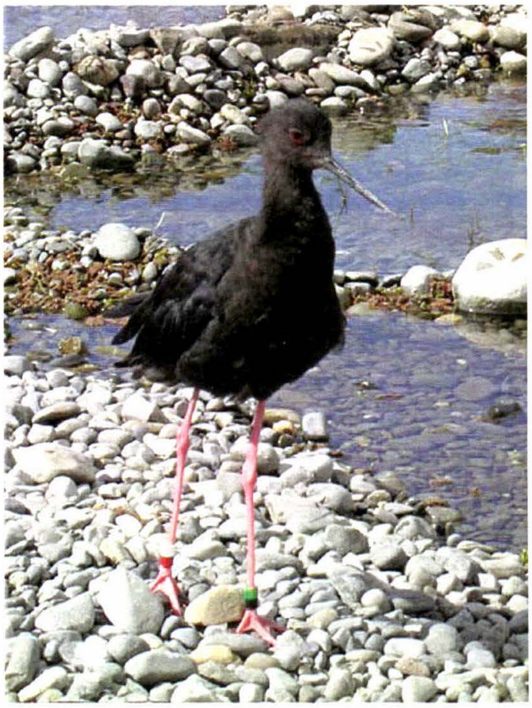
Limb growth and development in the endangered, captive reared, black stilt (*Himantopus novaezelandiae*)



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

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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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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 (28th 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_3 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_3, 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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