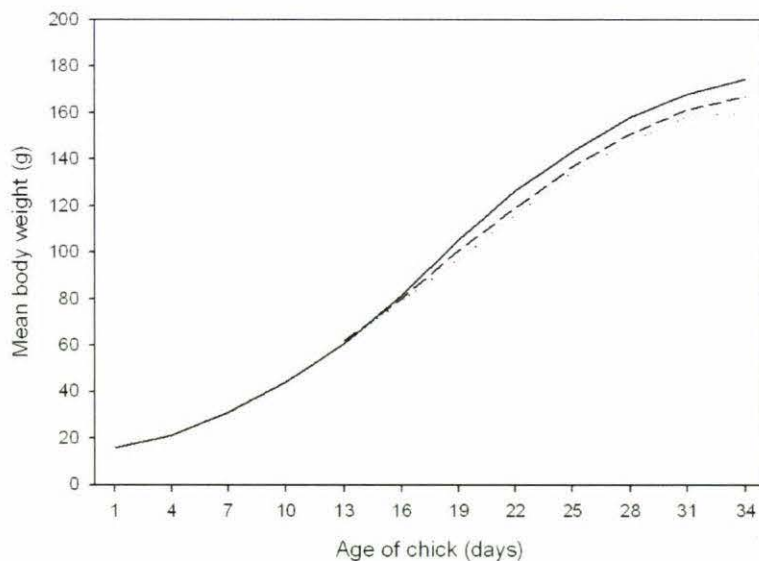


Fig. 2. Mean body weight. The solid black line represents stilts on an *ad libitum* diet, the dashed black line represents stilts on an 80% dietary restriction and the dotted black line represents stilts on a 70% restriction diet. SE are displayed in figure 1.



**Carpal**

Carpal growth had two best fitting models for predicting growth ( $\Delta_i = 0$ ) (table 7). These models are most parsimonious for the observed data and are the best models to use to predict future carpal lengths (mm) as the best fitting models indicates the most significant reduction in variance about the data. As there are two equally best fitting models for predicting carpal growth, the simplest model, random effect on the inflection point of the growth curve with the combined treatment effect on the overall carpal growth rate was chosen to use to predict carpal growth (figure 3). The predicted growth curve, had a relatively good fit to the observed carpal measurements for the black stilt on an *ad libitum* diet (figure 3 i). The predicted growth curve also has a relatively good fit to the observed measurements of stilts on restricted diets, but the model predicts some measurements, particularly on day 19, greater than the mean observed values (figure 3 ii and iii). The observed carpal measurements appear to take a slightly more sigmoidal growth pattern than those predicted by the growth model. There was an outlier in the 70% dietary restriction treatment group (figure 3 iii), this apparently healthy individual continually exhibited slower carpal growth than all other individuals.

Table 7. Growth models of carpal length. Smallest AIC<sub>c</sub> indicating the best model.

<sup>a</sup> Random effect accounting for random variation between individuals; treatment effect accounting for the combined effect of both the 80% and 70% dietary restriction; 80% and 70% treatment effect accounting for the individual effect of the 80% and 70% dietary restriction separately. a, k and i referring to the growth parameters in the growth model. <sup>b</sup> Number of parameters in model. <sup>c</sup> Corrected Akaike's Information Criterion adjusted for small sample size. <sup>d</sup> Delta AIC<sub>c</sub> indicating difference in AIC<sub>c</sub> value from that of the best model. <sup>e</sup> Akaike weight indicating relative support for the model ( $w_i = e^{-\Delta_i/2} / \sum e^{-\Delta_i/2}$ ).

Model <sup>a</sup>	K <sup>b</sup>	AIC <sub>c</sub> <sup>c</sup>	Δ <sub>i</sub> <sup>d</sup>	w <sub>i</sub> <sup>e</sup>
No random effect	4	3753.3	647	0.0000
Random effect on k	5	3626.8	520.5	0.0000
Random effect on a	5	3475.5	369.2	0.0000
Random effect on i	5	3158.7	52.4	0.0000
Random effect on i, treatment effect on i	6	3148.8	42.5	0.0000
Random effect on i, treatment effect on a	6	3122.9	16.6	0.0000
Random effect on i, 80% and 70% treatment effect on k	7	3107.6	1.3	0.1230
Random effect on i, treatment effect on k & i	6	3106.6	0.3	0.2028
Random effect on i, treatment effect on a, k & i	6	3106.6	0.3	0.2028
Random effect on i, treatment effect on a & k	6	3106.3	0	0.2356
Random effect on i, treatment effect on k	6	3106.3	0	0.2356

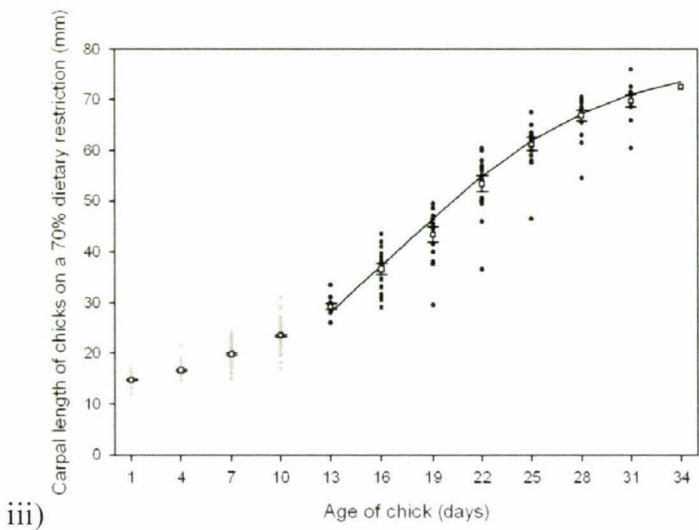
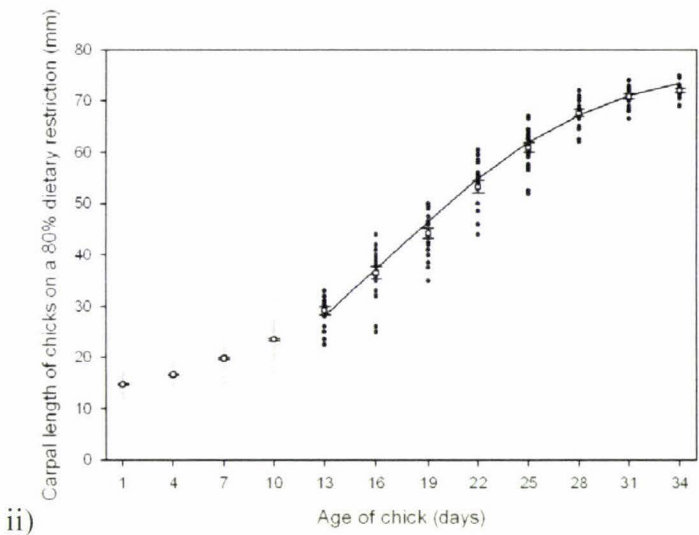
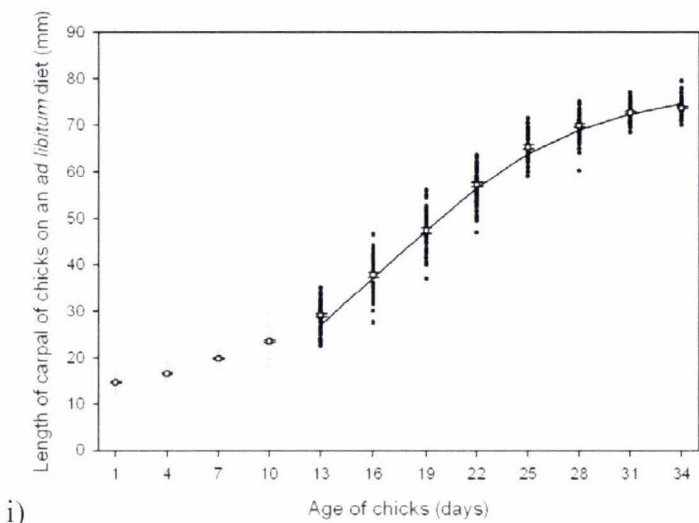


Figure 3 legend see over page.



Fig. 3. Observed, mean and predicted carpal length of birds on i) an *ad libitum* diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction. The  $\bullet$  represent the observed carpal lengths between days 1-10, they are the same for each graphed dietary treatment as these measurements were taken before the chicks were on the artificial diet and therefore before the age at which their diets were fed at a restricted rate or *ad libitum* for this study. The  $\bullet$  represent observed measurements of chicks once eating the captive mix (food restricted where appropriate), the  $\square$  represent mean body weight  $\pm$  1 SE and the solid black line represents the following predicted growth curve: Best model = Random effect on i, treatment on k;  $AIC_c = 3106.3$ . *Ad libitum* carpal length (mm) =  $78.13/[1+\exp^{-0.18(t-16.58)}]$ , 80% and 70% dietary restriction carpal length (mm) =  $78.13/[1+\exp^{-(0.18-0.02)(t-16.58)}]$ .

A comparison of the mean carpal length (mm) from the three dietary treatments is displayed (figure 4). Expected growth trends of the carpus were exhibited as black stilt on an *ad libitum* diet had the longest mean carpal bones in relation to stilts on food restricted diets. Carpal growth for both restricted diet groups were similar throughout the observed period.

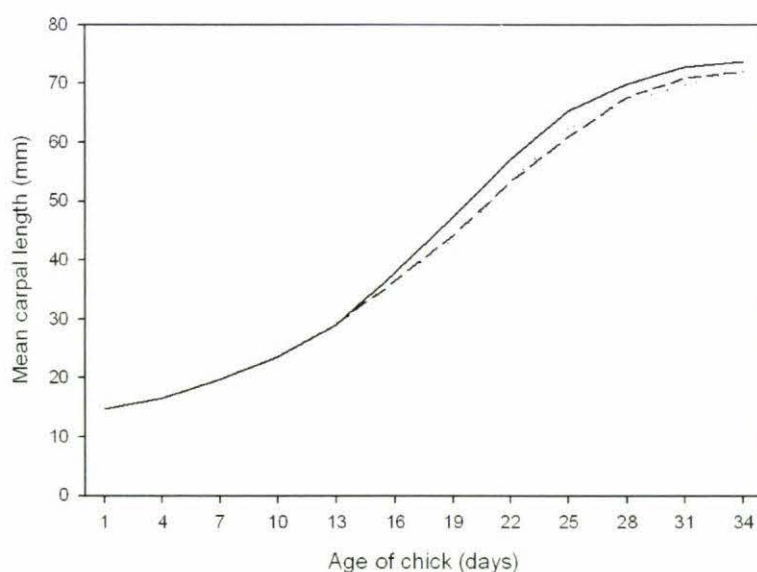


Fig. 4. Mean carpal length. The solid black line represents stilts on an *ad libitum* diet, the dashed black line represents stilts on an 80% dietary restriction and the dotted black line represents stilts on a 70% dietary restriction. SE are displayed in figure 3.

Remiges

The best fitting model to predict remex growth of captive reared stilts was random effect on the inflection point of the growth curve, combined treatment effect on the asymptote of the growth curve ( $\Delta_i = 0$ ) (table 8). This model is the most parsimonious for the data and is the best for predicting weight gain as it has the most significant reduction in variance about the data. The best fitting model fits the observed mean measurements of the remiges relatively well (figure 5). The growth model predicts remex length greater than the observed mean on day 13 for stilts under all dietary treatments. The growth model also over predicts remex growth on days 28-34 for stilts on a 70% dietary restriction (figure 5 iii). Remex growth continued at a relatively constant accelerated rate from 16-34 days of age. An outlier also appeared in stilts on a dietary restriction of 70% (figure 5 iii). The remiges of this apparently healthy individual continued to grow at a slower rate than all other chicks, this chick was the same individual which exhibited slower carpal growth than other chicks on a 70% dietary restriction (figure 3 iii).

Table 8. Growth models of remiges length. Smallest AIC<sub>c</sub> indicating the best model.

<sup>a</sup> Random effect accounting for random variation between individuals; treatment effect accounting for the combined effect of both the 80% and 70% dietary restriction; 80% and 70% treatment effect accounting for the individual effect of the 80% and 70% dietary restriction separately. <sup>a</sup>, <sup>k</sup> and <sup>i</sup> referring to the growth parameters in the growth model. <sup>b</sup> Number of parameters in model. <sup>c</sup> Corrected Akaike's Information Criterion adjusted for small sample size. <sup>d</sup> Delta AIC<sub>c</sub> indicating difference in AIC<sub>c</sub> value from that of the best model. <sup>e</sup> Akaike weight indicating relative support for the model ( $w_i = e^{-\Delta_i/2} / \sum e^{-\Delta_i/2}$ ).

Model <sup>a</sup>	K <sup>b</sup>	AIC <sub>c</sub> <sup>c</sup>	Δ <sub>i</sub> <sup>d</sup>	w <sub>i</sub> <sup>e</sup>
Random effect on k	5	5743.4	2370.2	0.0000
No random effect	4	4283.9	910.7	0.0000
Random effect on a	5	3733.0	359.8	0.0000
Random effect on i, treatment effect on a & i	6	3420.5	47.3	0.0000
Random effect on i	5	3419.3	46.1	0.0000
Random effect on i, treatment effect on i	6	3408.8	35.6	0.0000
Random effect on i, treatment effect on a, k & i	6	3406.0	32.8	0.0000
Random effect on i, treatment effect on k	6	3405.9	32.7	0.0000
Random effect on i, treatment effect on a & k	6	3405.9	32.7	0.0000
Random effect on i, 80% & 70% treatment effect on a	7	3375.1	1.9	0.2789
Random effect on i, treatment effect on a	6	3373.2	0	0.7211

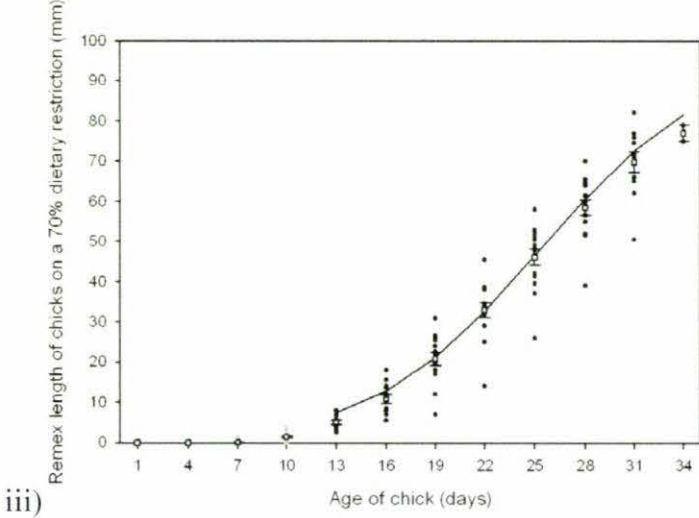
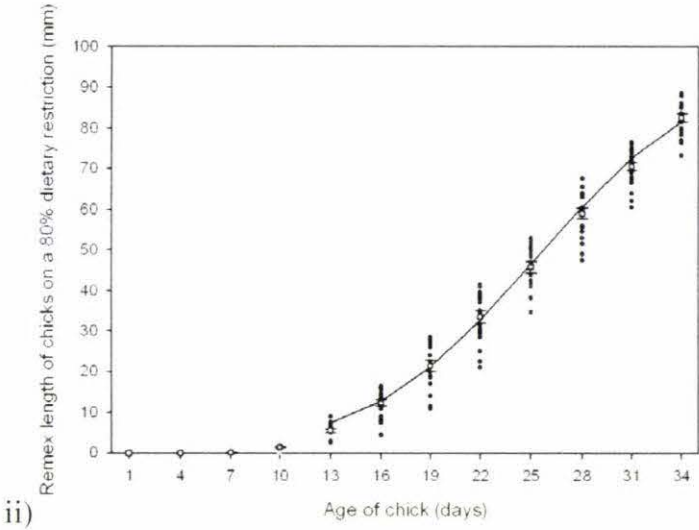
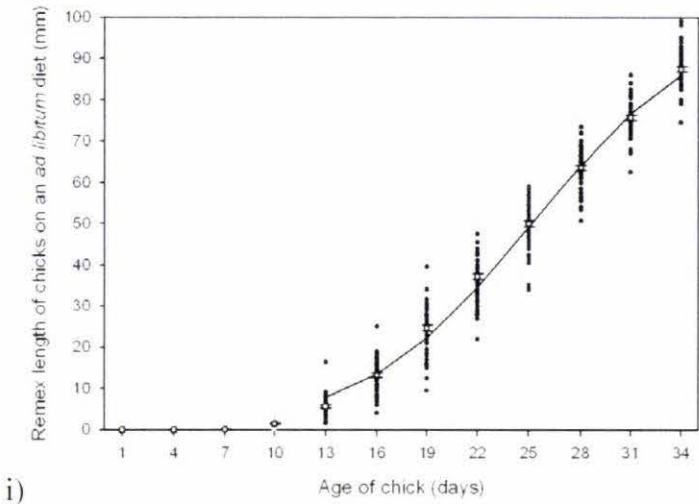


Figure 5 legend see over page.



Fig. 5. Observed, mean and predicted remex length of birds on i) an *ad libitum* diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction. The  $\bullet$  represent the observed remex lengths between days 1-10 are the same for each graphed dietary treatment as these measurements were taken before the chicks were on the artificial diet and therefore before the age at which their diets were fed at a restricted rate or *ad libitum* for this study. The  $\bullet$  represent observed measurements of chicks once eating the captive mix (food restricted where appropriate), the  $\square$  represent mean body weight  $\pm$  1 SE and the solid black line represents the following predicted growth curve: Best model = Random effect on i, treatment on a;  $AIC_c = 3373.2$ . *Ad libitum* remex length (mm) =  $100.58/[1+\exp^{-0.20(t-25.22)}]$  and 80% and 70% dietary restriction remex length (mm) =  $(100.58-5.16)/[1+\exp^{-0.20(t-25.22)}]$ .

A comparison of mean remex lengths from the three dietary treatments are displayed (figure 6). Remex length over the first 34 days of age follows the same general trend for all dietary treatments with the expected outcome that stilts on an *ad libitum* diet have faster feather growth than the feathers of stilts on a restricted diet. The remiges of stilts on an 80% and 70% dietary restriction grew at the same rate over the observed growth period, except for day 34 where the remiges of stilts on a 70% dietary restriction are shorter than the remiges of stilts on an 80% dietary restriction. This difference may occur as  $n = 2$  on day 34 for stilts on a 70% dietary restriction.

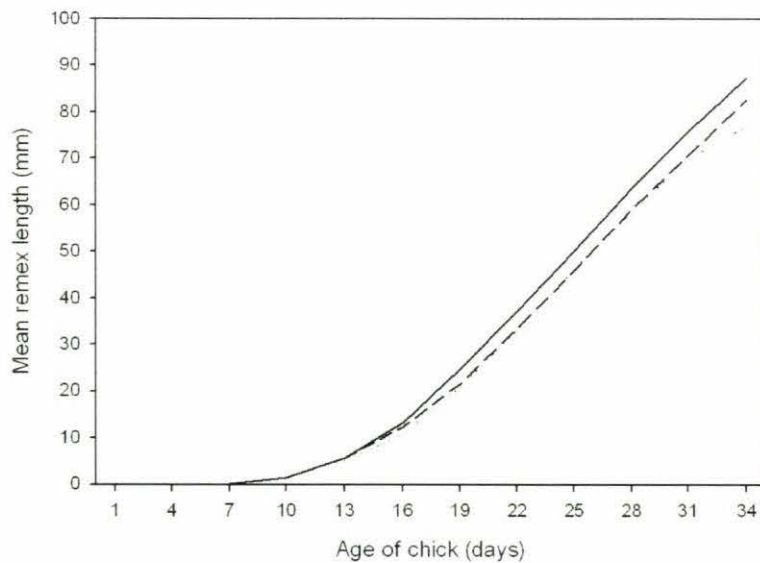


Figure 6. Mean remex length. The solid black line represents stilts on an *ad libitum* diet, the dashed black line represents stilts on an 80% dietary restriction and the dotted black line represents stilts on a 70% dietary restriction. SE are displayed in figure 5.

Tarsus

Tarsus length had three best models of equal fit for predicting tarsi growth of captive reared black stilt ( $\Delta_i = 0$ ) (table 9). As these models have the lowest  $AIC_c$  values, they are the most parsimonious for the observed data and are the best for predicting future tarsus lengths as they indicate the most significant reduction in variance about the data. The simplest best fitting model, random effect on the asymptote of the growth curve with the individual effect of the 80% and 70% treatment effect on the overall tarsi growth rate, was chosen to use to predict future tarsus growth. The best fitting growth model had an excellent fit to the mean tarsus length of captive raised black stilt for all dietary treatments (figure 7).

Table 9. Growth models of tarsus length. Smallest  $AIC_c$  indicating the best model.

<sup>a</sup> Random effect accounting for random variation between individuals; treatment effect accounting for the combined effect of both the 80% and 70% dietary restriction; 80% and 70% treatment effect accounting for the individual effect of the 80% and 70% dietary restriction separately. <sup>a</sup>, <sup>k</sup> and <sup>i</sup> referring to the growth parameters in the growth model. <sup>b</sup> Number of parameters in model. <sup>c</sup> Corrected Akaike's Information Criterion adjusted for small sample size. <sup>d</sup> Delta  $AIC_c$  indicating difference in  $AIC_c$  value from that of the best model. <sup>e</sup> Akaike weight indicating relative support for the model ( $w_i = e^{-\Delta_i/2} / \sum e^{-\Delta_i/2}$ ).

Model <sup>a</sup>	K <sup>b</sup>	AIC <sub>c</sub> <sup>c</sup>	$\Delta_i$ <sup>d</sup>	$w_i$ <sup>e</sup>
No random effect	4	3381.1	800.4	0.0000
Random effect on i	5	2807.4	226.7	0.0000
Random effect on k	5	2633.1	52.4	0.0000
Random effect on a, treatment effect on i	6	2598.9	18.2	0.0000
Random effect on a	5	2596.9	16.2	0.0000
Random effect on a, treatment effect on a	6	2593.0	12.3	0.0004
Random effect on a, treatment effect on a, k & i	6	2582.2	1.5	0.0966
Random effect on a, treatment effect on k & i	6	2582.2	1.5	0.0966
Random effect on a, treatment effect on a & k	6	2582.2	1.5	0.0966
Random effect on a, treatment effect on k	6	2582.2	1.5	0.0966
Random effect on a, 80% and 70% treatment effect on a & k	7	2580.7	0	0.2044
Random effect on a, 80% and 70% treatment effect on k & i	7	2580.7	0	0.2044
Random effect on a, 80% and 70% treatment effect on k	7	2580.7	0	0.2044

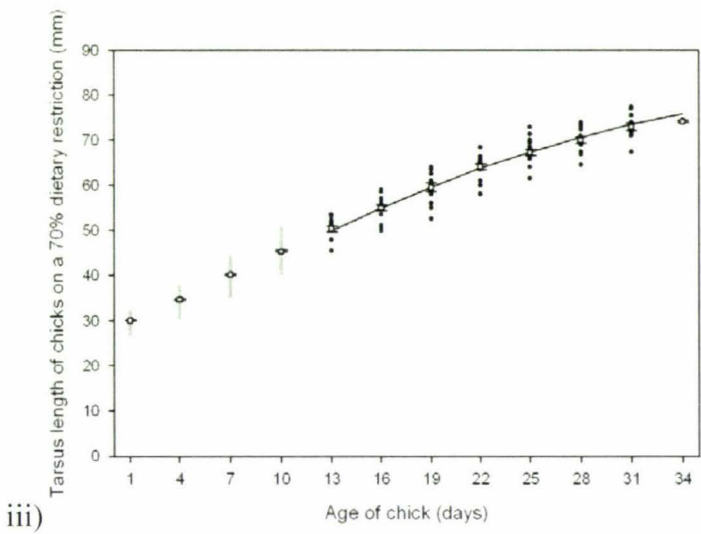
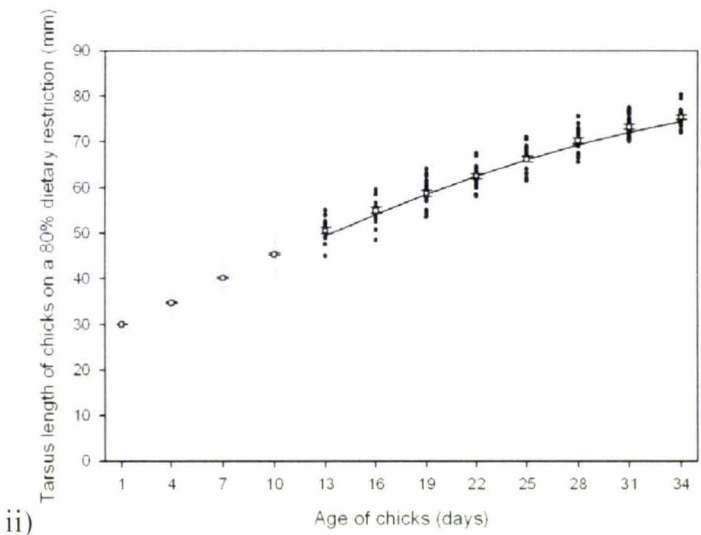
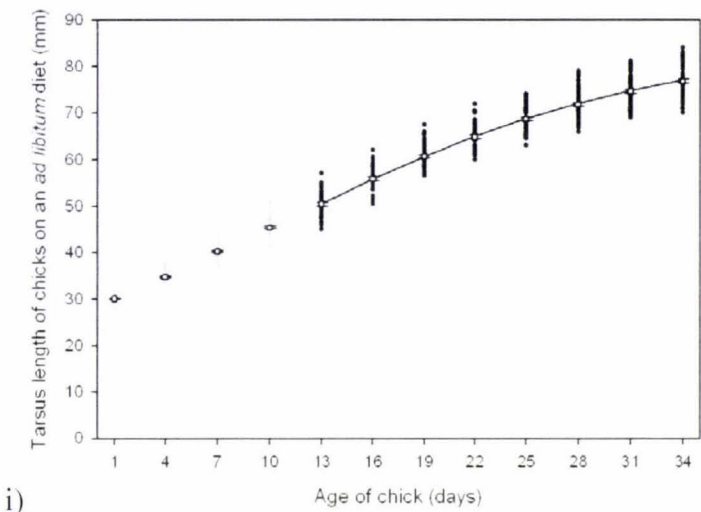


Figure 7 legend see over page.



Fig. 7. Observed, mean and predicted tarsus length of birds on i) an *ad libitum* diet, ii) a 80% dietary restriction and iii) a 70% dietary restriction. The  $\square$  represent the observed tarsus lengths between days 1-10, they are the same for each graphed dietary treatment as these measurements were taken before the chicks were on the artificial diet and therefore before the age at which their diets were fed at a restricted rate or *ad libitum* for this study. The  $\bullet$  represent observed measurements of chicks once eating the captive mix (food restricted where appropriate), the  $\square$  represent mean body weight  $\pm$  1 SE and the solid black line represents the following predicted growth curve: Best model = Random effect on a, treatment and 70% treatment effect on k;  $AIC_c = 2580.7$ . *Ad libitum* tarsus length (mm) =  $85.73/[1+\exp^{-0.09(t-8.83)}]$ , 80% dietary restriction tarsus length (mm) =  $85.73/[1+\exp^{-(0.09-0.01)(t-8.83)}]$  and 70% dietary restriction tarsus length (mm) =  $85.73/[1+\exp^{-(0.09-0.01+0.01)(t-8.83)}]$ .

A comparison of the mean tarsus length (mm) from the three dietary treatments are displayed (figure 8). The tarsus growth follows relatively the same trend for each dietary treatment over the growth period observed. Mean tarsus length of stilts on the 70% dietary restriction unexpectedly grew at a faster rate than stilts on an 80% dietary restriction between days 16-28, but by day 31, stilts on an 80% dietary restriction exhibited faster tarsus growth than stilts on a 70% dietary restriction.

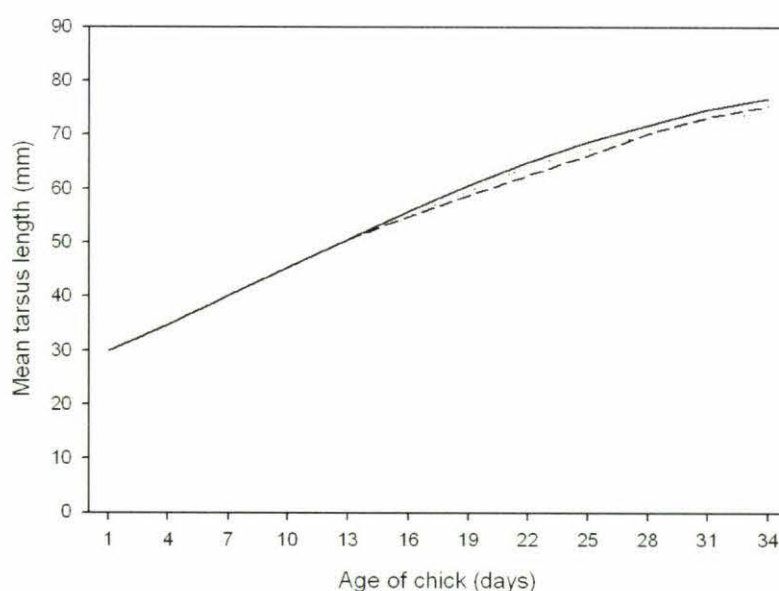


Fig. 8. Mean tarsus length. The solid black line represents stilts on an *ad libitum* diet, the dashed black line represents stilts on an 80% dietary restriction and the dotted black line represents stilts on a 70% dietary restriction. SE are displayed in figure 7.

Relative Growth

The relative growth of tarsus, carpus, and remiges indicated that each variable underwent growth at a different rate and time (figure 9). Tarsus length underwent a relatively constant growth rate from day one and only started to decrease at the age of 25 days. Twenty five days is also the same period at which carpal growth slowed, but at a relatively more dramatic rate in relation to the tarsus. The carpus never reached an equivalent length to the tarsus over the first 34 days of stilt growth. Remex growth did not begin until approximately day seven, growth was relatively slow until day 16 after which it continued at this rate throughout the rest of the observed measurement period, equaling tarsus and carpal length at approximately day 30. At the end of 34 days of age both the carpal and tarsal growth are beginning to level out, whereas the remex growth continued at its previous linear like rate.

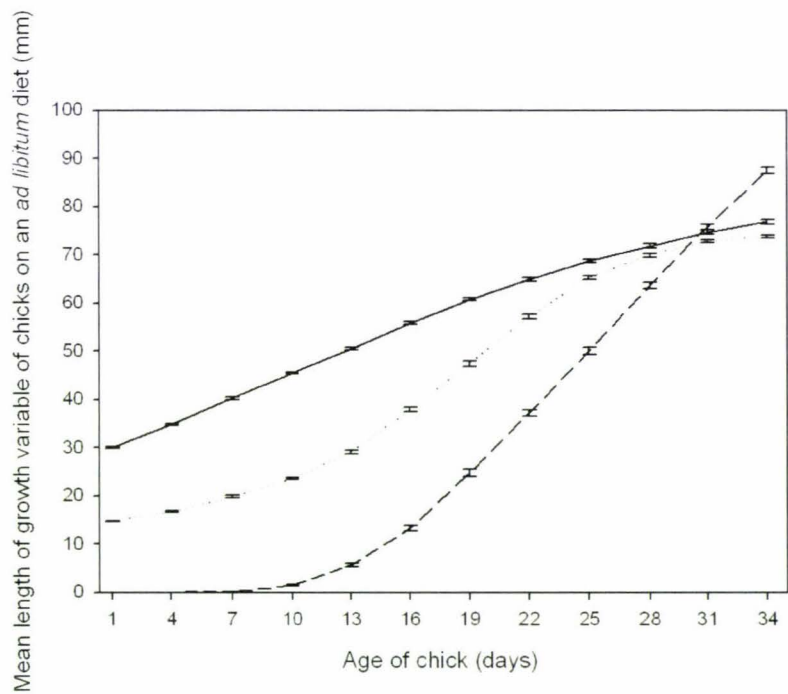


Fig. 9. Mean length ( $\pm 1$  SE) of tarsus, carpal and remiges of black stilt on an *ad libitum* diet. The solid black line represents the tarsus, the dotted black line represents the carpus and the dashed black line represents the remiges.

**Sub-adult growth**

*1. Sub-adult growth variables*

A previous 80% dietary restriction on captive reared black stilt had no significant effect on any of the measured growth variables as a sub-adult of 8-9 months of age (body weight  $P = 0.8421$ , tarsus  $P = 0.1161$ , carpal  $P = 0.3199$ , and remiges  $P = 0.1193$ ). The mean of each growth variable of the stilts that had been on an *ad libitum* diet as juveniles, were greater than the mean of each growth variable of the stilts that had been on an 80% dietary restriction as juveniles (table 10).

Table 10. Mean growth variables of sub-adults on an *ad libitum* diet or 80% dietary restriction as juveniles.

Growth variable	Juvenile dietary treatment	Mean	SE
Body weight (g)	<i>Ad libitum</i>	193.81	2.18
	80% restriction	193.17	2.39
Tarsus length (mm)	<i>Ad libitum</i>	94.95	0.58
	80% restriction	92.96	1.06
Carpus length (mm)	<i>Ad libitum</i>	74.83	0.57
	80% restriction	73.54	1.12
Remiges length (mm)	<i>Ad libitum</i>	146.26	2.20
	80% restriction	141.00	2.42

*2. Sub-adult growth variables in relation to gender*

Thirty one day old male black stilt that were on an *ad libitum* diet were significantly heavier in body weight ( $P = 0.0095$ ) and their tarsus ( $P = 0.0154$ ) and remiges ( $P = 0.0223$ ) were significantly longer than male black stilts that had previously been on an 80% dietary restriction. At 31 days old, there was not a significant difference in carpal length between males on the two dietary treatments ( $P = 0.1090$ ). As, sub-adults, the male black stilt that had been on an *ad libitum* diet as juveniles were still significantly heavier in body weight than males that had been on an 80% dietary restriction as juveniles ( $P = 0.0475$ ) (figure 10). There was no longer a significant difference in the other growth variables between male black stilts that had been on the two dietary treatment groups as juveniles (tarsus  $P = 0.1030$ ; carpal  $P = 0.1548$ ; and remiges  $P = 0.0810$ ).



Female 31 day old black stilt, which had been on the two dietary treatments had no significant difference in any of the growth variables (body weight  $P = 0.8412$ ; tarsus  $P = 0.8017$ ; carpal  $P = 0.5424$ ; and remiges  $P = 0.1855$ ). As sub-adults, females that had been on an *ad libitum* diet as juveniles were significantly lighter in body weight than females that had been on an 80% dietary restriction as juveniles ( $P = 0.0202$ ) (figure 11). There was still no significant difference in the other growth variables between female black stilt that had been on the two dietary treatments as juveniles (tarsus  $P = 0.8509$ ; carpal  $P = 0.3850$ ; remiges  $P = 0.9187$ ).

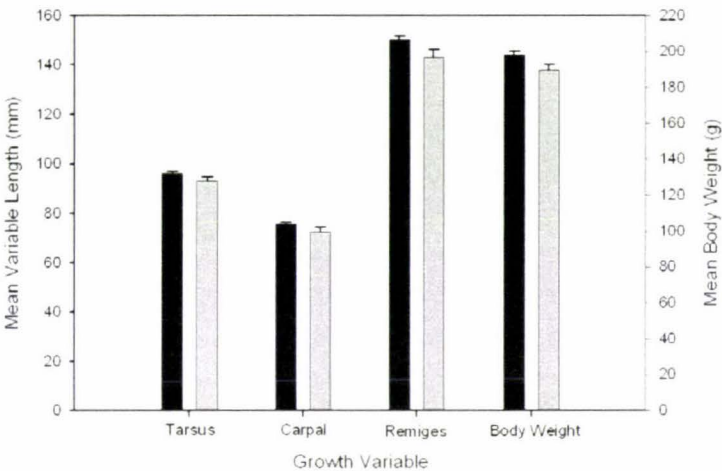


Fig. 10. Mean growth variables of male sub-adult black stilts on an ■ *ad libitum* diet or ■ 80% dietary restriction as juveniles ( $\pm 1$  SE).

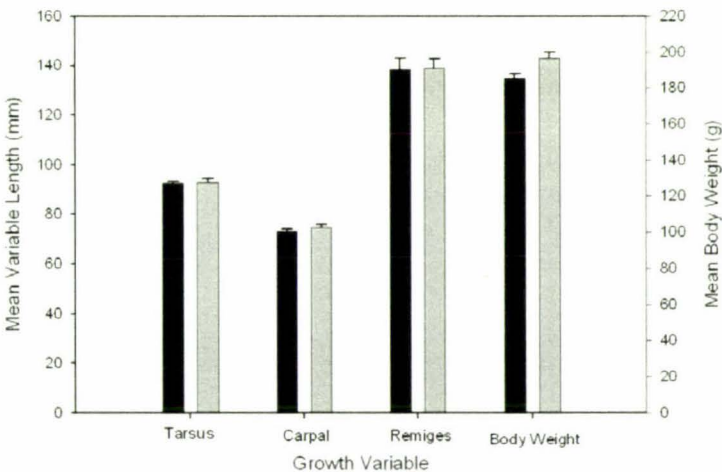


Fig. 11. Mean growth variables of female sub-adult black stilts on an ■ *ad libitum* diet or ■ 80% dietary restriction as juveniles ( $\pm$  SE).

3. Increase in growth between 31 days old and sub-adult stilts

For the growth variables, body weight, tarsus length and carpal length, the stilts that had been on an 80% dietary restriction as juveniles, increased on average, more than their counterparts that had been on an *ad libitum* diet as juveniles (table 11). Mean remiges length of stilts that had been on an *ad libitum* diet as juveniles (71.76mm), increased more than stilts that had been on an 80% dietary restriction (71.17mm). There was a significant difference in the increase in body weight between the two dietary groups ( $P = 0.05$ ). The differences in growth for the variables tarsus, carpal and remiges, were insignificant between stilts that had been on an *ad libitum* diet or 80% dietary restriction as juveniles (tarsus  $P = 0.8678$ , carpal  $P = 0.7342$  and remiges  $P = 0.8846$ ).

Table 11. Mean increase in growth variables between age 31 days and sub-adults.

Growth variable	Juvenile dietary treatment	Mean increase from age 31 days to sub-adult	SE
Body weight (g)	<i>Ad libitum</i>	27.71	1.77
	80% restriction	33.58	2.28
Tarsus length (mm)	<i>Ad libitum</i>	18.94	0.71
	80% restriction	19.13	0.86
Carpal length (mm)	<i>Ad libitum</i>	1.82	0.50
	80% restriction	2.25	1.14
Remiges length (mm)	<i>Ad libitum</i>	71.76	2.49
	80% restriction	71.17	3.21

Angel Wing Incident

During 2005/2006 black stilt chick rearing season one case of angel wing was observed. This occurred in the left wing of a chick on an 80% dietary restriction. This chick’s dietary restriction started at 16 days old as it was one of the oldest chicks in its brooder. Also at this age it was first noted that the left wing protruded ~5mm from the body. By 22 days old the wing had self corrected to the normal position of the wing (table 12 and figure 12). No other cases of angel wing were observed during our research season.

Table 12. Wing measurements of chick with initial signs of angel wing.

Age (days)	Carpal length (mm)		Carpal deviation (mm)		Remex length (mm)	
	Left	Right	Left	Right	Left	Right
10	24	23	0	0	3	2.5
13	29.5	30	0	0	6	7
16	40.5	38.5	5	0	15	15.5
19	48.5	51	4	0	25.5	27.5
22	58	58	0	0	39	40.5
25	67.5	65.3	0	0	52.2	55.2

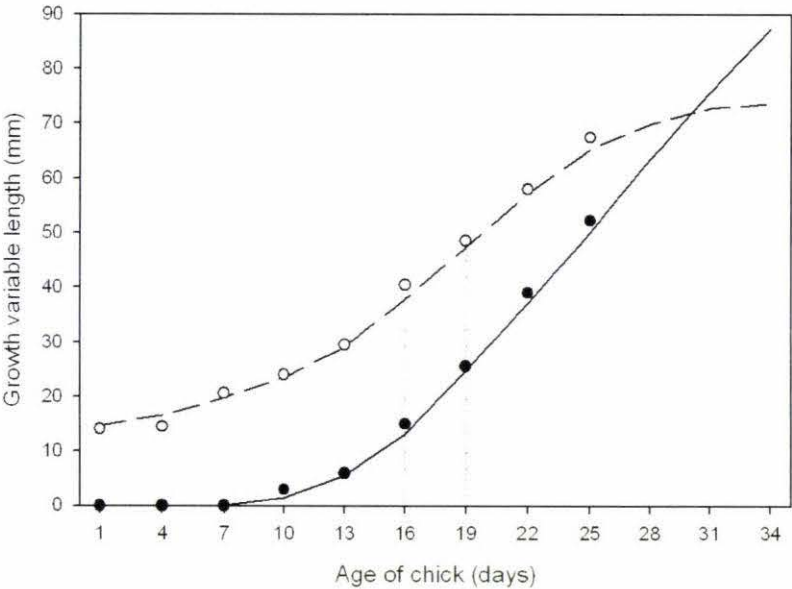


Fig. 12. Mean carpal and remex growth and the growth of the chick which developed the initial signs of angel wing. The solid black line represents the mean remex length of chicks on an *ad libitum* diet and the ● represent the remex length of the chick exhibiting angel wing. The dashed black line represents the mean carpal length of chicks on an *ad libitum* diet and the ○ represent the carpal length of the chick exhibiting angel wing. The dotted drop lines indicate the days in which the chick's carpal deviation was present. An 80% dietary restriction was implemented at 16 days of age for this chick. At 16 days of age the left wing first started to protrude from the body but by 22 days of age the wing had self corrected to its normal position.



Behavioural Observations

Food restriction had a significant effect on some aspects of black stilt behaviour (table 13). Feeding behaviour was influenced most by dietary restriction: stilts on a restricted diet foraged significantly more than stilts on an *ad libitum* diet ( $P = 0.0023$ ) (figure 13 i). Stilts on a restricted diet also spent significantly more time drinking than stilts on an *ad libitum* diet ( $P = 0.0106$ ) (figure 13 ii). Stilts on a restricted diet also spent significantly more time interacting than stilts on an *ad libitum* diet ( $P = 0.0376$ ) (figure 13 iii).

Table 13. Behaviour significantly affected by dietary restriction.

	Feeding Behaviour (% time)		Drinking Behaviour (% time)		Interactive Behaviour (% time)	
	$P = 0.0023$		$P = 0.0106$		$P = 0.0376$	
	<i>Ad libitum</i> diet	Restricted diet	<i>Ad libitum</i> diet	Restricted diet	<i>Ad libitum</i> diet	Restricted diet
Mean	21.64	29.68	2.56	4.05	0.28	0.89
SE	1.28	2.56	0.28	0.57	0.09	0.41
<i>n</i>	172	65	172	65	172	65

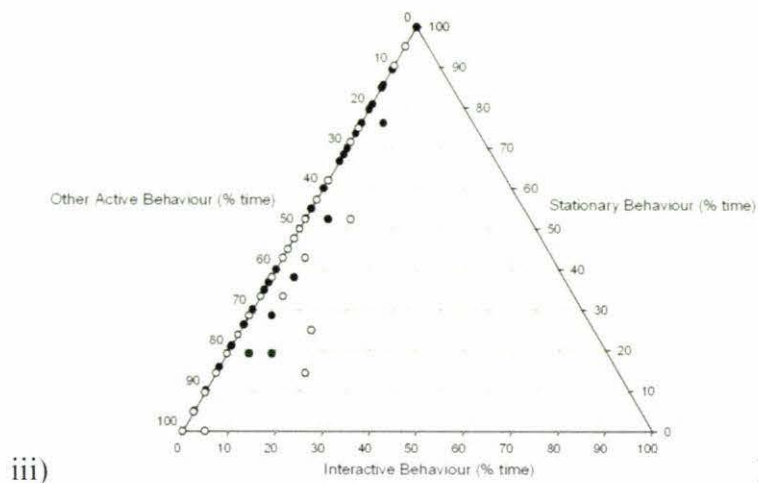
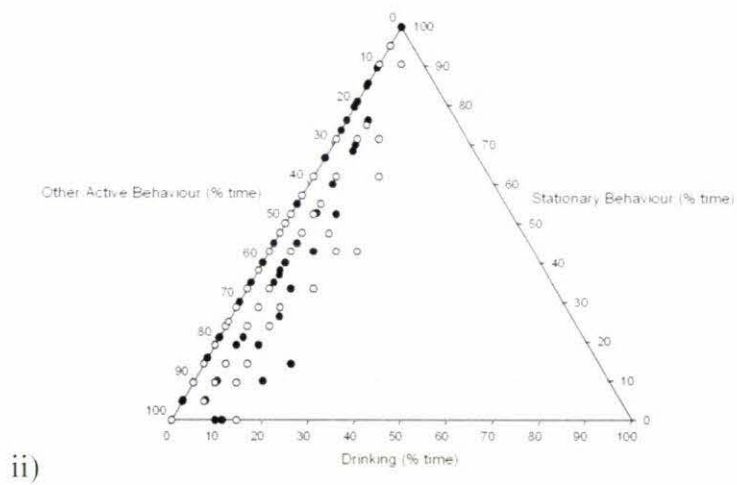
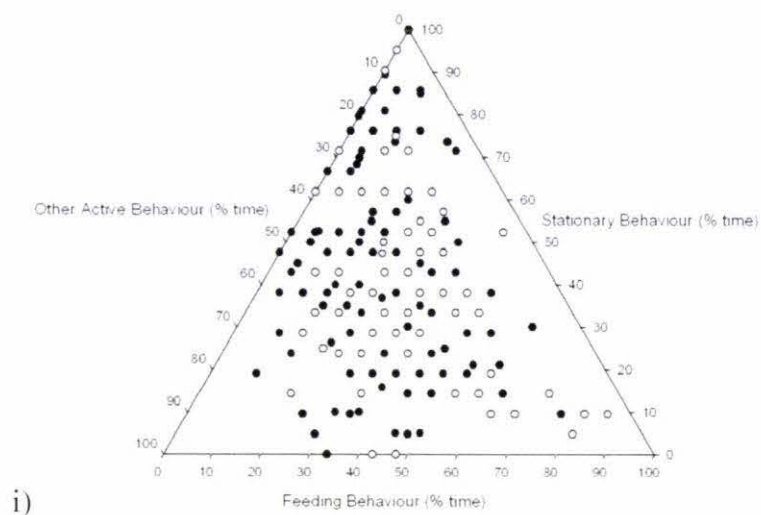


Figure 13 legend see over page.

Fig. 13. The interaction of behaviour of chicks on *ad libitum* and restricted diets. The ● represent chicks on an *ad libitum* diet and the ○ represent chicks on a restricted diet. i) Interaction between feeding, stationary and other active behaviour. ii) Interaction between drinking, stationary and other active behaviour. iii) Interaction between interactive, stationary and other active behaviour.



## Discussion

Black stilt survival against extinction in the wild currently relies on supplementation of the wild population with captive reared stock (Maloney and Murray 2001). Thus it is important that the birds being released are of the highest possible quality. It is therefore necessary to provide a captive environment which is going to encourage the growth of healthy chicks. Factors such as a relatively minimal occurrence of environmental stochastic events and *ad libitum* feeding in a captive environment encourage maximal growth rates of the black stilt.

A diet fed *ad libitum* will encourage high metabolisable energy intake and individuals will need less energy to forage for food. A species may respond to an *ad libitum* diet by gaining excess energy which will be allocated to tissues such as muscle, bone, feathers or fat. O'Connor (1984b) describes the natural feeding effect that parents may undertake in order to cope with wide spread hatching within a clutch. Parents may feed the youngest hatchlings at a more intensive rate in order to increase their growth so they are of a more similar size to their older siblings. The relatively natural *ad libitum* feeding strategy some parents may undertake will encourage excess growth of late hatchlings, relative to the early hatchlings, thus allowing the clutch to fledge at the same time. A high or excess food intake will increase metabolisable energy (Haye and Simons 1978), therefore resulting in increased energy allocated for growth.

In contrast a species on a dietary restriction may respond by altering rates of structural growth, maturation and metabolism. Schew and Ricklefs (1998) state that during food limitation an individual may continue to grow at a normal rate if they have stored fat reserves to use, once fat reserves are depleted growth will slow. If a chick continues to grow at a normal rate during food limitation their response may suggest that they have the ability to allocate resources to body parts essential for survival, resulting in the slowed growth and maturation of body parts of lesser importance for survival. Schew and Ricklefs (1998) also state that during food limitation a chick may have the ability to reduce energy expenditure by altering behaviour and physiological responses. A reduced growth rate may

result, which would reduce allocation of energy for tissue production and also minimise the increase of metabolically active tissues.

The dietary restriction imposed on captive reared black stilt significantly reduced body weight, and carpal and remex growth, relative to chicks fed on an *ad libitum* diet. There was not a significant reduction in weight gain or carpal and remex growth between chicks on an 80% and 70% dietary restriction. Dietary restriction had no significant effect on tarsal growth. This suggests that juvenile black stilts do not have significant body reserves of nutrients to compensate for reduced feed intake.

All measured growth variables of the black stilt were modeled relatively well by their corresponding best fitting growth model. Each model differed from all other variable growth models, indicating that each variable was affected differently by the dietary treatments. Body weight was best modeled by the combined effect of the 70% and 80% dietary restriction with the random variation between individuals on the body weight asymptote of the growth curve. Thus indicating that dietary restriction caused the most significant variation between individual body weight and had the strongest effect on growth at the end of the observed measurement period. Carpal length was best and most simply modeled by the random variation between individuals at the inflection point of the growth curve, with the combined effect of the dietary restriction treatments on the overall growth rate. This indicates that the most significant variation between individual carpal growth occurred at the point at which the growth rate was most rapid and declined beyond this point. The dietary restriction had the strongest effect on the overall carpal growth rate. Remex length was best modeled by the random variation between individuals at the inflection point of the growth curve, with the combined effect of dietary restriction treatments on the asymptote of the growth curve. Dietary restriction therefore had the most significant effect on variation between individual remex growth at the point at which the growth rate was most rapid and declined beyond this point, and it also had the strongest effect on growth on remex growth at the end of the observed measurement period. Tarsus length was not affected by dietary restriction but was best and most simply modeled by random variation between individuals on the tarsus asymptote, with the individual effect of



the 70% and 80% dietary restriction on the overall growth rate of the tarsus bone. This indicates that most variation between individual tarsal growth occurred at the end of the observed measurement period. There was also a slight, but insignificant, effect of the overall tarsal growth rate between chicks fed *ad libitum*, an 80% restriction and a 70% restriction.

It has been shown in many species, such as broiler chickens (Bradshaw, et al. 2002), that restricting feed during early growing periods can aid in reducing growth rates. High metabolisable energy density results from a diet fed *ad libitum*, this encourages thermoregulation, activity, maintenance and growth. A diet fed at a restricted rate encourages energy use for the most essential demands first, such as thermoregulation, activity and maintenance, thus resulting in reduced energy for growth (Schew and Ricklefs 1998). Klasing (1998b) also states that reduced metabolisable energy will likely result in slowed growth rates. Captive reared stilts on a dietary restriction would have had reduced metabolisable energy in comparison to *ad libitum* fed stilts, resulting in a reduction in weight gain and bone and feather growth. The artificial diet fed to black stilt *ad libitum* may encourage excessive energy intake, thus performing all essential tasks such as thermoregulation, maintenance and activity, leaving all remaining dietary energy for excessive growth and fat reserves.

Reducing feather growth rate was an important aim as excess weight from flight feathers can cause stress on carpal muscles encouraging immature carpal bones to hang and twist outwards (Flinchum 2006); a possible cause of the wing malformation observed in two captive black stilt in the 2004/2005 chick rearing season. It therefore may be important that the remiges emerge from bone and undertake their fastest growth during or after the developmental phase of wing bone and muscle maturation. It may be possible that birds have evolved to first start the maturation process of the skeleton, before developing primary feathers. This would ensure initial wing growth is encouraged prior to remex growth, thus wing abnormalities, such as angel wing, should not result from the additional weight of blood filled quills (O'Connor 1984a).



Tarsus was the only measured growth variable that was not significantly affected by dietary restriction, though the best fitting model for tarsus growth was 94% confident that dietary restriction did have an effect on tarsus growth. Therefore there may be a possibility that dietary restriction would have a significant effect and encourage optimal tarsus growth, rather than excessive growth while on an *ad libitum* diet, with a greater sample size. Leg bone maturation may be of priority at a juvenile age in precocial species. Skutch (1976) states that the brain, muscles and limbs of a precocial chick accounts for a larger proportion of body weight than those of altricial chicks. He also states that precocial chicks have a lighter digestive tract than altricial chicks as otherwise they would likely have trouble travelling with their parents. The fact that food restriction limits available energy for growth, may mean that other growth variables are spared, and priority is given during food restriction for tarsus growth.

Starck (1998) describes the development of the skeleton in precocial and altricial hatchlings. He suggests that in precocial species, such as the black stilt, the skeleton provides three main functions: support, resistance to mechanical forces from locomotion, and growth. In an altricial species, such as nestling parrots, the skeleton plays only a minor supportive function and does not need to resist mechanical forces from locomotion, as it is minimal in altricial species. He suggests the primary function of the skeleton in altricial birds is growth. Therefore the skeletal maturation of precocial species needs to be more advanced in maturation, relative to altricial species, as it has a greater number of functions at a juvenile stage.

The tarsus grew at an approximately constant rate slowing slightly at approximately 25 days of age. During the first two weeks of growth, carpal and remex growth rates were minimal relative to tarsus growth. This is likely to be an adaptive growth response as the legs are needed to support weight from hatch in precocial species, thus, the legs of precocial birds need to be more advanced in growth while other body parts such as the carpal and remex growth rates can be delayed; this is known as partitioning of growth. O'Connor (1984a) suggested that development occurs in a specific sequence in order to avoid growth abnormalities. Carpal growth followed a sigmoidal growth pattern in which

initial growth was small, with the most rapid growth occurring between days 13-25, before growth leveled off. The period of increased carpal growth coincided with the initial rapid remex growth phase, the carpus looked to be approaching its full length at day 34 while the feathers were still maturing. As black stilt are precocial birds they need to develop relatively quickly. It is important that all aspects of wing growth are coordinated, such as bone maturation, muscle development and feather growth, in order to avoid growth abnormalities. Again O'Connor (1984a) suggested the importance of this sequence of growth as wing bone structure is needed to support flight muscles and the development of remiges. The remiges did not emerge until 7-10 days of age. This growth strategy would allow bones to start the maturation process before being exposed to the increased weight from the development of emerging remiges. The rapid growth of the remiges continues past the slowed growth of both the tarsus and the carpus, at which time, the limb bones should be strong enough to support further gains in body weight.

An 80% dietary restriction as a juvenile did not have a significant effect on sub-adult (8-9 months of age) body weight, tarsus length, carpal length or remiges length. These results suggest that stilts previously on a dietary restriction were able to undergo compensatory growth, as the growth variables that were significantly reduced by dietary restriction as juveniles (body weight, carpal length and remiges length) reached similar weights and lengths to stilts that had previously been on *ad libitum* diets. Compensatory growth following an *ad libitum* diet after feed restriction as juveniles has mixed results in other research. Compensatory growth has been reported in turkeys and broiler chickens (Hurwitz, et al. 1992, Santoso 2002), whereas it has also been reported that broiler chickens did not exhibit compensatory growth after an *ad libitum* diet following the initial restricted fed diet (Yu, et al. 1990).

When analysing males and females separately, significant differences in body weight were observed between stilts on the two dietary treatments. Males that had been on an *ad libitum* diet as juveniles, were significantly heavier in overall bodyweight than males that had been on an 80% dietary restriction as juveniles. These results suggest that male black stilts that were on dietary restriction as juveniles were not able to undergo full compensatory growth,



as at 31 days of age *ad libitum* males were significantly heavier than restricted diet males, and this trend was still present when the birds had reached sub-adult stage.

In contrast, the female stilts that had been on dietary restriction as juveniles, were significantly heavier than females that had been on an *ad libitum* diet as juveniles. Brody et al. (1980) reported that female white rock broilers did not show full compensatory growth after severe food restriction (from hatch to 20 weeks) as they achieved approximately 25% of the weight of *ad libitum* fed birds. At 31 days of age, there was no significant difference in body weight between female black stilts raised on an *ad libitum* and restricted diet, suggesting dietary restriction did not have a strong effect on female black stilts. Sub-adult female black stilts raised on dietary restriction were significantly heavier than females raised on an *ad libitum* diet. This suggests that females on a restricted diet displayed the ability to undergo greater than full compensatory growth, as they reached weights above those of sub-adult females that were raised on *ad libitum* diets as juveniles. Plavnik and Hurwitz (1988a) and Santoso et al. (1993) respectively reported that turkeys and broiler chickens raised on a restricted diet, reached body weights greater than those raised solely on an *ad libitum* diet, after the change to *ad libitum* feeding.

The observed difference between males and females in our study may be due to different nutrient requirements between the sexes, and a differential response to food restriction as juveniles (Santoso 2002). Alternatively, this trend may have been a statistical anomaly through lack of power due to the small sample sizes.

Early dietary restriction had no significant lasting effect on skeletal or feather growth of sub-adult black stilt. As juveniles, at 31 days of age, dietary restriction resulted in stilts having significantly shorter carpal and remiges, than stilts on an *ad libitum* diet. Sub-adult measurements indicate that stilts that were on dietary restrictions as juveniles displayed some compensatory growth, as at 8-9 months of age, there was no longer a significant difference between stilts that had been on *ad libitum* and restricted diets as juveniles. Hurwitz et al. (1992) also found that both British United turkeys and white rock broiler chickens displayed compensatory growth of longitudinal bones after a few weeks of *ad*



*libitum* feeding, following the initial dietary restriction from days 7-13 for chickens and 7-21 for turkeys. The measurements of remiges taken at sub-adult stage were not considered reliable enough to compare to juvenile feathers due to a high incidence of damaged and frayed feather tips from wear in the aviaries.

A range of factors have been reported as influencing compensatory growth in broilers including the duration and timing of under-nutrition, the nature and severity of under-nutrition, the condition of re-alimentation, gender and the strain of a species (Zubair and Leeson 1996). The difference in the ability of males and females to compensate for the early dietary restriction may have been due to the severity of restriction, as the competition for food between individuals within each brooder may have resulted in some individuals receiving a more severe restriction than others (e.g. females being dominant over males). Hormones may also play a role in the differences observed in compensatory growth between genders (Wilson and Osborn (1960) in Plavnik and Hurwitz 1988b).

We are unable to conclude that reduced growth rates eliminated the incidence of limb abnormalities in captive reared black stilt as only one limb problem was observed during our field season. The left wing of the one chick that developed the initial signs of angel wing first started to protrude from its body on day 16, it was still abnormal on day 19 but had self corrected to the appropriate position by day 22. This chick was on an 80% dietary restriction but food restriction only commenced on day 16 as it was one of the oldest chicks in its brooder and feeding is carried out in relation to the youngest chick. It is possible that the dietary restriction implemented reduced carpal and remex growth and contributed to the self correction of the wing abnormality. To confirm this proposition, further dietary restriction trials would need to be put in place if angel wing arose again. During this trial we could conclude that an *ad libitum* diet does not cause greater than 2% wing abnormalities in captive reared black stilt.

Serafin (1982) reported that greater sandhill cranes (GSC) raised at the Patuxent Wildlife Research Center developed unilateral or bilateral twisting of the wing feathers, and twisting or drooping of the carpal bones that resulted in the displacement of remiges. These

abnormalities occurred between 10-28 days of age. Florida sandhill cranes (FSC) were reared on three of the same diets at the same Research Center, but did not develop any growth abnormalities. Diets trialled were 1. a diet containing 32% protein, 2. a diet containing 24% protein and 3. a diet the same as no. 2. but containing 17% less SAA (methionine and cystine). FSC grew slower than GSC, irrespective to which diet they were eating, and the author suggested that the FSC did not have the genetic capacity to grow at excessively fast rates, where as the GSC did, thus resulting in the rapidly growing feathers placing excess stress on the tendons and ligaments associated with the carpal joint causing abnormalities (Serafin 1982). During these trials food and water were fed *ad libitum* (Serafin 1982), thus it may be a possibility that over consumption of a balanced diet may have contributed to the rapid growths rates and associated skeletal deformities in the GSC. Like the GSC's, the black stilt may also possibly have a genetic predisposition for rapid growth. More likely, the excessive food intake captive black stilt consume on an *ad libitum* diet and the relatively stable captive environmental conditions are likely to encourage excessive growth, in contrast to the slower growing wild reared stilts which are exposed to a wider range of environmental fluctuations. Serafin (1982) concluded that diets with lowered protein and energy densities slowed the GSC growth and reduced the incidence of skeletal abnormalities. The black stilt is a wading bird and its natural primary source of food is aquatic invertebrates, these are high in protein (Klasing 1998a) and we therefore suggest that the protein content in the artificial diet would be an unlikely cause of the rapid growth observed in captive reared stilts.

Naldo *et al.* (1998) reported that angel wing was the most common skeletal abnormality observed in bustard chicks and typically occurred between 5-19 days of age. The cases of angel wing that occurred in the black stilt were recorded when the remiges were starting to develop. The abnormality has been recorded as first occurring between 16-21 days of age (E. Sancha, DoC, unpublished report). Only two cases of angel wing have been permanent in captive reared black stilt, but approximately 2-3 stilts per season, for the past three chick rearing seasons, have shown initial signs of feather protrusion, before being resolved by self-correction (E. Sancha, DoC, unpublished data). Angel wing has also been reported in a four week old raptor, the Northern goshawk (*Accipiter gentiles*) (Zsivanovits, et al. 2006).



This bird displayed valgus deformities of both wings causing dorsolateral rotation of the remiges. It was diagnosed as being caused by rapid growth causing rapid weight gain of the growing blood feathers resulting in the rotation of the immature metacarpal bones and dorsolateral deviation of the remiges (Zsivanovits, et al. 2006).

Increased aggression between individuals within a brooder was observed in dietary restricted chicks. Aggression was resolved by providing food on two plates and positioning a partition between plates. Behavioural observations indicated that dietary restriction (using the 80% and 70% dietary restriction as a combined data set) significantly increased the percentage of time chicks spent feeding, drinking and interacting. This could be viewed as having both a positive and negative influence on chicks. The beneficial point is that food restriction encourages increased activity, which may help to reduce the incidence of obesity, and abnormal, repetitive, or stereotypic behaviour, from developing in captive reared black stilt. Increased aggressive/submissive behaviour displayed by chicks on a restricted diet could possibly be argued as encouraging more natural behaviour, as it is unlikely that siblings have no aggressive behaviour towards each other in the wild. Increased foraging behaviour could also be considered a more natural behaviour as stilts do not have a constant food supply in the wild, and thus would have to continue to forage for long periods of time to ensure adequate intake. The increased period of time and the severity of interactive behaviours that arose between stilts on a restricted diet can also be viewed as negative. Severe aggressive behaviour exhibited by a small number of the stilts caused concern for the welfare of some individuals. As stilts on a restricted diet had increased activity levels, it is likely that food limitation encourages activity, thus increasing energy use contributing to slowed growth. It would be beneficial to carry out observations on chicks raised by their parent in the wild to establish the normal behavioural activity of black stilt chicks.

A factor that may have biased the behavioural results is the time of day behavioural observations were carried out. To fit in with other daily activities at the Kaki Captive Breeding Centre behavioural observations had to be carried out between 10:25am to 12:25pm. During this time period the chicks had received their morning feed and had food



available to forage on. Results may have been different if observations were carried out at different periods of the day. For example, it was observed that each morning when food was put out, chicks on a restricted diet would run to their food plates and start eating, whereas chicks on an *ad libitum* diet did not hurry to eat, they would continue behaviour such as preening and roosting.

Kaki management staff noted that during the chick rearing season of our research (2005/2006), more hand raised black stilts became sick than usual. Of six unwell chicks ( $\leq 35$  days of age) that recovered, three had been on a 70% dietary restriction, and three had been on an 80% dietary restriction. Two chicks died during the season, one had been on an *ad libitum* diet, and the other, which was euthanased, had been on an 80% dietary restriction. Two juveniles ( $> 35$  days of age) became unwell after being transferred to the main aviaries, both of these individuals had been on an *ad libitum* diet in the brooders. Increased stress on chicks on dietary restrictions may have resulted due to increased competition for food. This may have possibly contributed to the health problems exhibited by the individuals that had been on food restricted diets.

Management should remain aware of key aetiologies of angel wing and slipped tendon even though they have had minimal occurrences of these acquired limb disorders. Many published cases of angel wing and slipped tendon have been related to rapid growth rates. It is known that captive reared black stilt exhibit rapid growth in comparison to their wild reared counterparts (E. Sancha & R. Maloney, DoC, unpublished report). This has some management bonuses such as the ability to transfer juveniles to the main aviaries at a heavier weight and thus individuals having a likely increased ability to deal with environmental fluctuations and capture and transfer stress. The fast growth rates resulting in the ability to be able to transfer juveniles to the main aviaries by 34 days of age, allows management to move young chick into the brooders straight away, thus increasing the capacity of the captive breeding centre. The Kaki Captive Breeding Centre management should be aware that future changes to management procedures could potentially, unintentionally, encourage the development of further cases of limb abnormalities.

The growth models we have produced for each growth variable will allow future predictions of growth of hand raised black stilt to be made. This will aid management in a number of scenarios. A key benefit being the ability for management to predict 'normal' growth of an individual. This will help management to become more aware of critical time periods in which limb problems are likely to arise, for example, upon the commencement of rapid remex growth. The apparently normal growth curves I have produced will also allow management to monitor growth response if future changes are made to the captive diet. Growth curves will also give management the ability of possibly recognising at an early stage when growth is interrupted and could therefore give an indication that an individual is unwell.

At approximately 34 days of age, black stilt are transferred from the chick rearing brooders to the main aviaries (E. Sancha, DoC, unpublished report). At this stage chicks are weighed and a decision is made based on individual body weight as to whether the juveniles should be transferred. Based on the body weight growth model (growth on *ad libitum* diet =  $187.12/[1+\exp^{-0.16(t-17.50)}]$ ), an optimal weight at 34 days of age is 175g, thus our recommendation is that black stilt should be close to or above this body weight before being transferred from the brooders.

We have successfully shown that the growth rates and final body weight and final lengths of the carpal and remiges of captive reared black stilt can be reduced significantly by food restriction as a juvenile. By 8-9 months of age, previously dietary restricted male black stilt did not fully compensate in subsequent growth, whereas females compensated in subsequent growth to greater than that of females previously on an *ad libitum* diet, neither response resulting in any reported adverse problems. Food restriction will be recommended to the Kaki Captive Breeding Centre as an option to consider if limb deformities become more frequent in captive reared black stilt in the future.

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## Chapter 3

### A histological description of bone maturation in the black stilt (*Himantopus novaezelandiae*)



A 33 day old captive reared black stilt. Photo: Bridget Wrenn.



## **A histological description of bone maturation in the black stilt (*Himantopus novaezelandiae*)**

Bridget J. Wrenn and Brett D. Gartrell

### **Abstract**

Histology can be used to identify normal patterns of bone maturation and the critical periods where birds are most susceptible to acquired limb deformities. Longitudinal sections of the tarso-metatarsus (tarsus) and the first phalanx of the major digit of the carpo-metacarpus were taken from fresh and archived black stilt. An estimation of the length of the zones of proliferation, hypertrophy and ossification zones were measured ( $\mu\text{m}$ ) in stilts ranging in age from one day to five months (tarsus) and one day to three months of age (first phalanx). Both the tarsus and the first phalanx show the distinct maturation zones of epiphyseal cartilage, proliferation, hypertrophy and ossification. We found that the distal growth plate of the tarsus regresses at approximately one month of age, whereas its proximal growth plate regresses at approximately five months of age. The proximal growth plate of the first phalanx regresses between approximately 32 days of age to two months of age. We identified the most critical periods of growth in which black stilt captive managers need to be aware of limb deformities arising by relating the bone maturation of the tarsus and first phalanx to physical measurements of limb growth. These were identified as: the first three weeks of age for leg abnormalities and between 13-25 days of age for wing abnormalities.

## Introduction

Acquired limb deformities in birds generally occur during the rapid, early, growth phase following hatching (Kirkwood, et al. 1989a) and are associated with the period of most active bone growth. Histology can be used to identify normal patterns of bone growth and the critical periods where birds are susceptible to acquired limb deformities. The determination of the cause of limb deformities in avian species is not always straight forward, as information is lacking on growth plate morphology and maturation rates (Kirkwood, et al. 1989b). Growth plate morphology and maturation varies within and between mammal and avian species (Kirkwood, et al. 1989b) and also altricial and precocial avian species (Kirkwood and Webster 1984).

The black stilt, or kaki (*Himantopus novaezelandiae*), is a critically endangered wading bird endemic to New Zealand (Maloney and Murray 2001) in which patterns of normal bone maturation are unknown. The species was once distributed throughout New Zealand but like many of New Zealand's endemic species, introduced predators and habitat degradation have led to a severe reduction in its natural range (Atkinson and Cameron 1993, Reed, et al. 1993, Craig, et al. 2000). The instigation of the black stilt recovery and captive management plans, when the population reached an estimated low of c.28 individuals in the wild (Reed, et al. 1993), has improved the status of the black stilt, leading to a recovery of the wild population with the most recent wild population count (28 August 2006) residing at a total of 182 black stilt, 100 being adults (E. Sancha, Department of Conservation, unpublished data). A key goal of captive management to improve on the species current critically endangered status, is to aid in the recovery of the wild population through supplementation with captive reared juveniles (Maloney and Murray 2001). Early captive management practices had mixed success (Reed, et al. 1993) but advancements in the development of the captive diet and husbandry techniques saw 115 stilts fledge in captivity during the 2005/2006 chick rearing season (Sancha, et al. 2006).

A number of nutritional related abnormalities have been identified in captive held and reared black stilt resulting in adverse affects on stilt recruitment (Sancha, et al. 2004,



Sancha, et al. 2005). The most recent abnormalities of concern have included: low incidences of long bone limb deformities including fibrous osteodystrophy (Sancha, et al. 2005); outward rotation of the carpo-metacarpus (angel wing); and luxation of the gastrocnemius tendon from the condyles of the tibio-tarsus (slipped tendon or perosis).

Fibrous osteodystrophy appears to have been eliminated from captive reared black stilts after ultra violet fluorescent bird lamps have been installed in the brooders and additional calcium carbonate has been added to the captive diet (Sancha, et al. 2005). The causes of angel wing and slipped tendon in captive reared black stilt have not been confirmed but literature has suggested a number of possible aetiologies for these disorders in other avian species. These included a high protein intake (Serafin 1982, Macwhirter 1994, Smith 1997, Mushi, et al. 1999, Naldo and Bailey 2001, Bradshaw, et al. 2002, Flinchum 2006, Zsivanovits, et al. 2006), lack of exercise (Smith 1997, Klasing 1998a, Mushi, et al. 1999), over feeding (Flinchum 2006), vitamin deficiencies (Naldo and Bailey 2001, Flinchum 2006) and mineral deficiencies and imbalances (Wiese, et al. 1938, Wedekind and Baker 1990, Baker and Oduho 1994, Macwhirter 1994, Mushi, et al. 1999, Naldo and Bailey 2001, Flinchum 2006). Genetic factors (Naldo, et al. 1998, Mushi, et al. 1999, Naldo and Bailey 2001) and temperature influences during incubation (Kear 1973, Serafin 1982, Naldo and Bailey 2001) and early rearing stages (Kear 1973, Naldo and Bailey 2001) have also been suggested as possibly contributing to limb abnormalities.

Limb deformity most commonly occurs in the epiphyseal growth plate at the ends of the bone where active generative – degenerative activity is taking place (Klasing 1998b). For this reason, a sound knowledge of the normal maturation of bone and soft tissues of the limbs is essential in understanding the pathogenesis of limb abnormalities (Hahulski, et al. 1999).


Bone modelling is the process that allows bones to grow in both diameter and length (Klasing 1998b). Bones expand in width by appositional growth, where new layers form on top of the inner periosteal layer, and a bone expands in length by cartilage growing on the epiphyseal plate (Aughey and Frye 2001). Once bone stops growing, remodelling



continues. Bone resorption and accretion result in continual turn over of minerals which make them available during dietary shortages (Klasing 1998b).

Growth of epiphyseal cartilage occurs in distinct zones. They have been identified in a red-necked wallaby as the zone of reserve cells, the zone of proliferation, the zone of hypertrophy, and the zone of ossification (Kirkwood and Kember 1993). Kirkwood, et al. (1989b) identifies them in a juvenile Rhode Island red cockerel as the epiphyseal cartilage zone, the proliferation zone (flat cell zone), the maturing cell zone, and the hypertrophic chondrocyte zone. The growth zones are described working from the bone ends to the centre of the long bone shaft (table 1) and some zones are documented in a Rhode Island red cockerel (figure 1).

Table 1. Description of the epiphyseal growth zones in bone (Kirkwood, et al. 1989b, Kirkwood and Kember 1993, Ross, et al. 1995, Zhang 1999)

	Epiphyseal Zone	Description
Closest to epiphysis (end of bone)	Zone of epiphyseal cartilage (zone of reserve cartilage)	In this zone chondrocytes (cartilage cells) are randomly orientated and undertake no proliferation (cell formation). A small number of cells may occur in pairs.
	Zone of proliferation	In this zone cell division is undergone by the chondrocytes and they are arranged in obvious columns parallel to each other and the bone shaft. These cells are flattened in shape and are larger than the cells in the previous zone. Vascular channels may penetrate this zone.
	Zone of maturation and hypertrophy	In this zone cell division has stopped. Chondrocytes are greatly enlarged in size, due to the accumulation of glycogen and lipid in their cytoplasm, but they still remain in parallel columns.
Closest to centre of diaphysis (bone shaft)	Zone of calcification and ossification (zone of resorption)	In this zone the enlarged chondrocytes degenerate and die. The cytoplasm and nuclei shrink and cartilage matrix becomes calcified. Blood vessels maybe found in the bone marrow cavity and osteoclasts absorb dead chondrocytes.

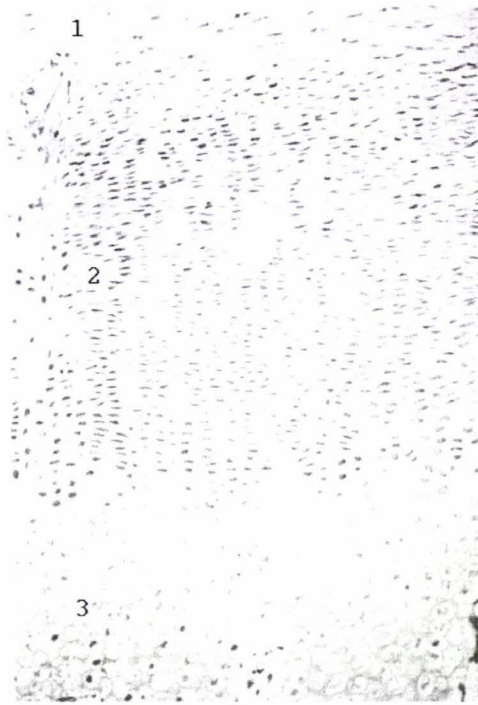


Figure 1. Longitudinal section through the growth plate of the distal tip of the tibio-tarsus of a Rhode Island red cockerel age four weeks of age. 1 = Zone of epiphyseal cartilage, 2 = Zone of proliferation, and 3 = Zone of maturation and hypertrophy. Haematoxylin and eosin x 150 (Adapted from Kirkwood, et al. 1989b).

Kirkwood et al. (1989a) state there is little variation in the mechanisms of longitudinal growth between mammals and birds. The only factor they highlight as being slightly different, is that in birds, the columnar arrangement of cells can be less pronounced. Kirkwood and Kember (1993) state that regardless of mammalian species, the growth development zones also undergo the same general pattern. Bone growth rates of mammals are generally lower than the growth rates of birds (Kirkwood and Kember 1993).

Growth rate is determined by the number of cells dividing in the zone of proliferation, thus influencing the size of this zone (Kember, et al. 1990, Kirkwood and Kember 1993). Growth is also influenced by the frequency in which chondrocytes divide in this zone and the size they are prior to ossification (Kirkwood and Kember 1993). Kirkwood et al. (1989a) proposed that deep cartilaginous growth plates may be associated with rapid

growth rates, and if so, may explain why the rapidly growing tarso-metatarsus in some avian species is susceptible to deformity.

The aims of this study were to document the normal tarso-metatarsus and the first phalanx of the carpo-metacarpus bone maturation of the endangered black stilt and correlate these to morphological measures of limb growth. This will identify the critical phase in which the black stilt grows most rapidly and is most likely develop acquired limb abnormalities.



## **Methods**

### **Collection and Preparation of Specimens**

We took longitudinal sections of the tarso-metatarsus (from here on referred to as the tarsus) and the first phalanx of the major digit of the carpo-metacarpus (from here on referred to as the first phalanx) from fresh and archived black stilt fixed in 10% buffered formalin held at the Institute of Veterinary Animal and Biomedical Sciences (IVABS), Massey University. The sections were taken from black stilts ranging in age from hatch to 2.5 years of age. One longitudinal section was taken from both the distal and proximal ends of the tarsus from one leg in each specimen and of proximal end of the first phalanx from one wing in each specimen.

Sections were placed facedown in plastic lid cassettes and were stored in 10% buffered formalin until ready for decalcification. Sections were decalcified in DeltaCal (DeltaCal Products Group, Aurora, IL) overnight to soften the bone. They were processed as large samples under program three of the Processor Jung TP 1050 (Leica Instruments, Nussloch, Germany) the following night for 14 hours. This process consisted of using absolutes to dehydrate the sections, then xylene as an intermediate stage before processing in Paraplast wax.

Sections were embedded in wax using the histo embedder centre, Jung Histo Embedder (Leica Instruments, Nussloch, Germany). They were trimmed on the microtome, Microtec 4055 (Microtec, Walldorf, Germany), to expose the full section of bone before undergoing surface decalcification in DeltaCal for a further 10 minutes. Bone sections from older stilts sometimes needed further decalcification before being suitable to cut. The sections were cut at a thickness of  $3\mu\text{m}$  using recognized trimming procedures, dried for one hour at  $60^{\circ}\text{C}$  and stained with Haematoxylin and Eosin for examination.

### **Histological Measurements**

An estimation of the length of the zones of proliferation, hypertrophy and ossification zones were measured in  $\mu\text{m}$  using image analysis software (Soft Imaging System 2003) and rounded to the nearest  $50\mu\text{m}$ . Measurements were taken in approximately the centre of the bone shaft, down the longest, most continuous fragment of each zone (parallel to the bone diaphysis) from both the distal and proximal growth plates of the tarsus and the proximal growth plate of the first phalanx. Areas containing processing artifacts such as fissures and folds were avoided for all measurements. Measurements were carried out on black stilts ranging from one day to five months of age (tarsus) and one day to three months of age (first phalanx). Only one specimen was available for each age, therefore these measurements only give an estimation of bone maturation as it does not account for variation of growth between individuals of the same age. A further limitation is that individual variation is not accounted for, as the plane each section was cut on will result in variation within each individual.

## Results

### Tarsus Maturation

The distal and proximal growth plates of the tarsus show the same growth zones as previously published including the zones of epiphyseal cartilage, proliferation, hypertrophy and ossification. The distal growth plate regresses at approximately one month of age, whereas the proximal growth plate regresses at approximately five months of age.

At hatch, black stilts show a large amount of epiphyseal cartilage in which chondrocytes are randomly orientated (figure 2), this zone recedes as the stilts age. The zone of proliferation follows the epiphyseal cartilage, and this zone was characterised by flattened cells that are orientated in columns (figure 2). Vascular channels sometimes penetrate the zone of proliferation, becoming more frequent as black stilts age. At the proximal growth plate the zone of proliferation generally appears to enlarge in size from hatch until approximately one month of age (750-3400 $\mu$ m). There was some variation between individuals showing a fluctuating pattern over this age period (figure 3). Thereafter, the size of the zone of proliferation recedes in size to full regression by three to five months of age. The zone of proliferation of the distal growth plate increases slightly in size over the first two weeks of age (500-700 $\mu$ m). Over the following two weeks the zone recedes dramatically in size (50-100 $\mu$ m) and by 40 days of age the zone of proliferation in the distal growth plate regresses completely (figure 4).

The next zone of growth was the zone of hypertrophy which is characterised by greatly enlarged chondrocytes which were mostly ordered in distinct columns (figure 2). Vascular channels were common in the zone of hypertrophy (figure 2) and generally increased in size as the stilts age. At the proximal growth plate the zone of hypertrophy generally increased in size over the first month of age (5500-7550 $\mu$ m). By 78 days of age the zone of hypertrophy recedes dramatically (2200 $\mu$ m) and continues to decrease thereafter (figure 3). At the distal growth plate the zone of hypertrophy peaks closest to hatch (4950 $\mu$ m, 5950 $\mu$ m, zero days and one day of age respectively). Thereafter the zone of hypertrophy recedes and fully regresses by 40 days of age (figure 4). The zone of hypertrophy is consistently larger



than the zone of proliferation as stilts age, and the zones of proliferation and hypertrophy in the proximal growth plate are consistently larger than the corresponding zones of growth in the distal plate, with the exception of the chick at one day of age where the two zones are of approximately equal size.

The zone of ossification is present in the stilts from one day of age. It is no greater than 200 $\mu$ m but is mostly close to 50 $\mu$ m in length. Beyond this zone, calcified bone and medullary cavities are present (figure 5).

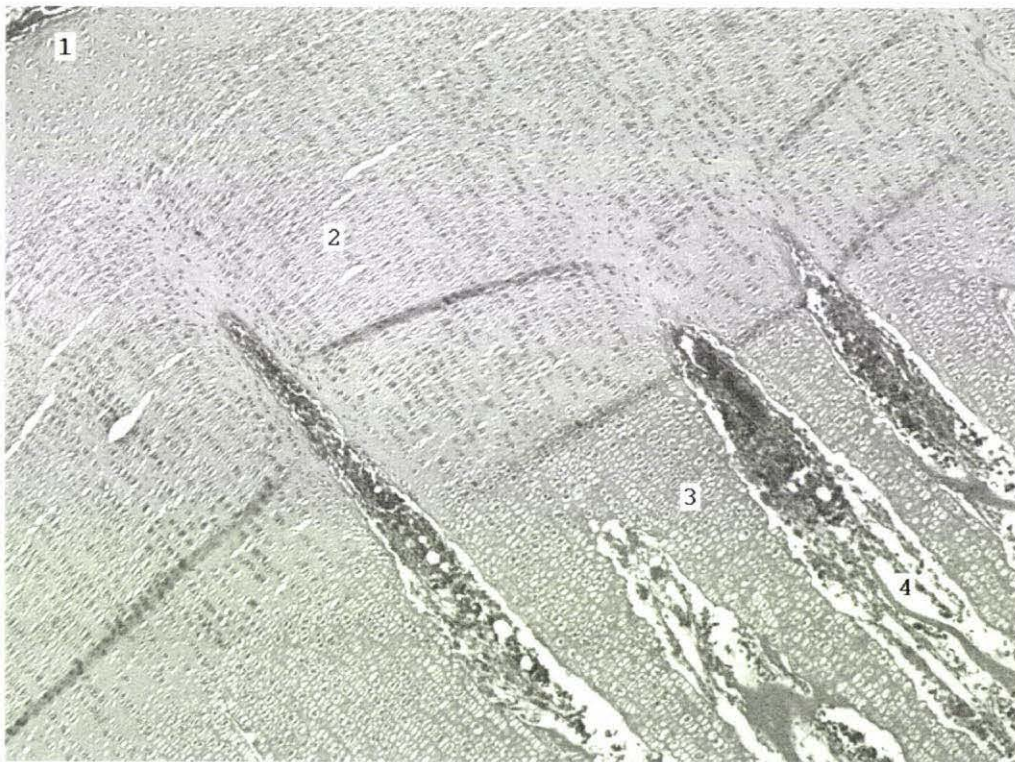


Figure 2. Longitudinal section through the proximal growth plate of the tarso-metatarsus of a black stilt aged 40 days of age. 1 = Zone of epiphyseal cartilage, 2 = Zone of proliferation, 3 = Zone of hypertrophy, 4 = Vascular channel. Haematoxylin and Eosin. Magnification  $\times 100$ .

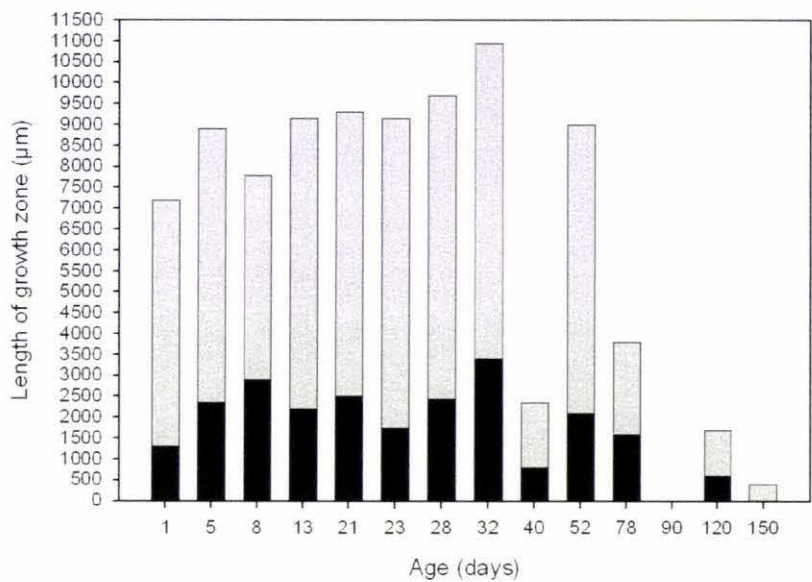


Figure 3. Estimation of lengths of the zones of proliferation and hypertrophy in the proximal growth plate of the tarsus of black stilts aged one day to five months (150 days) of age. ■ = zone of proliferation; □ = zone of hypertrophy.

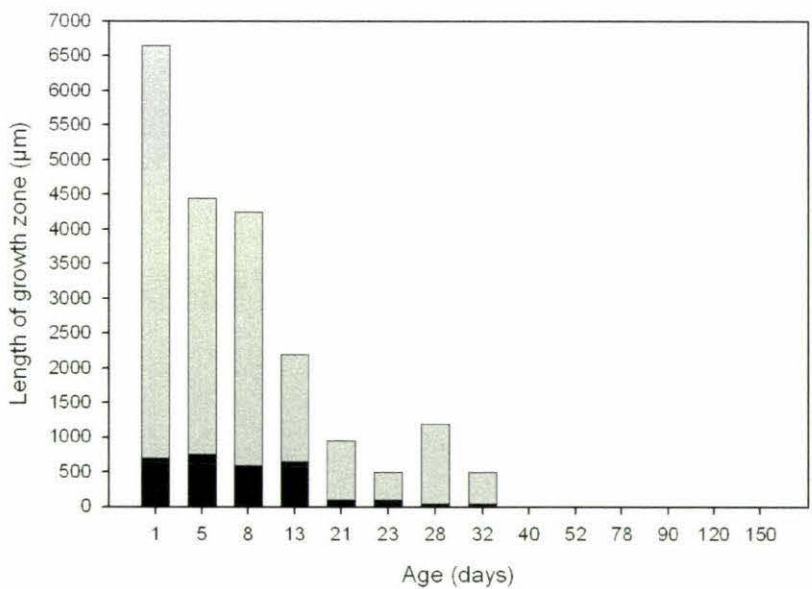


Figure 4. Estimation of lengths of the zones of proliferation and hypertrophy in the distal growth plate of the tarsus of black stilts aged one day to five months (150 days) of age. ■ = zone of proliferation; □ = zone of hypertrophy.



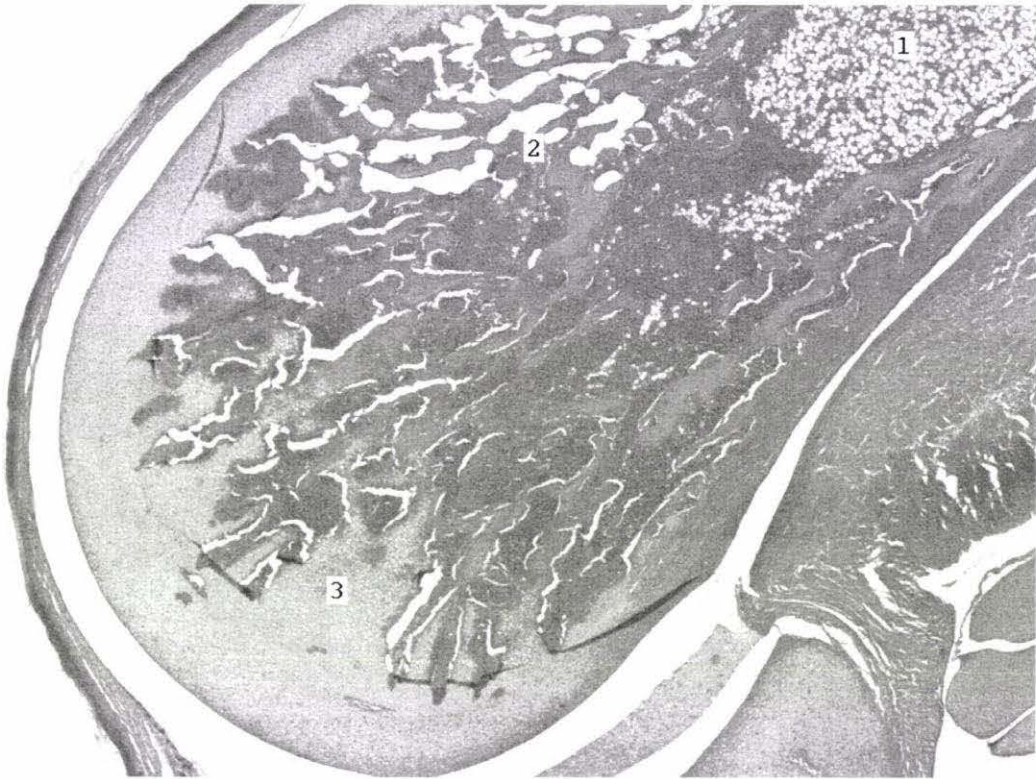


Figure 5. Longitudinal section through the distal growth plate of the tarso-metatarsus of a black stilt aged 21 days of age. 1 = Medulla, 2 = Trabecular bone, 3 = Zone of hypertrophy. Haematoxylin and Eosin. Magnification x 40.

### **First Phalanx Maturation**

The proximal growth plate in the first phalanx regresses between 32 days and two months of age. The zone of epiphyseal cartilage, proliferation and maturation and hypertrophy are identified as described in table 1 and are documented in a stilt aged one day of age (figure 6).

The proliferation zone of the proximal growth plate of the first phalanx gradually increases in length from approximately 200 $\mu$ m at one day of age to approximately 500 $\mu$ m at 25 days of age. By 32 days of age the proliferation zone decreases to approximately 150 $\mu$ m and regresses between 32 days and two months of age as the zone was absent (figure 7). The zone of hypertrophy displays a fluctuating pattern in length between the stilt aged one day



to the stilt aged 32 days of age, but the general pattern is increasing in length over this age period. By two months of age the zone of hypertrophy has also completely regressed as the zone was absent and the proximal phalanx consisted of medullary bone (figure 7). Generally, the zone of hypertrophy was greater in length than the zone of proliferation and vascular channels were not common in the proximal growth plate of the first phalanx. The zone of ossification was present in the stilts from one day of age but was not greater than approximately 200µm in length.

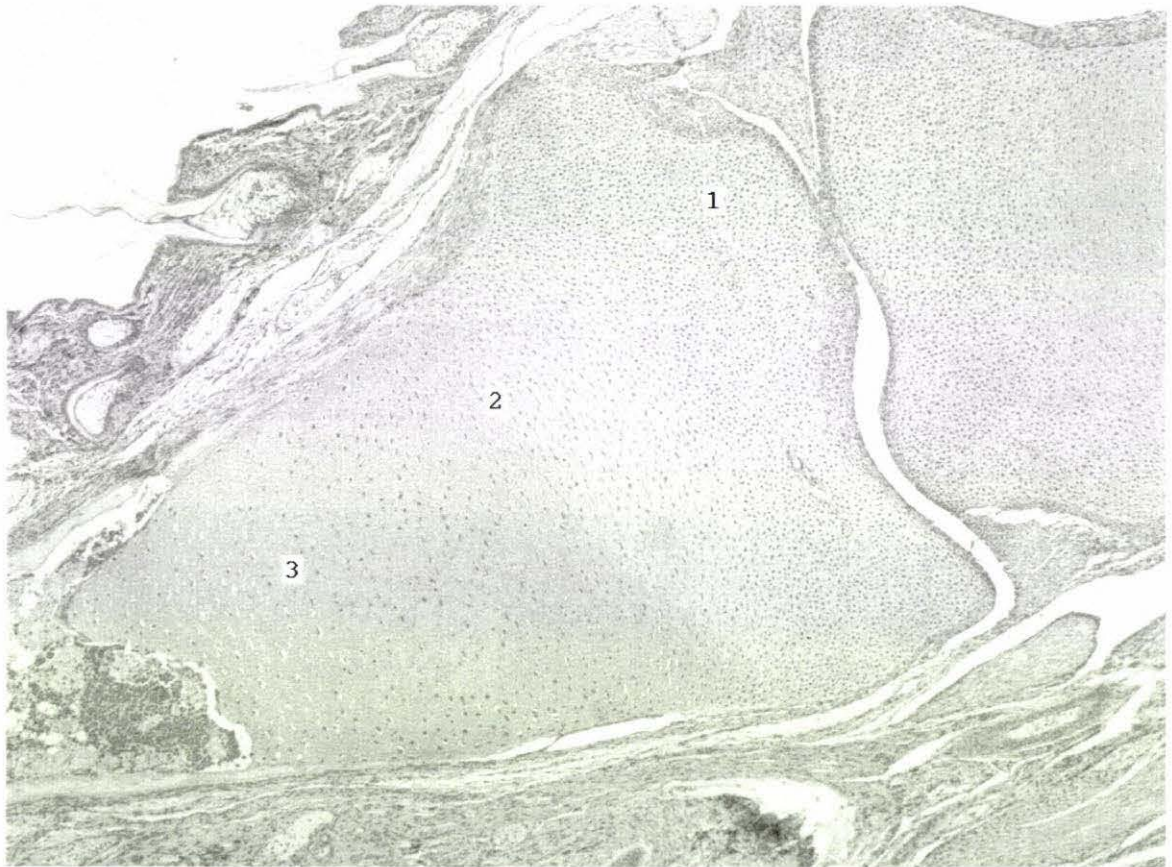


Figure 6. Longitudinal section through the proximal growth plate of the first phalanx of a black stilt aged one day of age. 1 = Zone of epiphyseal cartilage, 2 = Zone of proliferation, 3 = Zone of hypertrophy and maturation. Haematoxylin and Eosin. Magnification x 100.

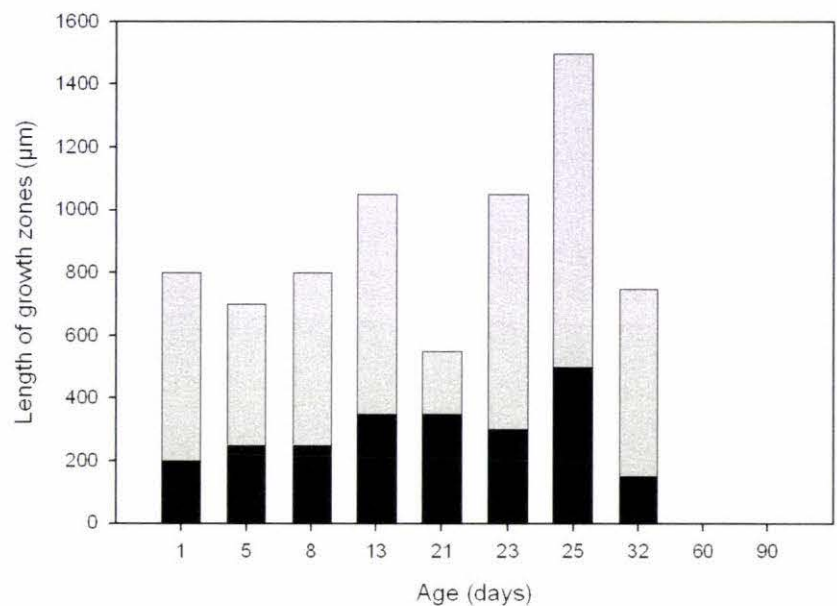


Figure 7. Estimation of lengths of the zones of proliferation and hypertrophy of the proximal growth plate in the first phalanx of black stilt aged one day to three months of age. ■ = zone of proliferation; □ = zone of hypertrophy.

## Discussion

Both the tarsus and the first phalanx show the distinct maturation zones of epiphyseal cartilage, proliferation, hypertrophy and ossification. We found that the distal growth plate of the tarsus regresses at approximately one month of age, whereas its proximal growth plate regresses at approximately five months of age. The proximal growth plate of the first phalanx regresses between approximately 32 days to two months of age.

Morphological measurements of the tarsus we have carried out investigating growth rates of captive reared black stilt during their first month of age (B Wrenn, Massey University, unpublished data), indicate that the tarsus grows at a relatively constant rate from 1-25 days of age. This trend corresponds to the bone maturation documented here as the zone of proliferation in the distal growing tip gradually decreases in length during this time period, whereas the zone of proliferation in the proximal growing tip generally slowly increases in length. Thus resulting in a relatively constant grow rate, as growth rate is determined by the number of cells dividing in the zone of proliferation (Kember, et al. 1990, Kirkwood and Kember 1993). Morphological measurements carried out on the carpo-metacarpus (B. Wrenn, Massey University, unpublished data) also suggest that the growth plate of the carpo-metacarpus regress soon after 32 days of age, as the morphological growth rate is nearing a horizontal line at approximately 30 days of age.

Morphological measurements of the tarsus of the black stilt at 8-9 months of age suggest that the continued growth of the proximal growth plate after one month is much slower than during the first month. The growth plate histology also suggests this, as only a single growth plate (the proximal) was active after approximately one month of age. Growth rates slow with age as resources are no longer concentrated on growth, they are allocated to functional maturity (Ricklefs, et al. 1998).

A traditional hypothesis that had been proposed to account for initial growth rates in chicks is that growth rates are rapid in order to reduce the time that individuals are most vulnerable to predation (Lack (1968) in Ricklefs 1973). This hypothesis has been rejected as variation



in mortality rates in avian species have not been correlated to changes in growth rates (Ricklefs 1973). A further hypothesis that has been postulated is that slower growing individuals have been selected as they require less energy and will therefore permit a greater number of siblings (Lack (1968) in Ricklefs 1973). This hypothesis does not apply to the black stilt as they are precocial species so the parents do not feed their young. The internal constraints of a growing organism has been postulated as a hypothesis to affect growth rates (Ricklefs 1973). Internal constraints are closely linked to a chicks mode of development. The mode of development may be constrained by the demand of cells and tissues for muscle, skeleton, nervous system and integument (Ricklefs, et al. 1998). Ricklefs et al. (1998) suggests that the evolution of post-natal growth diversification is restricted by an antagonism between growth and the function of tissues. The growth rate – functional maturity trade-off is important for precocial birds as they grow 3-4 times slower than altricial species (Ricklefs (1979) in Ricklefs, et al. 1994). In precocial birds functional maturity and animal size should be negatively correlated, because an increase in growth would only result from reduced tissue function (Ricklefs, et al. 1998), therefore, decreasing growth rates in the black stilt, should be correlated to the increase in tissue maturation as the stilts near adult size. Cell and tissue maturity increases with increasing adult size, and this varies considerably between precocial species due to differences in functional maturity during development (Ricklefs, et al. 1998).

The genotype of a growing individual, as well as their parents, will also have an effect on growth (van Noordwijk and Marks 1998). The genotype of precocial chicks will delay growth in relation to altricial chicks, and the environment provided by the parents at the chosen nest site will influence foraging ability in precocial chicks, thus having an influence on food consumption and the resulting growth (Schew and Ricklefs 1998).

The zone of proliferation regulates growth rates by the number of chondrocytes dividing within it (Kember, et al. 1990, Kirkwood and Kember 1993). In the adjacent zone of hypertrophy, chondrocytes mature, before being ossified into bone matrix by osteoblasts (Ross, et al. 1995) in the zone of calcification and ossification. Osteoclasts are also present here, and undergo bone resorption during injury (Ross, et al. 1995) and continually turnover

minerals (Klasing 1998b). Bone formation and resorption is undergone at different rates in different locations within a bone, thus resulting in a bone's specific morphology in response to its functional demands (Klasing 1998b). Wing formation and growth is delayed in relation to the legs. Earlier leg maturation is essential for precocial species as they are needed for locomotion and mechanical strength soon after hatch (Starck 1998), whereas wing maturation is of lesser importance initially in a precocial species as they do not fly straight after hatch. But, wing maturation is essential later in development to support flight muscles and flight feathers (O'Connor 1984a). Therefore different rates of bone formation (growth partitioning), are prioritised in relation to the function of each skeletal element.

Both the documented bone maturation and the morphological measurements indicate that the tarsus is growing most rapidly over the first three weeks of age and the first phalanx, is growing most rapidly between approximately days 13-25, as the zones of proliferation are greatest during these time periods. These critical periods during the first three and a half weeks of age are of importance to wild black stilts as young forage for themselves and are most vulnerable to predation while confined to the ground. Tarsus growth is most rapid initially, and over a longer period of time as legs provide one of the most essential mechanisms in precocial species, ground locomotion (O'Connor 1984b), which assist's with more productive foraging and may aid with predator avoidance. Rapid growth of the first phalanx, and other wing bones, is also an essential phase of development, as while confined to the ground, stilts are most vulnerable to predation. Bone maturation, followed by flight muscle development and flight feather growth is an important sequence of growth to establish the ability to fly (O'Connor 1984a). Black stilts fledge between 39-55 days of age (Reed, et al. 1993) and flight is an essential response in order to help stilts avoid both ground dwelling and aerial predators and increase the habitats used for foraging. These identified time periods are critical phases of rapid growth, and are important to captive management facilities, as they are the most likely phase of growth after hatch in which individuals may develop limb deformities (Kirkwood, et al. 1989a), resulting in adverse affects on the essential life function, locomotion.



It has been proposed that rapid growth rates may be associated with deep cartilaginous growth plates, and this factor has been suggested as possibly contributing to the susceptibility of the growing tarsus in some bird species to develop twisting abnormalities (Kirkwood, et al. 1989a). Altricial species grow faster than precocial species and have larger cartilaginous growth plates in their long bones (Starck 1998). Large cartilaginous growth zones are likely to encourage bone growth, but may impose a cost of reduced mechanical strength (Kirkwood, et al. 1989b). If faster than normal growth rates are encouraged in precocial species such as the black stilt, cartilaginous growth zones may be enlarged, which will result in reduced mechanical strength to support a heavier than normal chick. This may possibly result in weight bearing skeletal structures, such as leg and wing bones, being more susceptible to deformity.

It should be noted that this research was limited by sample size, as the black stilt is a critically endangered species and thus we were limited in the availability of archived species at each specific age. Classic studies of this sort have consisted of a larger sample size of the same aged individuals and have been undertaken on poultry (Hahulski, et al. 1999), another species that can grow rapidly in captive environments and can develop long bone limb deformities.

Despite these limitations, the description of the normal bone maturation of the tarsus and first phalanx will be beneficial to the black stilt and other species that exhibit similar bone morphology and growth rates. It will aid pathologists in recognising skeletal abnormalities that may arise in the future, as they will have the ability to compare malformed bones to normal bones. This will likely result in an increased understanding of bone abnormality and pathogenesis, and may possibly aid in reducing the incidence of limb deformity in the captive black stilt population, by altering management practises if appropriate.

We have confirmed the most rapid periods of tarsus and first phalanx growth by taking both physical measurements of limb growth in previous work and by documenting bone maturation in this study. The critical periods of rapid growth in which Kaki Captive



Management need to be aware of limb deformities developing are: the first three weeks of age for leg abnormalities and between 13-25 days of age for wing abnormalities.

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## **Chapter 4**

# **Manganese concentration in bone and feathers of the black stilt**



Juvenile black stilt sleeping in a brooder aviary. Photo: Josie Galbraith.



## **Manganese concentration in bone and feathers of the black stilt**

Bridget J. Wrenn, Brett D. Gartrell and Nick Cave

### **Abstract**

Captive management is essential for the survival of the endangered wading bird, the black stilt (*Himantopus novaezelandiae*). Slipped tendon (perosis) and angel wing have been seen in captive reared chicks. A possible cause for these limb deformities is manganese deficiency. Bone and feather samples were taken from archived, captive reared black stilt and wild reared hybrid stilts to investigate the role of manganese deficiency in the incidence of limb abnormalities in black stilt. The primary objectives of this research were to determine if: 1. manganese concentrations vary between stilts with and without limb deformities; and 2. captive reared black stilt are deficient in manganese in comparison to wild reared hybrid stilts. The secondary objectives of this research were to better understand the variables involved with manganese body stores. We examined the effect of age on bone and feather manganese, and wild and captive parentage on neonatal feathers. We also assessed whether feather concentrations of manganese were a useful indicator of manganese bioavailability. Captive reared black stilt were not deficient in bone manganese in relation to wild reared hybrids, but wild reared hybrid stilts had significantly greater concentrations of feather manganese than both captive hand and parent raised black stilt ( $P = 0.001$ ). Bone manganese was found to have a small, but definite positive trend as stilts aged ( $r^2 = 0.345$ ). There was no significant difference in the manganese of the neonatal feathers of captive reared black stilt with wild or captive parents, and we found that there was no correlation between bone and feather manganese in captive reared black stilt, therefore feathers can not be used as a non-invasive technique to monitor manganese bioavailability.

## Introduction

Skeletal abnormalities are a problem in non-domesticated, juvenile, avian species reared in captivity. Affected species include both meat-type fowl (Cherel, et al. 1991, Hester 1994, Lilburn 1994, Orth and Cook 1994, Bradshaw, et al. 2002, Waldenstedt 2006) and endangered species such as the greater sandhill crane (Serafin 1982) and a range of bustard's (Naldo, et al. 1998). The black stilt, or kaki (*Himantopus novaezelandiae*), is a critically endangered wading bird endemic to New Zealand (Maloney and Murray 2001) that has also had a low incidence of skeletal deformities in captive reared settings reported (Sancha, et al. 2005). Due to its population status, it is of importance that abnormalities observed in captive reared stilts limiting population recruitment are eliminated, as the survival of the species in the wild is assisted by the supplementation of captive reared juveniles (Maloney and Murray 2001). Further, sub-clinical bone deformity may reduce the survival of birds released to the wild.

The black stilt was once distributed throughout New Zealand but like many of New Zealand's endemic species, introduced predators and habitat degradation have led to a severe reduction in its natural distribution (Atkinson and Cameron 1993, Reed, et al. 1993, Craig, et al. 2000). The black stilt is now restricted to the braided rivers of the Upper Waitaki Basin, with its population reaching a record low of c.28 individuals in 1980 (Reed, et al. 1993). Due to the species' small population, hybridization with its close relative, the pied stilt (*H. h. leucocephalus*), is also having a detrimental effect on black stilt recruitment, as hybrids are fertile and interbreed with black stilts, pied stilts, and other hybrid stilts (Reed, et al. 1993). The instigation of the black stilt recovery and captive management plans has improved the status of the black stilt, leading to a recovery of the wild population with the most recent wild population count (28<sup>th</sup> August 2006) residing at a total of 182 black stilt, 100 being adults (E. Sancha, Department of Conservation, unpublished data). Early captive management practices had mixed success in stilt recruitment back to the wild (Reed, et al. 1993), but advancements in the development of the captive diet and husbandry techniques saw 115 stilts fledge in captivity during the 2005/2006 chick rearing season (Sancha, et al. 2006).



During the 2003/2004 and 2004/2005 chick rearing seasons at the Kaki Captive Breeding Centre, Twizel, a low incidence of long bone deformity was evident in captive reared black stilt chicks. This manifested as either an outward rotation of the wing (angel wing) or luxation of the gastrocnemius tendon from the condyles of the tibio-tarsus (slipped tendon or perosis) (B. Gartrell, Massey University, unpublished report).

Survival of the black stilt in the wild is assisted by supplementing the wild population with captive reared stock (Maloney 2003). The critically endangered species is in danger of declining to extinction without the continued management outlined in the black stilt recovery plans, in which captive management is an important aspect (Reed, et al. 1993, Maloney and Murray 2001). For this reason it is important that the stilts reared at the Kaki Captive Breeding Centre are of high quality to increase their chance of survival in the wild. The cases of angel wing and slipped tendon observed in captive reared stilts are of unknown aetiology, thus it is important to establish or eliminate possible causes, such as a manganese (Mn) deficiency.

A number of factors have been proposed as being linked to both angel wing and slipped tendon in captive reared avian species. These include a wide range of nutritional factors such as high protein intake (Serafin 1982, Macwhirter 1994, Smith 1997, Naldo and Bailey 2001, Bradshaw, et al. 2002, Flinchum 2006, Zsivanovits, et al. 2006), lack of exercise (Smith 1997, Klasing 1998a), over feeding (Flinchum 2006), vitamin deficiencies (Naldo and Bailey 2001, Flinchum 2006), and mineral deficiencies and imbalances (Wiese, et al. 1938, Wedekind and Baker 1990, Baker and Oduho 1994, Macwhirter 1994, Naldo and Bailey 2001, Flinchum 2006). Individuals may also be genetically predisposed to the conditions (Naldo, et al. 1998, Naldo and Bailey 2001), and it has been proposed that interruption during incubation and high temperatures during early rearing stages may contribute to limb abnormalities (Kear 1973, Naldo and Bailey 2001).

Mn deficiency has been highlighted as a common contributing factor in the displacement of the gastrocnemius tendon from the tibio-tarsus – tarso-metatarsus (hock) joint, known as



slipped tendon or perosis (Serafin 1982, Wedekind and Baker 1990, Macwhirter 1994, Klasing 1998c, Naldo and Bailey 2001). It has also been suggested, but not confirmed, as possibly contributing to the outward rotation of the carpo-metacarpus, also known as angel wing (Olsen 1994, Naldo and Bailey 2001, Flinchum 2006). Klasing (1998c) described Mn deficiency in growing chicks as causing shortened and thickened long bones, deformed hock joints and weakened cartilage. Mn is required for new skeletal muscle and other tissue growth over the maturation of connective tissues (Klasing 1998c); it is essential for cartilage development (Underwood and Suttle 1999). Mn is important for epiphyseal cartilage metabolism, in which its involvement with the biosynthesis of proteoglycans is most vital. Proteoglycans make up a major part of the cartilage extracellular matrix (Leach (1986) in Liu, et al. 1994). A deficiency of Mn reduces the total amount of proteoglycans and also causes qualitative changes in the ones present in the epiphyseal growth plate cartilage (Liu, et al. 1994). Proteoglycans provide hydration and swelling pressure to the tissue, which allow the tissue to withstand compressional forces (Yanagishita 1993), thus proteoglycans are essential to avoid deformation. Klasing (1998c) reports that a deficiency results in joint cartilage and associated tendons becoming weak in relation to the skeletal muscles pulling on them. Mn deficiency has been associated with the detachment of the gastrocnemius (Achilles) tendon from abnormal condyles of the hock joint, causing inflammation and twisting of the distal tibio-tarsus and proximal tarso-metatarsus. Klasing (1998c) reports that this disorder is common in captive raised waterfowl fed an *ad libitum* diet high in energy, protein and calcium (Ca) but deficient in Mn, and suggests that sufficient concentrations of dietary Mn may not prevent this limb disorder unless growth is slowed by daily food restriction.

Limb abnormalities in another of New Zealand's captive reared endangered species, the takahe (*Porphyrio [Notornis] mantelli*), has possibly been eliminated by the supplementation of potassium permanganate (KMnO<sub>4</sub>) solution to their captive diet (Eason and Willans 2001). From 1985-1994 a total of 10% of captive reared takahe chicks between the ages of 2-7 weeks old suffered from medial dislocation of one or both patellas (Eason and Willans 2001), the patella being associated with the tendon of the femoro-tibialis muscle (King and McLelland 1984). During the same period, some chicks also

developed constricted toe tendons.  $\text{KMnO}_4$  was added to their diet and following this, neither limb abnormality has reoccurred. It was suggested that they were caused by a Mn deficiency (Eason and Willans 2001).

The natural diet of the black stilt consists of aquatic invertebrates. Dominant species found at nest sites include mayfly larvae (*Deleatidium* spp.), midge larvae and beetle larvae (family Elmidae) (Marchant and Higgins 1993, Sanders 1997). Due to the high number of chicks raised each breeding season and the number of captive breeding adults held at the Kaki Captive Breeding Centre, it is not suitable to replicate this diet in captivity, and thus an artificial diet has been formulated. This currently consists of ox-heart mince, a commercial insectivore rearing mix, calcium carbonate and iodised salt (table 1). The current artificial diet has a Mn content of 27.9  $\mu\text{g/g}$  (diet DM) (Cottam, et al. 2004), whereas the Mn requirement of white-egg laying leghorn-type chickens between 0-6 weeks of age is 60  $\mu\text{g/g}$  (National Research Council 1994). Few studies have reported the nutritional composition of wild invertebrates, thus making it difficult to establish the nutritional requirements of insectivorous species. A nutritional analysis on mayfly larvae has been carried out for the Kaki Captive Breeding Centre (table 3). It needs to be emphasized that mayfly larvae only make up one part of the black stilt's natural diet, therefore the nutrition received from other dietary items such as, Elmidae beetle larvae, midge larvae and water sources, are unknown.

Table 1. Components of the captive mix fed to captive reared black stilt (Cottam, et al. 2004). \* Refer to table 2.

<b>Ingredient</b>	<b>Amt (%)</b>
Ox-heart mince	65.6
Wombaroo Insectivore Rearing Mix*	32.8
Iodised salt	0.1
Calcium carbonate	1.5



Table 2. Approximate composition of the Wombaroo Insectivore Rearing Mix/kg (Insectivore Rearing Mix, Wombaroo Food Products, Australia, unpublished data).

Ingredient	Rate	Ingredient	Rate	Ingredient	Rate
Protein	520g	Inositol	400mg	Phosphorus	11g
Fat	120g	Thiamine (Vit B <sub>1</sub> )	45mg	Potassium	5g
Fibre	50g	Riboflavin (Vit B <sub>2</sub> )	12mg	Sodium	2g
ME	19MJ	Nicotinamide (Vit B <sub>3</sub> )	150mg	Magnesium	1g
Retinol (Vit A)	240µg	Pantothenic Acid (Vit B <sub>5</sub> )	70mg	Iron	40mg
Cholecalciferol (Vit D <sub>3</sub> )	80µg	Pyridoxine (Vit B <sub>6</sub> )	15mg	Manganese	40mg
α-Tocopherol (Vit E)	70mg	Cyanocobalamin (Vit B <sub>12</sub> )	150µg	Zinc	22mg
Phytomenadione (Vit K <sub>1</sub> )	8mg	Folic Acid	5mg	Copper	4mg
Ascorbic Acid (Vit C)	150mg	Biotin (Vit H)	600µg	Iodine	600µg
Choline	600mg	Calcium	16g	Selenium	120µg

Table 3 Nutritional analyses of the artificial captive black stilt diet and mayfly larvae, N.Z. \*(Cottam, et al. 2004). \*\* (G. Guilford, Massey University, NZ, unpublished data). \*\*\* (W. Martin, Grasslands, New Zealand Department of Scientific and Industrial Research, unpublished data).

Ingredient	Artificial Captive Diet*	Mayfly Larvae
Protein	62%	45%**
Lipid	12%	15%**
Carbohydrates	16%	24%**
Ash	10%	3%**
Manganese	7.812 µg/g (fresh sample)	0.63 µg/g (fresh sample)***

The primary objectives of this research were to investigate the role of Mn in the incidence of limb abnormalities observed in captive reared black stilt by determining:

1. if Mn concentrations vary between stilts with and without limb deformities; and
2. if captive reared black stilt are deficient in Mn in to comparison wild parent reared hybrid stilts.

The secondary objectives of this research were to better understand the variables involved with Mn body stores. For this we examined the effect of age on bone and feather Mn, and wild and captive parentage on neonatal feather Mn. We also aimed to determine whether



feather Mn reflected skeletal Mn. A positive correlation would support a non-invasive technique to monitor Mn concentration and possibly other essential minerals in individuals, as it is not appropriate to use invasive techniques, such as liver or bone analysis, to monitor mineral concentrations in a critically endangered species.

## Methods

### Procedure

We took samples from the tibio-tarsus bone and feathers, from both fresh and archived (fixed in 10% formalin) black stilts held at the Institute of Animal, Veterinary and Biomedical Sciences, Massey University. We took feather samples from the remiges when available, but in stilts that had not yet developed these feathers (< seven days of age), the samples were of covert feathers from any tracks on their bodies. Where available, we took both bone and feather samples from the same stilt. We included seven hybrid stilts (offspring from a black stilt  $\times$  pied stilt pair or black stilt  $\times$  hybrid pair) in the analysis as no wild raised black stilt were available for the research. We also included two stilts with limb deformities, one with slipped tendon and the other with angel wing, to determine whether Mn concentrations varied between stilts with and without limb deformities. Samples ranged in age from hatching to 2.5 years of age. In total we analysed 42 bone samples and 42 feather samples in the analysis. Samples were assayed for Mn by a commercial laboratory (Hill Laboratories, Hamilton, New Zealand). Approximately 0.5 g of sample tissue, 2.5 mL of nitric acid [69%], and 0.5 mL of fuming hydrochloric acid [37%] were added to a clean 50 mL polypropylene capped tube. Tubes were swirled to ensure samples and acids were thoroughly mixed before being left to sit on a heating block to digest the samples at 85°C for one hour. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) determination (according to the methods described by Niu and Houk (1996)) was carried out using a Perkin Elmer Sciex Elan 6100 Dynamic Reaction Cell (DRC) II. This was calibrated at zero and 50 ppb for Mn and used internal standard correction to compensate for sample introduction and matrix effects. The intra-assay co-efficient of variation was 2.26%.

### **Statistical Analysis**

Data was analysed using SAS version 9.1 (2003). We determined whether 1. there was a linear correlation between feather and bone Mn in both captive reared black stilts and wild reared hybrid stilts using a Pearsons correlation. 2. captive hand raised and captive parent raised black stilt were deficient in Mn in comparison to the wild reared hybrid stilts using a one-way ANOVA and the post-hoc test, Tukey's Studentised Range Test. 3. Mn concentrations varied in the neonatal feathers of captive reared stilts with either captive or wild parents using a t-test. 4. neonatal feathers as being from chicks of seven days of age or less as determined by previous work we have carried out on the growth of captive reared black stilt. And 5. Mn concentrations change linearly as captive reared stilts age using a regression analysis.



## Results

### Manganese concentrations in captive reared black stilts with and without limb deformities

No statistical tests were carried out to test the significance of Mn concentrations between stilts with and without limb deformities due to the small sample size of stilts with angel wing and slipped tendon ( $n = 1$  for both groups). The sample size of stilts unaffected by limb deformities (bone Mn  $n = 31$ ; feather Mn  $n = 33$ ) was much greater than stilts with limb deformities, thus, a larger sample size of stilts with limb deformities would have allowed significance test to be carried out. Bone Mn appears to be similar between stilts without limb deformities ( $\bar{x}$  bone Mn = 6.41 mg/kg) and the stilt with angel wing (bone Mn = 7.35 mg/kg). The stilt with slipped tendon has a bone Mn concentration of greater than double that of either the stilt with angel wing and the stilts without limb deformities (bone Mn = 16.0 mg/kg) (figure 1). Mean feather Mn of stilts without limb deformities ( $\bar{x}$  feather Mn = 1.66 mg/kg) was similar to the stilt with angel wing (feather Mn = 0.54 mg/kg). No feather Mn data was available for the stilt with slipped tendon.

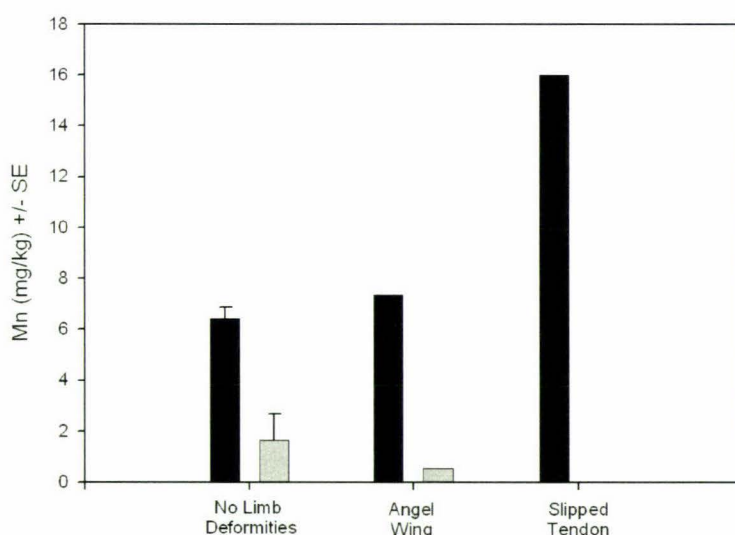


Figure 1. Bone and feather Mn in captive reared black stilt with and without limb deformities. Chicks without limb deformities is presented as mean Mn,  $\pm 1$  SE (bone Mn  $n = 31$ , feather Mn  $n = 33$ ). For both angel wing and slipped tendon  $n = 1$ ; note there was no feather Mn data available for the chick with slipped tendon. ■ = bone Mn; and ■ = feather Mn.

### Manganese concentrations in captive reared black stilt in comparison to wild reared hybrid stilts

Bone Mn did not differ significantly ( $P = 0.67$ ) between captive hand raised stilts ( $\bar{x} = 5.763$  mg/kg,  $n = 19$ ); captive parent raised stilts ( $\bar{x} = 6.20$  mg/kg,  $n = 4$ ) and wild parent raised hybrid stilts ( $\bar{x} = 6.714$  mg/kg,  $n = 7$ ) (Figure 2). The mean feather Mn of both captive hand raised ( $n = 21$ ) and captive parent raised ( $n = 5$ ) stilts was  $<1.0$  mg/kg. The Mn in the feathers of hybrid chicks was significantly greater than the Mn in the feathers of captive reared stilts ( $\bar{x} = 10.14$  mg/kg,  $n = 7$ ,  $P = 0.001$ ). Captive hand raised stilts included individuals aged zero days to 2.5 years of age, captive parent raised stilts included stilts  $< 32$  days of age, and wild parent raised hybrid stilts included individuals  $< 5$  days of age.

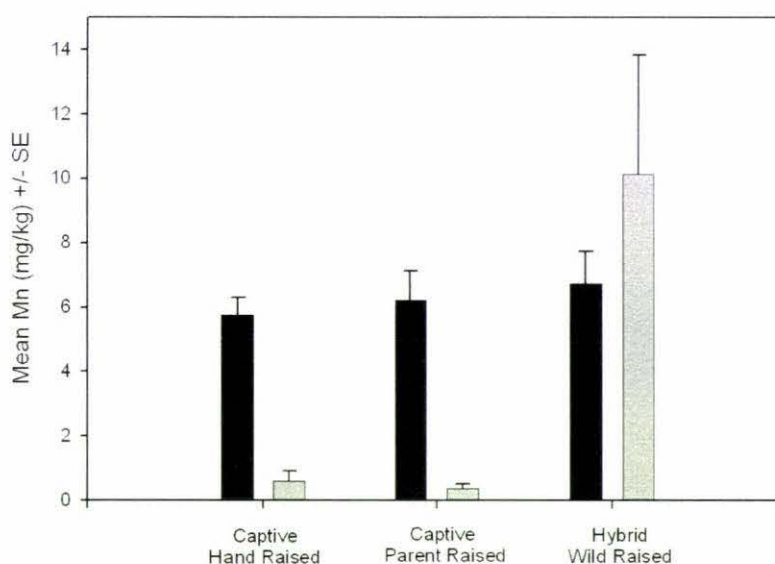


Figure 2. Mean Mn concentrations in captive reared black stilt in comparison to wild reared hybrid stilts,  $\pm 1$  SE. ■ = bone Mn; and ■ = feather Mn.

### Manganese concentrations as captive reared stilts age

There was no correlation between feather Mn and age ( $r^2 = 0.056$ ), one individual appears as an outlier at one month of age (feather Mn = 6.5 mg/kg) (figure 3). There was a low correlation with a small but definite relationship between bone Mn and age ( $r^2 = 0.345$ ) (figure 4). Bone Mn increases at a steady trend over the first 33 days of age, with the exception of a cluster of three outliers at approximately nine days of age ( $r^2 = 0.183$ )

(figure 5). Both feather and bone data sets have clusters of points at less than one month of age, if more data was available throughout the rest of the 16 month period observed, the positive correlations may have been stronger.

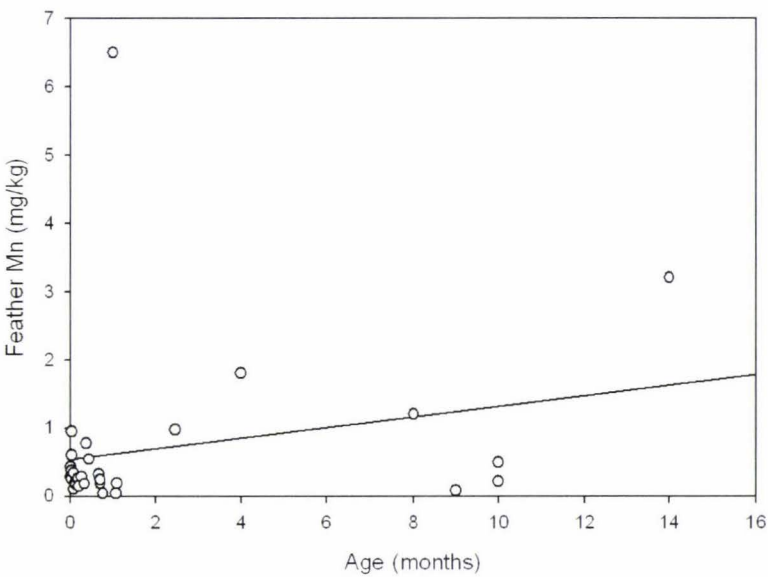


Figure 3. Correlation between feather Mn and age of captive reared black stilts over the first 16 months of age. Fitted with a linear regression line ( $r^2 = 0.056$ ).

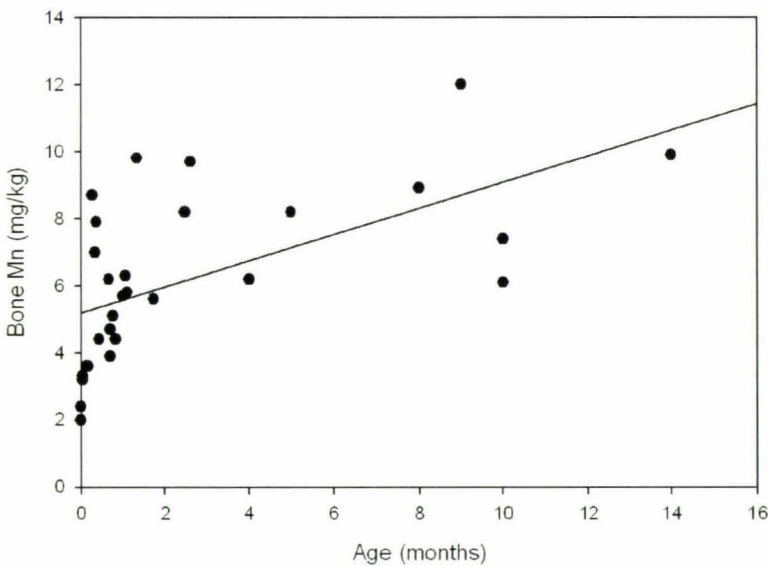


Figure 4. Correlation between bone Mn and age of captive reared black stilts over the first 16 months of age. Fitted with a linear regression line ( $r^2 = 0.345$ ).



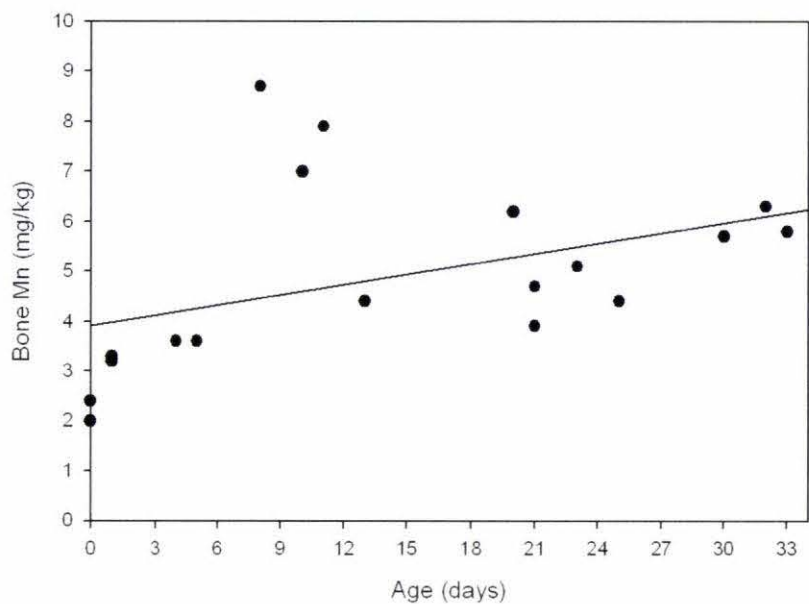


Figure 5. Correlation between bone Mn and age of captive reared black stilts over the first 33 days of age. Fitted with a linear regression line ( $r^2 = 0.183$ ).

**Manganese concentrations in neonatal feathers of captive black stilts**

Stilts with captive parents do not have a significantly higher mean Mn ( $P = 0.43$ ) in their neonatal feathers ( $\bar{x} = 0.398$  mg/kg,  $n = 6$ ) than captive stilts with wild parents ( $\bar{x} = 0.285$  mg/kg,  $n = 6$ ) (Figure 6).

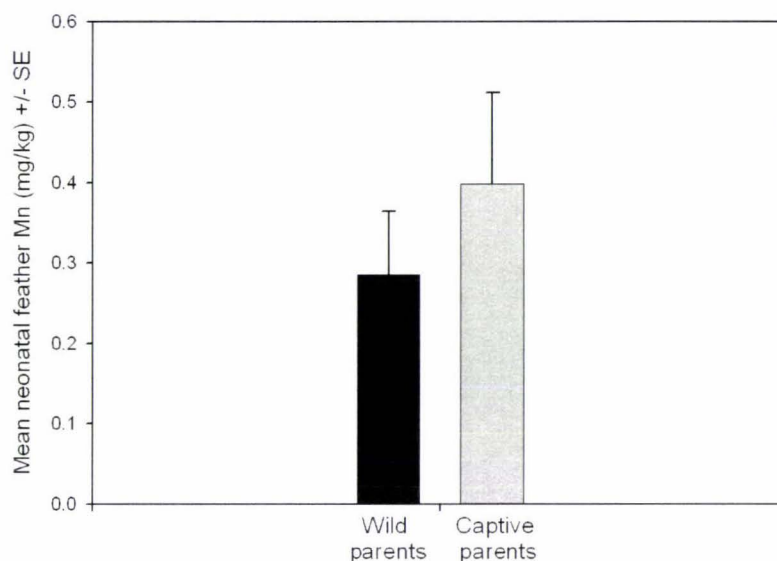


Figure 6. Mean Mn concentrations in neonatal feathers of captive raised black stilt with wild and captive parents,  $\pm 1$  SE.

**Correlation between feather and bone manganese**

There was a significant positive relationship between wild hybrid stilt bone and feather Mn (Pearsons correlation co-efficient = 0.8418,  $P = 0.0175$ ) (figure 7). This group included seven wild raised hybrid stilts < five days of age. There was not a significant relationship between captive reared black stilt bone and feather Mn (Pearsons correlation co-efficient = 0.3102,  $P = 0.1312$ ,  $n = 25$ ) (figure 8). This group included 25 captive reared black stilt aged between hatching to 2.5 years of age.

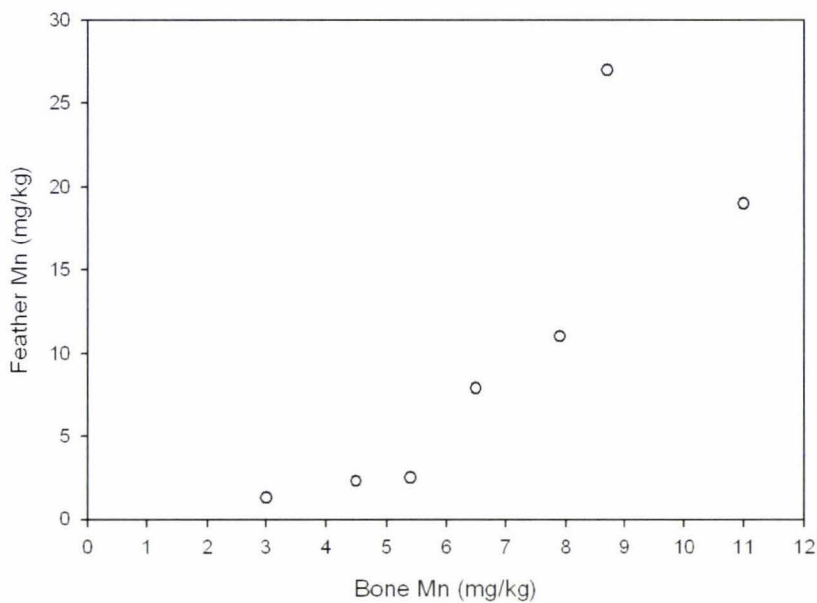


Figure 7. Correlation between bone and feather Mn (mg/kg) in hybrid stilts.

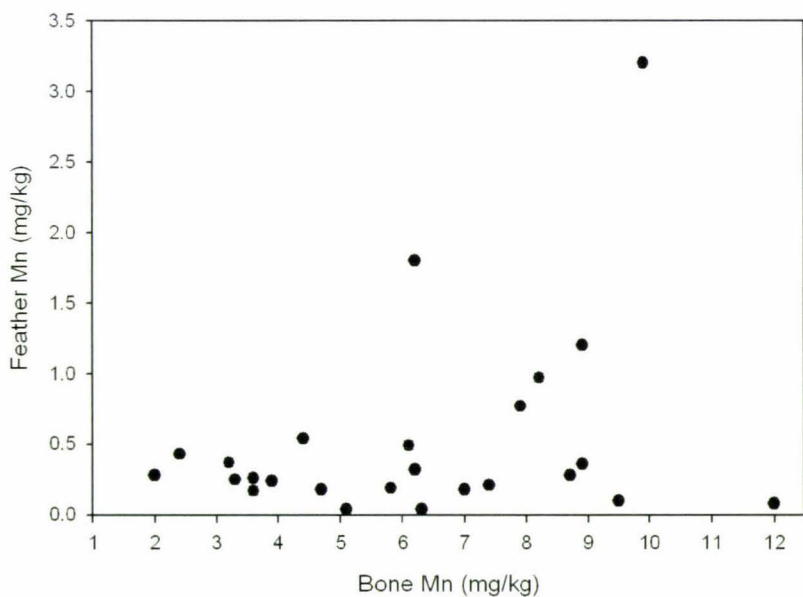


Figure 8. Correlation between bone and feather Mn (mg/kg) in captive black stilts.



## Discussion

This research found no evidence that Mn deficiency is contributing to the bone deformities in growing, captive reared, black stilts. Our results are significant in characterizing the differences in Mn deposition that occurs between species, or in our case, hybrids and pure black stilts. Our results suggest that using feathers to assess trace mineral components of avian diets (Hanson and Jones (1968) and Muller et al. (1984) in Altmeyer, et al. 1991) needs to be carefully validated for each species, as the black stilts showed no correlation between Mn concentrations in feather and bone, whereas a significant correlation was observed in the hybrid stilts.

Captive reared black stilts with limb deformities did not appear to be deficient in Mn in comparison to unaffected captive reared stilts. Statistical analysis was not appropriate due to the sample size of stilts with limb deformities, but the stilt with slipped tendon had bone Mn of greater than double that of the chick with angel wing and the mean of chicks without limb deformities. This may suggest that Mn deficiency is not responsible for the slipped tendon seen in captive reared black stilt. We can conclude that the elevated concentration of Mn observed in this individual is not likely to cause any toxicity effects as Klasing (1998c) reports that Mn is one of the least toxic minerals and is unlikely to cause toxicity at concentrations up to 1,000mg/kg. The individual that suffered from angel wing had similar bone Mn to the mean of the stilts without limb deformities. Its feather Mn was lower than the feather Mn of stilts unaffected by limb deformities, but within 1 SE of the mean. Lower feather Mn may indicate that this stilt may have been deficient in Mn at the time of feather formation, as feathers indicate element accumulation during the time of their formation (Altmeyer, et al. 1991). A larger sample size would provide less biased results. We could not confirm that a Mn deficiency is responsible for this case of angel wing, but if further cases of angel wing arise in captive reared black stilt, it may benefit to test Mn concentrations in order to eliminate this possible aetiology.

There was no significant difference between the bone Mn of captive hand raised black stilt, captive parent raised black stilt and wild parent raised hybrid stilts. This suggests that there

are sufficient concentrations of Mn in the artificial captive diet in relation to what the hybrids feed on in the wild. The artificial captive diet consists of 7.812µg/g Mn (fresh sample) (Cottam, et al. 2004), whereas mayfly larvae consist of 0.63µg/g (fresh sample) (W. Martin, Grasslands, New Zealand Department of Scientific Research, unpublished data). Mayfly larvae make up only part of the black stilt natural diet, and no nutritional analyses have been found on other components of their natural diet, such as Elimidae larvae or midge larvae. The Mn requirement of white-egg laying leghorn-type chickens between zero-six weeks of age is 60 µg/g (National Research Council 1994), but our study found no direct evidence that the lower dietary intake of black stilts is resulting in deficiency within the bone. Birds fed a purified diet have considerably lower Mn requirements, as there is an absence of factors that reduce Mn absorption (Klasing 1998c), such as high concentrations of phosphorus (P) (Wedekind, et al. 1991).

Bone Mn increased with age over the observed 16 month period. This may suggest that Mn is required in higher concentrations as adults for maintenance, than as juveniles during growth. It is of interest that over the first 33 days bone Mn increased at a steady rate, with the exception of the group of outliers at approximately 10 days of age. These data points may be outliers but there is also a possibility that Mn concentrations in growing chicks do not follow a linear relationship, as they may peak around 10 days of age as the individuals grow. Previous work we have carried out on bone histology indicates that around day 10, the proximal and distal ends of the tarso-metatarsus are growing at approximately similar rates. After this time period, growth of the distal end of the bone slows and ceases, before the proximal end of the tarso-metatarsus. These factors may suggest that at around 10 days of age, when the tarso-metatarsus is growing most rapidly, the Mn requirement may increase as it is vital for cartilage development (Underwood and Suttle 1999), thus accounting for the cluster of outliers at approximately 10 days of age. Previous work we have carried out on the growth rates of captive reared black stilt indicates that during a similar time period, 10-13 days of age, the carpal growth rate increases, therefore increasing cartilage formation, and possibly the Mn requirement of captive reared black stilt. Mn is important for epiphyseal cartilage metabolism, in which its involvement with the biosynthesis of proteoglycans is most vital. A deficiency of Mn reduces the total



amount of proteoglycans and also causes qualitative changes in the proteoglycans present in the epiphyseal growth plate cartilage (Liu, et al. 1994). Proteoglycans are important as they provide hydration and swelling pressure to the tissue in the cartilage extracellular matrix, which allows the tissue to withstand compressional forces (Yanagishita 1993), thus proteoglycans and therefore Mn are essential to avoid bone deformation.

Feather Mn did not show a strong trend with age. Dmowski et al. (1984) *in* (Altmeyer, et al. 1991) states that different pigmentations of feathers can alter the metal concentrations of different feathers from individual birds. With a larger data set, a stronger correlation between feather Mn and age may be demonstrated. If so, it may be possible that variation of feather Mn with age could be correlated with feather pigmentation, as stilt chicks have mottled brown plumage, juvenile and sub-adult black stilts have black and white plumage and adults have only black plumage.

There was no significant difference between the Mn concentrations in the neonatal feathers of captive reared black stilt with wild and captive parents. The ability of the laying mother to forage freely in the wild, in comparison to being restricted to foraging in an aviary and consuming the provided artificial captive diet, had no significant effect on the Mn concentrations in the egg and therefore the development of the hatchlings neonatal feathers. The concentration of Mn of the feathers of wild parent reared hybrid stilts was significantly greater than the concentration of Mn in the feathers of captive reared stilts. As discussed above, wild reared stilts have the ability to alter their diet in relation to nutritional needs, where as captive reared stilts are restricted to foraging in their aviary and the provided artificial diet. It therefore may be that dietary requirements i.e. Mn concentrations, are increased during feather development. It has been reported that there are differences in metal concentrations in different feathers of individual birds and also between different parts of the feather, the vane having higher mineral concentrations than the shaft (Altmeyer, et al. 1991). These factors may have influenced the results we obtained on black stilt feather Mn bioavailability. All feathers were submitted for analysis as whole feathers, but stilts of < seven days of age had a number of neonatal feathers submitted from all tracks, where as stilts greater than this age had a small number (depending on feather mass) of



primary wing feathers submitted. Differing numbers of feathers submitted from each individual may have influenced the Mn results in the feathers.

As there was not a significant correlation between bone and feather Mn in captive reared black stilt, we have established that using feathers as a non-invasive monitoring technique of Mn, and therefore most likely other minerals, would not be a suitable method to confirm concentrations in individuals. Feather Mn remained relatively constant as bone Mn increased, limiting its usefulness as a diagnostic aid. It may have been expected that there should be limited correlation between bone and feather Mn as the concentrations of minerals in a feather should be similar to that of the concentration in the body at the time the feather grew (Altmeyer, et al. 1991), whereas, Mn concentration in bone is a reliable indicator of the relative bioavailable Mn (Baker and Oduho 1994). In other avian species, such as penguins (Metcheva, et al. 2006) and the white-tailed eagle (Altmeyer, et al. 1991), feathers have been used as bioindicators of their environment. Feathers have been deemed as providing reliable information of element accumulation during the whole process of feather growth (Altmeyer, et al. 1991). This was not the case in black stilt as majority of our samples were < one month of age, the time in which feathers were undertaking rapid growth (as established from previous work we have carried out on captive reared black stilt growth rates), and feather Mn concentrations did not correlate to bone Mn, which is considered the most responsive tissue to dietary intake (Pond, et al. 2005). Differences in element accumulation have been related to molting patterns, changes of the diet during molting, different pigmentation and also external contamination (Goede and de Bruin (1984), Struger et al. (1987), Hanson and Jones (1968), Muller et al. (1984), Dmowski et al. (1984) and Weyers et al. (1988) in Altmeyer, et al. 1991).

In contrast to the black stilts, there was a significant strong positive correlation between the bone and feather Mn of wild reared hybrids stilts, suggesting that there are differences in the deposition of Mn in the feathers of hybrid stilts from those of black stilts. This may reflect that wild stilts have the ability to alter their diet to account for different nutritional needs during molts or feather development (Hanson and Jones (1968) and Muller et al. (1984) in Altmeyer, et al. 1991). During feather molt birds increase their requirement of

amino acids for synthesis of replacement feathers and also increase protein and energy requirements (Klasing 1998b, Klasing 1998d). The hybrid stilts in this analysis were all < five days of age. Therefore, the feathers submitted for analysis would have been produced from the nutrients in the egg and embryo, initially received from their parents. Klasing (1998c) states that the trace minerals are transferred to the hatchling from the liver of the laying female. The status of the trace minerals in the laying female determines the trace minerals transferred to the egg and storage for the hatchling. This suggests that the high concentration of Mn in the feathers of the hybrid chicks occurred as the mothers of the hybrid chicks had higher concentrations of Mn. This may be possible due to their parents change in dietary needs during egg production, or a difference in nutrient requirements of the mother (a hybrid, pied or black stilt, of which is unknown). It may also be possible that hybrid stilts show different patterns of Mn deposition into feathers from the black stilts.

As the aetiology of the two limb deformities of concern have not been confirmed, it is of importance to consider other possible factors that may have caused the abnormalities. Wedekind and Baker (1990) reported that in chickens, when P is fed at rates that reduce growth, Mn supplementation can partly overcome this effect, they went onto conclude that Ca and P can induce slipped tendon by increasing the Mn requirement, thus excess P intensifies Mn deficiencies (Baker and Oduho 1994). Wedekind et al. (1991) reports that excess Ca has no effect on Mn utilization in chickens. We found no evidence that Mn deficiency is contributing to the bone abnormalities in the black stilt but, we should be aware of the influence other minerals have on Mn utilization.

Other common causes of both slipped tendon and angel wing are a high protein intake, over feeding and lack of exercise (Serafin 1982, Macwhirter 1994, Smith 1997, Naldo and Bailey 2001, Bradshaw, et al. 2002, Flinchum 2006, Zsivanovits, et al. 2006). These factors can all be inter-related in a captive situation, so one single factor may be unlikely as causing the limb abnormalities in the black stilt. A high protein intake and over feeding will result in a faster than normal growth rate, and accompanied by being confined to a captive environment, likely resulting in a lack of exercise, will encourage increased weight gain. Rapid growth rates from a juvenile age can lead to musculoskeletal disorders such as



osteodystrophy (Mushi, et al. 1999) or result in weak proximal and distal portions of limb bones from weight stress, both resulting in deformation of joints, such as the hock joint (Serafin 1982), which may result in luxation of the gastrocnemius tendon. The rapid growth of the remiges in relation to muscle development also places excess stress on the wing resulting in outward twisting of the carpus (Flinchum 2006). Rapid growth rates in captivity resulting in increased weight stress on both the hock and wing joints, are a possible cause for the limb abnormalities observed in captive black stilts.

### **Management Implications**

We found no evidence that Mn has been a factor in the limb abnormalities observed in captive reared black stilt. Small sample sizes were the major limitation of our study. Low sample size is a problem which is frequently encountered when working with endangered species. the limited number of limb abnormalities also compounded this problem. A further limitation to our study was that the concentration of body Mn at the time of cartilage development when the growth abnormality was triggered, may be quite different to what it was at the time of our analysis. The captive diet appears sufficient in Mn as it contains much greater concentrations than that of mayfly larvae, a common natural food source of the black stilt. We would recommend carrying out a nutrient analysis on other natural food sources such as Elmidae and midge larvae to establish nutrients offered by other common natural food sources. We found that there was no correlation between bone and feather Mn in captive reared black stilt, therefore feathers can not be used as a non-invasive technique to monitor Mn bioavailability.

As bone Mn is a reliable index of Mn bioavailability, it seems likely that a Mn deficiency in captive reared black stilt can be eliminated as a potential cause of slipped tendon, as this individual had significantly higher bone Mn than stilts unaffected by limb deformities. We were unable to confirm the role Mn played in the incidence of angel wing in captive reared black stilt. Further research into the incidence of limb deformities in captive reared black stilt is recommended to confirm the aetiologies of both angel wing and slipped tendon.



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## **Chapter 5**

### **General discussion, implications for conservation and future research options**



Captive reared black stilt in the wild at the Ahuriri release site. Photo: Josie Galbraith.



## General discussion and implications for conservation

The cause of slipped tendon and angel wing in captive reared black stilt was not resolved by my research. I was successful in reducing body weight, carpal length and remex length, by food restriction, in captive reared juveniles by 31 days old. By 8-9 months old, after previously food restricted stilts had changed to an *ad libitum* diet at approximately 35 days, male sub-adults had not fully compensated in all aspects of subsequent growth, whereas females generally compensated in subsequent growth to greater than that of females previously on an *ad libitum* diet. Neither growth response resulted in any reported adverse effects, suggesting that stilts fed a restricted diet to slow growth during their rapid growth phase as a juvenile, have the ability to respond suitably to their needs for subsequent growth.

Both the physical growth measurements taken, and the histological documentation of bone maturation indicate that the tarsus is growing most rapidly over the first three weeks of age and the first phalanx, is growing most rapidly between approximately days 13-25. The most rapid growth of the carpus coincided with the initial rapid remex growth phase. These identified time periods are critical phases of growth, as the management team should be most aware of limb abnormalities developing during rapid growth periods (Kirkwood, et al. 1989a). Kirkwood et al. (1989a) proposed that rapid growth rates may be associated with deep cartilaginous growth plates, and this factor has been suggested as possibly contributing to the susceptibility of the growing tarsus in some bird species to develop twisting abnormalities. If faster than normal growth rates are encouraged in precocial species such as the black stilt, cartilaginous growth zones may be enlarged, which will result in reduced mechanical strength (Kirkwood, et al. 1989b) to support a heavier than normal chick. This may result in weight bearing skeletal structures, such as leg and wing bones, being more susceptible to deformity.

Partitioning of growth or development that occurs in a specific sequence in order to avoid growth abnormalities is an important aspect of growth (O'Connor 1984). The black stilts

tarsus grew at an approximately constant rate slowing slightly at approximately 25 days of age, and during the first two weeks of age, carpal and remex growth rates were minimal relative to tarsus growth. This is likely to be an adaptive growth response as the legs are needed for mechanical strength from hatch in precocial species, thus, the legs of precocial birds need to be more advanced in growth while other body parts such as the carpal and remex growth rates can be delayed. This growth strategy would allow bones to start maturing and flight muscles to strengthen before being exposed to the increased weight from emerging remiges (O'Connor 1984). It is important for management of the Kaki Captive Breeding Centre to be aware of the implications that the rapid growth observed in captive reared stilts may have on the disruption of their natural growth partitioning pattern, thus possibly resulting in weight related abnormalities.

I was unable to conclude that reduced growth rates eliminated the incidence of limb abnormalities in captive reared black stilt as only one limb abnormality was observed during the field season. The left wing, of the chick that developed the initial signs of angel wing during the field season, self corrected, as have most captive reared black stilt that have developed initial signs of the deformation in other chick rearing seasons (E. Sancha, Department of Conservation, personal communication). This chick was on an 80% dietary restriction but food restriction only commenced on day 16, which happened to be the first day that I noted its wing protruding from its body. It is possible that the dietary restriction implemented, reduced carpal and remex growth and contributed to the self correction of the wing abnormality by 22 days of age. To confirm this proposition, management would need to enforce further dietary restriction trials if angel wing arises again in the future.

Food restriction resulted in a significant increase in the time chicks spent foraging and interacting with each other. This factor has both positive and negative implications for management. Beneficial points include the encouragement of increased activity, thus possibly reducing the incidence of obesity, and abnormal, repetitive, or stereotypic behaviour, from developing in captive reared black stilt. These behavioural abnormalities have not been reported as problems in captive reared black stilt. Pododermatitis has been recorded as a common problem in captive black stilt (E. Sancha, Department of



Conservation, unpublished report), and has been related to obesity in captive poultry (Macwhirter 1994), penguins (Reidarson, et al. 1999) and raptors (Rodriguez-Lainz, et al. 1997, Muller, et al. 2000), therefore increased activity may result in reduced body weights and possibly a reduction in pododermatitis. Increased time spent foraging may be more natural, as stilts do not have a constant food supply in the wild, and thus would have to continue to forage for long periods of time to ensure adequate food intake. Management were concerned about the increased period of time and the severity of aggression observed in stilts on restricted diets. The small number of incidences where severe aggression was apparent, raised concern for the welfare of the receiving individuals. The aggression was not a long-term problem, as differences between individuals were generally sorted out within a couple of days. If food restriction was implemented as apart of management of captive reared black stilt in the future, aggressive problems may possibly be more easily resolved by providing extra food during aggressive situations (if dietary restriction research was not a factor to consider), as both our behavioural data and Mench (2002) found birds on *ad libitum* diets to be less aggressive.

I found no evidence that manganese (Mn) deficiency contributed to slipped tendon, as this individual had significantly higher bone Mn than stilts unaffected by limb deformities. I was unable to confirm the role Mn played in the incidence of angel wing. The captive diet appears to be sufficient in Mn as it contains much greater concentrations than that of the common natural food source of the black stilt, mayfly larvae. I also found that feathers are not an appropriate method to use as a non-invasive technique to monitor Mn bioavailability in black stilt.

In the 2003/2004 chick rearing season, two chicks were diagnosed with fibrous osteodystrophy, a metabolic bone disease associated with nutritional imbalances in the diet (Cottam, et al. 2004). In order to correct these imbalances, an analysis of the mineral content of the diet fed to juvenile chicks was undertaken. Following the mineral analysis, calcium carbonate was added at a rate of 1.5% (as fed) to increase the calcium (Ca) to phosphorus (P) ratio in the 2004/2005 chick rearing season, to make the diet more suitable for growing chicks (Cottam, et al. 2004). During the 2004/2005 chick rearing season,



chicks were fed chick-rearing poultry mash from a bowl from day 3 until day 11-12, when they started eating the Wombaroo mix (Sancha, et al. 2005). This was to ensure that chicks received good levels of Ca and a good Ca:P ratio while feeding during the transition phase from eating aquatic invertebrates (up until seven days old) to eating solely the Wombaroo mix (approximately 12 days old) (Sancha, et al. 2004, Sancha, et al. 2005). The introduction of a tray of chick-rearing poultry mash to the juvenile diet coincided with the development of the first incidences of angel wing in the black stilt. The wing abnormality, in the first stilt, developed as the feathers started emerging. The feathers began to protrude from the chicks body and appeared as a broken wrist so the individual was euthanased. The second stilt that developed the abnormality still had the ability to fly and was released into the wild as planned on September 9<sup>th</sup> 2005 (E. Sancha, Department of Conservation, personal communication). The increase in Ca in the chick diet may have resulted in a Ca:P imbalance. Imbalances can result in skeletal abnormalities (Doneley 2006), which may have possibly contributed to the incidences of angel wing in the black stilt.

I was unaware during our research season that the captive diet being fed to chicks omitted one component, the chick-rearing poultry mash, which was present during the previous season in which two cases of angel wing completely developed. As I have no growth measurements of chicks raised during the 2004/2005 season, I am unsure as to whether the chick-rearing poultry mash increased growth rates between days 3-12 of age and thus could have potentially contributed to the cases of angel wing observed.

An issue that arose during my research which hindered my data collection was the occurrence of chicks falling ill. Unwell individuals were removed from their brooder after falling ill. Once deemed well enough the individual was returned to their original brooder. If it had previously been on a restricted diet the entire brooder was removed from the dietary trial as feeding was changed to *ad libitum*. During our research season, the Kaki management staff noted that more hand raised black stilts became unwell than usual. In total, seven of the sick chicks had been on a dietary restriction. It may be possible that the chicks on restricted diets had elevated stress levels caused by increased competition for food between fellow brooder mates (Ness 2006), thus possibly resulting in a lowered

immunity (Dahlhausen 2006). Kaki management increased the use of the quarantine unit throughout our research season and found that it was feasible to treat unwell chicks in the unit for up to two weeks and return them to original brooders without aggression problems arising (Sancha, et al. 2006).

One limitation to the majority of my research, mainly to the data gathered on bone maturation and Mn concentrations is the limited sample size. As the black stilt is a critically endangered species, it was unfeasible to enlarge sample sizes as I had to rely on the limited specimens available.

The causes of angel wing and slipped tendon in captive reared black stilt were not resolved by our research. I have been able to eliminate a Mn deficiency as contributing to slipped tendon, but am unsure as to whether it may have contributed to angel wing. Rapid growth rates have neither been confirmed nor eliminated from contributing to the low incidences of both slipped tendon and angel wing. I successfully restricted stilt growth by food restriction and recommend that management consider restricting food if limb deformities become more frequent in captive reared stilts in the future. If limb abnormalities do not arise again in the black stilt, management still need to be aware of other implications of rapid growth rates, such as heart failure (Konarzewski, et al. 2000). Our research has also allowed pathologists to improve their understanding of tarsus and first phalanx maturation, thus giving them the ability to recognise skeletal abnormalities more confidently. I have developed growth models which will aid management in recognising possible growth abnormalities and immunocompromised chicks, as they can now refer to reference charts of normal chick growth. I have also identified the critical growth stage of black stilts in which limb problems related to rapid growth are likely to arise, thus indicating to management the time period of which they should be most aware of growth abnormalities occurring. I recommend that stilts should be approximately 175g before being transferred from the brooders. All of these factors have improved the knowledge and understanding of aspects of the black stilt, with the aim of contributing to increased health of captive reared stilts and the recovery of the wild population.



## **Future research options**

As the aetiologies of angel wing and slipped tendon were not determined during my research, further exploration into the possible causes of these skeletal abnormalities in captive reared black stilt should be investigated. Avenues of research that would be beneficial to proceed with in relation to the possible causes of slipped tendon and angel wing include:

### **1. Further research into rapid growth**

As rapid weight gain was not eliminated as a possible cause of the limb deformities, further research into the relationship between the growth rates of captive reared black stilt and the incidence of angel wing and slipped tendon should be undertaken. The key focus should be on the body weight, and the tarsus, carpal and remex lengths of effected stilts and the quantity of food consumed during the critical growth period. Other bones that may be of importance to follow their growth rate include: the humerus, ulna/radius bones, and the tibio-tarsus, as they are likely to be associated with limb deformities. If angel wing continues to arise in captive reared stilts, I recommend further research into food restriction, as it possibly contributed to the self correction of the deformity during our research season.

### **2. Exercise**

Lack of exercise is another possible cause of rapid growth rates in captive reared avian species (Klasing 1998). Increased exercise is an option which could potentially reduce energy levels and thus reduce growth rates of the captive reared black stilt. An increase in activity level during rapid growing periods may increase the metabolisable energy allocated to maintain activity, leading to a decrease in the energy allocated to growth, because growth is of lesser importance for survival (Schew and Ricklefs 1998). Bradshaw *et al.* (2002) reported that increased activity may have helped to decrease growth rates in broilers and thus helped to reduce leg problems. The same principle may apply to the black stilt as they are both precocial species.



The dusky parrot (*Pionus fuscus*) is an altricial species that does not normally exhibit exercise until they leave the nest. Harcourt-Brown (2004) reported that too much exercise at a young age may contribute to skeletal malformations in hand-reared parrots. Harcourt-Brown (2004) noted that when removing individual dusky parrots from their nest for photographing, the chicks would stand up and walk around, but when returned to their nest it would resume normal huddling behaviour. An altricial bird normally has no need to walk or fly during initial growth phases as it is confined to the nest. The strength of their bones would therefore not be expected to be strong enough to support their weight (Harcourt-Brown 2004). Hand reared parrots are generally friendly and active from a young age and are therefore exposed to an abnormal amount of increased movement, thus predisposing immature growing leg bones to uncharacteristic weight bearing activities (Harcourt-Brown 2004). Precocial birds, such as the black stilt, would be expected to react differently from altricial species to activity from a young age, as precocial species hatch with relatively advanced development and have the ability to forage for themselves, thus their leg bones should be strong enough to support weight bearing activities and exercise at a young age (Harcourt-Brown 2004), therefore too much exercise at a young age is unlikely to have adverse effects on black stilt skeletal growth. However, not enough exercise should be further examined as my behavioural observations found stilts on an *ad libitum* diet to be more sedentary than stilts on a restricted diet.

### **3. Natural diet**

I feel detailed research into the nutrient composition of the natural diet of the black stilt is necessary to gain full understanding of their nutritional requirements, and thus provide possible leads into further nutritional research that should be carried out in order to possibly diagnose the cause of the limb abnormalities observed in the black stilt. A nutritional analysis has been carried out on mayfly larvae (G. Guilford, Massey University, NZ, unpublished data, W. Martin, Grasslands, New Zealand Department of Scientific and Industrial Research, unpublished data), the main component of their diet (Pierce 1986, Sanders 1997), but it would be beneficial to carry out analysis on other aquatic invertebrates they eat such as midge and Elmidae beetle larvae (Sanders 1997), and water sources and substrates in their environments. It may also be beneficial to analyse the

nutritional components of the gut of archived wild black stilt, in order to establish exactly what they are consuming in their wetland habitats. Faecal analysis both black and pied stilts and autopsies of pied stilt have been used to establish the nature of the *Himantopus* sp. diet (Pierce 1982), but it would be beneficial to carry out a nutritional analysis of the gut of black stilt, as they are a closely related, but a different species to the pied stilt. Key components of interest include protein, fat, mineral and vitamin content of components of the natural diet.

#### **4. Utilization of manganese**

If Mn is being provided at a suitable concentration in a diet, it may be possible that it is not being utilized as expected. Wedekind and Baker (1990a) found that in chickens, the most efficient absorption of Mn occurs when it is at lower dietary levels than when it is present at an excess, due to homeostatic mechanisms. It may be possible that Mn in the captive diet is in excess (7.812 µg/g fresh sample (Cottam, et al. 2004)), as Mn in the stilts common, natural food source, mayfly larvae, it is only 0.63 µg/g (fresh sample) (W. Martin, Grasslands, New Zealand Department of Scientific and Industrial Research, unpublished data). Excess phosphorus reduces Mn utilization (Wedekind and Baker 1990a) and gut absorption of Mn (Wedekind, et al. 1991). Wedekind and Baker (1990b) have suggested that excess P may possibly contribute to a decrease in Mn uptake by avian bone, therefore bone Mn in the stilts may be lower than that of Mn dietary intake, but should be a reliable indicator of Mn bioavailability. It may be possible that Mn is provided in an appropriate amount, and excess P is inhibiting successful utilization of the mineral.

#### **5. Genetic pre-disposure**

Black stilt may be genetically pre-disposed to the abnormalities through inheritance from their parents. This seems unlikely for slipped tendon as only two cases have been reported, but a possibility for angel wing as two stilts have developed permanent wing deformation and 2-3 chicks a year from the past three chick rearing seasons, have developed initial signs before self correcting the problem (E. Sancha, Department of Conservation, personal communication). In 1980 the black stilt population reached a low of c.28 birds (Reed, et al. 1993), resulting in a limited gene pool, thus leading to possible inbreeding and the



possibility of exposing detrimental recessive genes (Wallis 1999), genes that may have possibly contributed to the limb deformities observed in captive reared black stilt. The low number of black stilt has also enhanced interspecific breeding with the pied stilt resulting in hybrid offspring (Reed, et al. 1993, Wallis 1999). Hybrids are coded according to their genetic closeness to the black stilt. Dark hybrids considered to contain significant amounts of black stilt genetic material (nodes G, H and I) are managed as black stilts (Reed, et al. 1993). These dark hybrid stilts may have contributed to health problems seen in captive reared black stilt as hybrid stilts have reduced fitness (Wallis 1999). Research into the parentage lines of the captive reared stilts that developed skeletal deformities should be considered.

## **6. Stocking density**

A factor discussed by Bradshaw *et al.* (2002) that has been related to leg disorders is stocking density. They state that there is evidence for high stocking levels to negatively affect bird growth and quality. This is therefore another factor that management of the Kaki Captive Breeding Centre should be aware of, as black stilt chicks are housed in groups of 2-6 individuals.

## **7. Temperature influences**

Temperature influences have also been suggested as a possible cause for both slipped tendon and angel wing (Kear 1973, Mushi, et al. 1999, Naldo and Bailey 2001). It seems unlikely that temperature influences during incubation stages would have resulted in the observed limb deformities as over 100 eggs are incubated each season (Sancha, et al. 2004, Sancha, et al. 2005, Sancha, et al. 2006), and only a minute proportion of individuals have developed abnormalities. White (1985) in Naldo and Bailey (2001) reported that excessive heat during early growing periods may possibly contribute to angel wing. The region in which the Kaki Captive Breeding Center is located, receives extreme temperatures of up to 35°C (January 2006 Tara Hills Research Station, Omarama, NIWA, unpublished data) during the chick rearing season (chicks are exposed to outside temperature after seven days of age), therefore extreme temperature influences during early rearing stages should not be eliminated from causing the limb abnormalities in captive reared black stilt.



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## Appendices



Juvenile black stilt bathing in a brooder pond. Photo: Josie Galbraith.

Appendix 1.

Approximate composition of the Wombaroo Insectivore Rearing Mix per kg (Insectivore Rearing Mix, Wombaroo Food Products, unpublished data).

Ingredient	Rate	Ingredient	Rate	Ingredient	Rate
Protein	520 g	Inositol	400 mg	Phosphorus	11 g
Fat	120 g	Thiamine (Vit B <sub>1</sub> )	45 mg	Potassium	5 g
Fibre	50 g	Riboflavin (Vit B <sub>2</sub> )	12 mg	Sodium	2 g
ME	19 MJ	Nicotinamide (Vit B <sub>3</sub> )	150 mg	Magnesium	1 g
Retinol (Vit A)	240 µg	Pantothenic Acid (Vit B <sub>5</sub> )	70 mg	Iron	40 mg
Cholecalciferol (Vit D <sub>3</sub> )	80 µg	Pyridoxine (Vit B <sub>6</sub> )	15 mg	Manganese	40 mg
α-Tocopherol (Vit E)	70 mg	Cyanocobalamin (Vit B <sub>12</sub> )	150 µg	Zinc	22 mg
Phytomenadione (Vit K <sub>1</sub> )	8 mg	Folic Acid	5 mg	Copper	4 mg
Ascorbic Acid (Vit C)	150 mg	Biotin (Vit H)	600 µg	Iodine	600 µg
Choline	600 mg	Calcium	16 g	Selenium	120 µg



## Appendix 2

Images of taking measurements of the tarsus, carpal and remiges. Photo's: Emily Sancha.

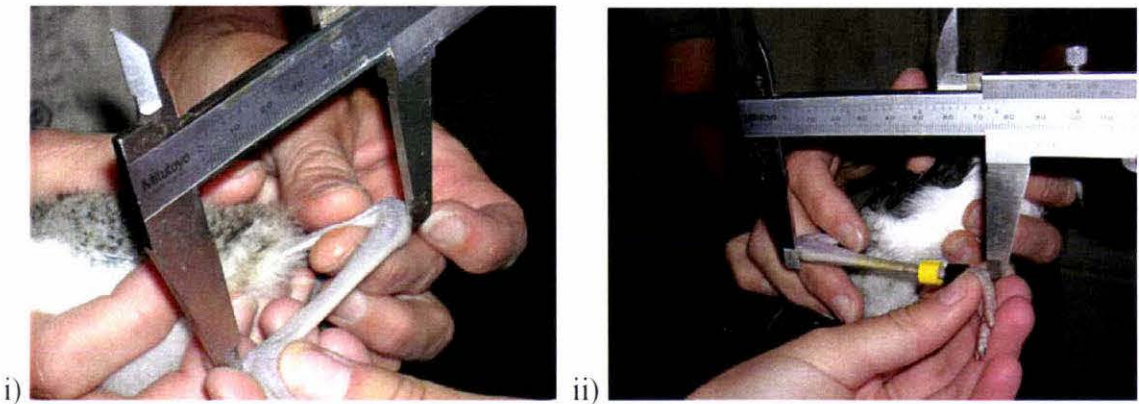


Fig. 1. Measuring the tarsus from the distal end of the bone with the foot contracted, to the cotyles of the tarso-metatarsus when this joint was in a flexed position on i) a black stilt aged 19 days of age and ii) a black stilt aged 28 days of age.

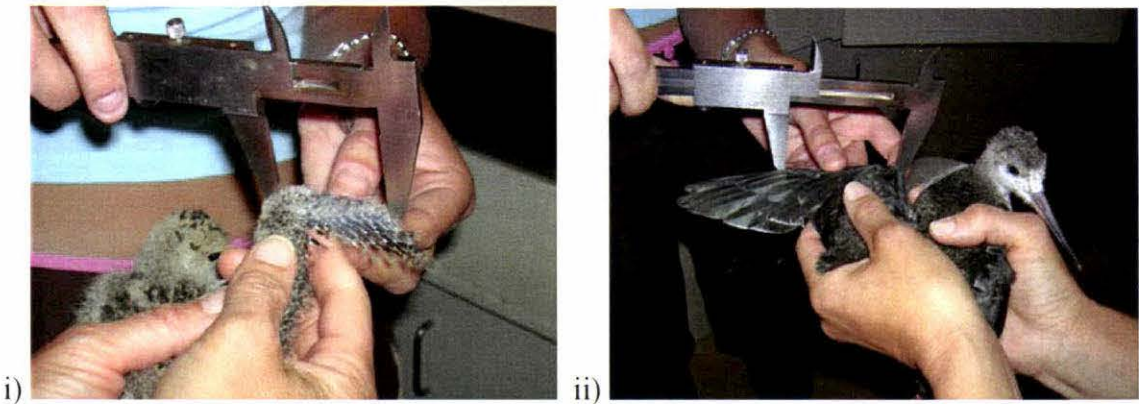


Fig. 2. Measuring the carpal from the tip of the carpo-metacarpus to the ridge of the carpo-metacarpal- radio-ulnar joint on i) a black stilt aged 19 days of age and ii) a black stilt aged 28 days of age..



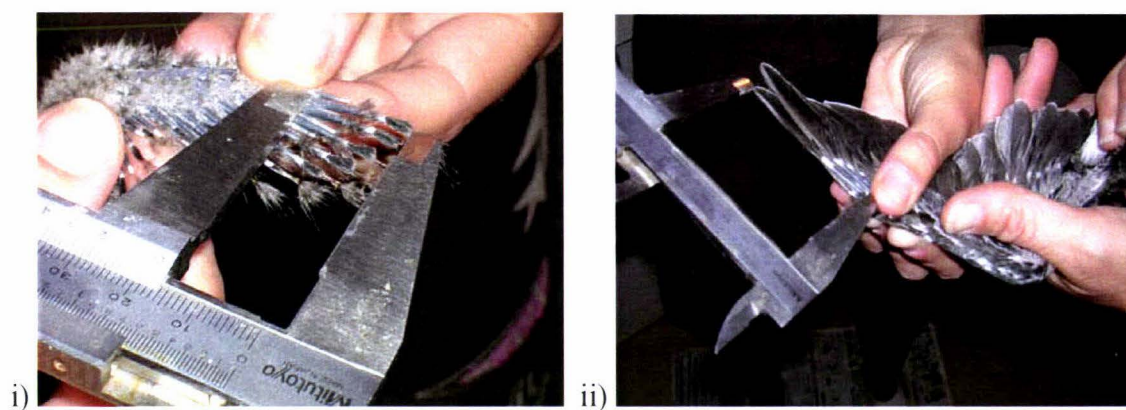
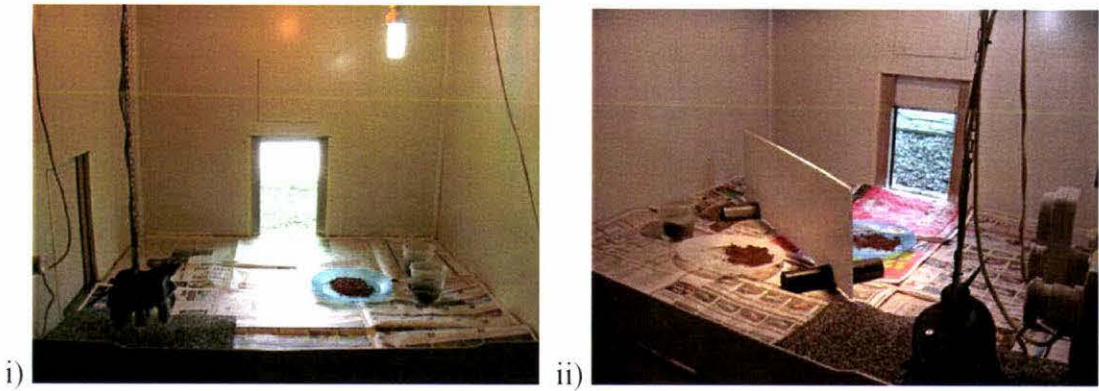


Fig. 3. Measuring the first remex from the point in which the feather shaft emerged from the skin to the tip of the feather on i) a black stilt aged 19 days of age and ii) a black stilt aged 28 days of age.

### Appendix 3.

Images of brooder setup. Photo's: Bridget Wrenn.



i) Normal brooder feed setup ii) Brooder feed setup for restricted feed groups that developed aggressive behaviour towards other chicks within their brooder.

## Appendix 4.

Composition of Kiwi Premix, Carlyle Veterinary Clinic, Napier. Daily dose based on 0.16 g/stilt/day (Cottam, et al. 2001)\*.

Active Ingredient	Daily Dose Per Stilt	Units	Source Material
Calcium	46.3	mg	Calcium carbonate
Vitamin A	123.5	iu	Rovimix A 500
Vitamin D <sub>3</sub>	9.3	iu	Rovimix D <sub>3</sub> 400
Vitamin B <sub>12</sub>	0.0004	mg	Vitamin B <sub>12</sub> conc.
Vitamin E <sub>25</sub>	0.93	iu	Rovimix E <sub>25</sub>
Thiamine	0.068	mg	Thiamine hydrochloride
Riboflavin	0.046	mg	Riboflavin
Ferrous Iron (Fe <sup>++</sup> )	0.386	mg	Ferrous fumarate
Vitamin C	0.772	mg	Ascorbic acid
Vitamin K	0.062	mg	Menadione sodium bisulphate
Vitamin H	0.003	mg	Biotin 1% (Vit H%)
Zinc	0.39	mg	Zinc oxide
Manganese	0.39	mg	Manganese sulphate
Copper	0.039	mg	Copper carbonate
Iodine	0.0039	mg	Potassium iodate
Selenium	0.0023	mg	Selenium stock conc.
Cobalt	0.004	mg	Cobalt carbonate
Pyridoxine	0.039	mg	Pyridoxine hydrochloride
Nicotinic acid	0.309	mg	Nicotinic acid
Pantothenic acid	0.232	mg	Calcium D-pantothenate 80%
Choline	5.79	mg	Choline chloride 50%
Folic acid	0.015	mg	Folic acid
Inositol	0.154	mg	Inositol
BHT	0.15436	mg	Butylated hydroxy toluene

\*Cottam, Y., Hendriks, W. and Sancha, E. 2001. Captive diet of New Zealand black stilt held at Twizel. Conservation advisory science notes 337. - Wellington, Department of Conservation.