Investigating the Physiological Impacts of Capture and Handling on Threatened Avian Species by Using Surrogate Species as Models

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Abstract

The conservation management of many threatened species requires the capture and handling of wild individuals for monitoring, translocation or research purposes. However whenever wild animals are captured and handled there is the potential for these procedures to negatively impact the animal and result in altered behaviour or physiology, injury and even death. Therefore this thesis aimed to investigate what physiological impacts routine capture and handling may be having on threatened avian species in New Zealand by using surrogate species of birds as models for threatened birds.

Layer hens (*Gallus domesticus*) were used as surrogates to model the physiological impacts of capture and handling on kiwi (*Apteryx spp*.). A treatment and control group of hens were serially blood sampled over 72 hours. Hens in the control group were placed in a box between blood samples and hens in the treatment group went through a simulation of a kiwi chase, capture and handling scenario. After 72 hours all birds were euthanized and their muscles examined histopathologically.

Wild pūkeko (*Porphyrio porphyrio melanotus*) captured using a net-gun at the Awapuni Sustainable Development Centre in Palmerston North were used as surrogates to model the physiological impacts of capture and handling on takahē (*Porphyrio hochstetteri*). Wild mallard ducks (*Anas platyrhynchos*) captured using a net-gun at Massey University's Turitea campus were used as surrogates to model the physiological impacts of capture and handling on threatened waterfowl such as pateke/brown teal (*Anas chlorotis*), or whio/blue duck (*Hymenolaimus malachorhynchos*). All mallards and pūkeko captured were serially blood sampled at capture (0 minutes), 30 and 120 minutes. Within each species there was a control group that was held in a box between samples and a treatment group which was handled according best practice protocol for takahē (for pūkeko) or pateke (for mallards). A further group of pūkeko was also shot using a rifle as comparison.

To assess the physiological impact of capture, biochemical analytes measured included plasma concentrations of the enzymes creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and the stress hormone corticosterone (CORT). In mallards and pūkeko capture using the net-gun the plasma concentrations of uric acid (UA) were also measured.

Capture was found to elicit a stress response in all three of the species studied as shown by elevated plasma CORT; however there were differences between species on the effect of capture on plasma CK, AST, GLDH and UA. The handling protocol was found to have minimal impact on the physiological response of any of the species and the impact of capture either overrode the effects of handling or handling protocol was simply not a significant factor on any of the biochemical analytes measured.

Layer hens were found to have altered physiology at the commencement of the study, probably due to the high metabolic demands of egg production. There was also significant variation in their 'normal' physiology and physiological response between the two weeks they were studied. Layer hens are therefore considered to be inappropriate surrogates for kiwi or any wild bird.

Baseline levels of the biochemical analytes of pūkeko that were captured using a net-gun and those that were shot were similar. The time of day the pūkeko were captured caused significant variation in the concentration of plasma GLDH and UA. Capture did cause significant elevations in plasma CK and AST showing subclinical muscle damage was occurring in the pūkeko and this damage and the stress response was greater when the pūkeko were captured in flight. Capture also had a significant if less clearly defined impact on renal and gastro-intestinal physiology.

Seasonal variation and some time of day variation were observed in the concentration of CK in mallard ducks. While capture caused a significant stress response in captured mallards it did not have a significant effect on CK, GLDH or UA. Plasma AST concentrations decreased significantly following capture albeit by a very small amount.

The difference found between species in their physiological response to similar procedures highlights that surrogate species may not be appropriate and validation between the surrogate and threatened species is required. Small differences in the capture technique may have a significant impact on the animal's physiological response. In conclusion the handling protocol has a minimal physiological impact on these birds following capture and further research should focus on capture techniques and protocols. If surrogate species are used for further research then there should be some attempt to validate that the physiological response observed is similar in the threatened species.

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I dedicate the following work to my late Grandparents, and thank them for their love and support throughout my life and for the many pictures and videos about birds and nature that helped sparked my interest in the natural world.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 CAPTURE AND HANDLING OF THREATENED SPECIES FOR CONSERVATION

The conservation management of many threatened species require the capture and handling of wild individuals for monitoring, translocation or research purposes. While indirect sampling and monitoring are preferred, there is certain data that can only be obtained by the capture and handling of an animal (Powell & Proulx, 2003) and certain management procedures that can only be performed by the capture and handling of an animal (O'Connor *et al.*, 2007; Holzapfel *et al.*, 2008; Wickes *et al.*, 2009; Glaser *et al.*, 2010). Translocations are a valuable tool in conservation which involves the relocation of animals to new areas to start new or re-establish extirpated populations or to supplement existing populations (Anonymous, 1987). In New Zealand translocations are a critical tool in the conservation management of threatened taxa (Armstrong & McLean, 1995).

Animals used in translocations may be captured from the wild or sourced from captive breeding programs (Holmes & Caskey, 2001). Captive populations of species that have been established for breeding programmes, as insurance populations, or for advocacy may also require capture from within the enclosure and handling for various procedures or monitoring (Gummer & Evans, 2003; Bassett, 2012). While these procedures may be required, capture and handling techniques have the potential to injure, alter normal physiology or behaviour and even kill the animals in question (Southern & Southern, 1983; Kreeger *et al.*, 1990; Williams & Thorne, 1996; Cattet *et al.*, 2008; Jacques *et al.*, 2009). Therefore we should investigate current and new techniques for capturing and handling wildlife in order to explore how the techniques affect wildlife and how/if we can improve them. This is important for animal health, welfare and because of the potentially confounding affect it may be having on research.

The capture and handling of wildlife may also be required as part of the emergency response management of species. In environmental disasters such oil spills, the pre-emptive capture of threatened species or the capture of sick animals for treatment may be required (Massey, 2006; Wolfaardt *et al.*, 2009). It is important that capture and handling techniques have a minimal impact on the animals as the animals pre-emptively captured are likely to be highly threatened species or animals with already compromised health.

The impact of capture and handling is particularly important when it involves highly threatened species with small populations because the health of each individual has that much more conservation value and the individuals captured often belong to the most at risk populations. Additionally if capture and handling has an impact on survival, body condition or breeding then it may bias data and result in inappropriate management decisions (Williams & Thorne, 1996). In New Zealand many of the threatened birds' recovery plans require wild or captive birds to be captured and handled as of part of their management (O'Connor *et al.*, 2007; Holzapfel *et al.*, 2008; Wickes *et al.*, 2009; Glaser *et al.*, 2010). The impact of the procedures used on New Zealand birds should be assessed and if necessary refined to ensure that they are minimally affecting the animals and thus not hindering conservation goals.

1.2 CAPTURE IMPACTS

1.2.1 Capture Myopathy

Capture myopathy is a condition where the stressful nature of capture and the associated struggling and exertion of animal during the process, can cause degenerative or necrotising damage to the animals' muscles (Williams & Thorne, 1996). Capture myopathy is the most common term used to describe the disease but it is known under a range of names including but not limited to exertional rhabdomyolysis, stress myopathy, leg paralysis, overstraining disease, white muscle stress syndrome, capture disease and even simply referred to as 'cramp' in waders (Basson & Hofmeyr, 1973; Green, 1980; Minton, 1993; Spraker, 1993; Williams & Thorne, 1996; Herraez *et al.*, 2007). It is thought to be a natural occurring phenomenon that animals may experience during events such as predator-prey interactions (Spraker, 1993; Williams & Thorne, 1996). However as far as conservation management is concerned this condition is caused during capture, handling and transport procedures. The pivotal factor in the development of the disease it thought to be physiological stress and while the condition may occur in the absence of over-exertion, exertion is considered to still be a significant factor (Basson & Hofmeyr, 1973).

A wide range of species across the vertebrate groups are susceptible to capture myopathy and preventing its occurrence should be one of the most important considerations when capturing wild animals (Williams & Thorne, 1996). Capture myopathy has been documented in a range of avian species including: wild turkey (*Meleagris gallopavo*), (Spraker *et al.*, 1987); red-

legged partridge (*Alectoris rufa*), (Hofle *et al.*, 2004); whooping cranes (*Grus americana*), (Hanley *et al.*, 2005); little bustard (*Tetrax tetrax*), (Marco *et al.*, 2006); greater sandhill cranes, (*Grus canadensis tabida*), (Businga *et al.*, 2007); razorbills (*Alca torda*), (Czujkowska et al., 2009); bar-tailed godwits (*Limosa lapponica baueri*), (Ward *et al.*, 2011) and pileated woodpeckers (*Dryocopus pileatus*), (Ruder *et al.*, 2012). The behavioural and physical characteristics of some species may make them more susceptible to capture myopathy (Basson & Hofmeyr, 1973), although this may due to the particular capture and handling techniques that are typically used with these species (Williams & Thorne, 1996). Under the appropriate conditions involving capture, stress and/or exertion it is likely that capture myopathy could develop in any or all avian species (Williams & Thorne, 1996).

The condition can either be considered to be made up of four discrete forms (capture shock, ataxic myoglobinuric, ruptured muscles, or delayed-peracute) based on different pathophysiology between the syndromes (Spraker, 1993), or the condition can be considered as a continuum and classified into four stages (hyperacute, acute, subacute or chronic) based on pathological and physiological changes over time following capture (Harthoorn, 1973 as cited by Williams & Thorne, 1996). What is clear is that the clinical condition may be fatal and can occur soon after capture or in the hours, days or even weeks after the capture event (Basson & Hofmeyr, 1973; Spraker, 1993). Capture myopathy may also occur at a subclinical level where even in the absence of clinical signs, large numbers of animals may experience some muscle damage following capture and handling (Spraker *et al.*, 1987). This is shown by gross or microscopic lesions in the skeletal muscle or cardiac muscle. However the term subclinical may not always be entirely accurate because some of these animals may go on to develop clinical signs later on (Spraker *et al.*, 1987).

The earliest signs of capture myopathy are those that may be expected after capture and handling such as increased respiratory and cardiac rates and often elevated body temperature. Early clinical signs may include unsteadiness, muscle stiffness, tremors, wing cramp, ataxia, fever, a lack of response to stimulus or an inability or reluctance to move normally. These symptoms may develop in the hours or days following capture and may progress to recumbency, become insensitive to the environment, paresis, paralysis and even death. Other signs may include dark coloured urine due to the presence of myoglobin from the damaged muscle and weight loss in chronically affected animals (Basson & Hofmeyr, 1973; Bartsch *et al.*, 1977; Spraker, 1993; Williams & Thorne, 1996).

The exact pathophysiology of capture myopathy may depend on a number of factors such as the species, techniques used, stress experienced by the animal and exertion experienced by the animal. It is a dynamic and complex process but the central idea is that stress and exertion of capture and/or restraint causes many biological mechanisms operating to maintain homeostasis in an emergency to fail. These can include a build-up of heat and lactic acid in the muscles which results in metabolic or localised acidosis. The causes increased cell permeability and lysis resulting in the degeneration and necrosis of muscular tissue. The initial damage to muscle tissue may cause localised edema which increases local pressure and promotes ischemia in the muscles which may cause more damage. Secondary to this the myoglobin released from the damaged muscles may cause renal tubular necrosis which may lead to renal failure (Bartsch *et al.*, 1977; Spraker, 1993; Williams & Thorne, 1996).

As mentioned clinical signs of capture myopathy may not be present and without assessment by clinical pathology or histopathology the condition may be overlooked (Spraker *et al.*, 1987). However, pathological examination is not a suitable diagnostic tool in field conservation for obvious reasons. Therefore in the absence of clinical signs, physiological indicators of muscle damage are often used to assess the presence and extent of muscle damage and therefore capture myopathy (Williams & Thorne, 1996).

Two of the most useful physiological indicators are high concentrations of enzymes such as creatine kinase (CK) and aspartate aminotransferase (AST). These two enzymes are released from muscle tissue when it is damaged and they leak out of the cells in to the blood stream (Williams & Thorne, 1996). Of the two enzymes, creatine kinase is the most valuable clinical signal of muscle damage as it is primarily specific to skeletal and cardiac muscle tissue and will rise relatively rapidly following damage to muscle tissue (Cardinet, 1989; Williams & Thorne, 1996) making it a sensitive indicator of muscle damage in birds (Franson *et al.*, 1985; Lumeij *et al.*, 1988). Unlike CK, AST is found in significant levels in cells beside muscle tissue and elevated AST may reflect damage to other organs particularly the liver. Therefore it is usually looked at in conjunction with CK when assessing muscle damage (Franson *et al.*, 1985; Cardinet, 1989; Williams & Thorne, 1996). The concentrations of CK and AST that reflect debilitating or fatal muscle damage are not known (Bollinger *et al.*, 1989; Dabbert & Powell, 1993; Wobeser, 1997). Although Bollinger et al. (1989) suggests birds with myopathy will exhibit CK concentrations in excess of 1000 IU/L. While capture myopathy was never confirmed histologically in one study on Northern bobwhites (*Colinus* virginianus) the survival probability of the birds making it to 16 weeks following capture

went down by 14 % for every 1000 IU/L increase in plasma CK on the day of capture (Mueller, 1999). There appears to be no specific concentration of the enzymes that necessarily correlates with clinical signs of capture myopathy. Even within a species a specific range of muscle enzyme concentrations may be associated with the clinical syndrome in some birds but not in others (Bailey et al., 1997). Regardless of the exact concentration of the enzymes the correlation between elevated concentrations of plasma CK and AST and clinical signs of capture myopathy in birds is well established (Bollinger et al., 1989; Ward et al., 2011). The circulating concentrations of these enzymes are therefore measured to assess muscle damage in live animals.

In addition to directly harming the birds, capture myopathy may predispose animals to predation or accident (Williams & Thorne, 1996). For example, it has been associated with increased rates of predation in birds due to reduced flight ability (Cox & Afton, 1998). These impacts may be short term in the weeks following release as they heal or could be long term, as myocardial lesions could result in a decreased tolerance to exercise (Spraker *et al.*, 1987). There is evidence that subclinical exertional myopathy may result in reduced performance in draught animals (Sharma & Murthy, 2007). It is therefore possible that subclinical capture myopathy in wild birds may result in an increased risk of predation as they are less able to escape or fight off predators. Mueller (1999) found that CK concentrations in asymptomatic northern bobwhites (*Colinus virginianus*) on the day of capture were negatively correlated with survival probability at 8 to 20 weeks following capture. Capture may also cause reduced movement rates in animals which may result in reduced foraging and cause a loss of body condition which may affect reproduction or survival (Cattet *et al.*, 2008). Another more subtle consequence is that if capture and handling is affecting animal survival, movement or reproduction in the days week or months following capture, it can cause bias and confound research or monitoring data resulting in inaccurate conclusions and potentially inappropriate management (Bollinger *et al.*, 1989; Williams & Thorne, 1996; Abbott *et al.*, 2005).

1.2.2 Capture Stress

1.2.2.1 The Avian Stress Response

One component of wildlife capture is the stress caused to the wild animal by the event. Stress can be defined as the state of animal when it is responding to a stressor through the activation of the hypothalamo-pituitary-adrenal axis (Cockrem, 2004). When birds are faced with a stressful event or situation (stressor), such as capture, they respond with both a specific and non-specific regulatory process. The way in which the specific response manifests will depend on the particular stressor, while the non-specific response will initiate a state of general stress irrespective of the environmental stimulus (Siegel, 1980).

In birds this general response to a stressor is highlighted by an increase in the secretion of corticosterone from the adrenal glands (Siegel, 1980; Fudge, 1999; Carsia & Harvey, 2000). Therefore measuring the concentration of corticosterone (CORT) in the bloodstream can be an effective measure of stress in birds (Cockrem, 2004). Corticosterone is the major adrenal glucocorticoid in birds (Carsia & Harvey, 2000) and it performs a range of actions in the body promoting changes in behaviour and physiology (Cockrem, 2004). It increases blood glucose levels, increases locomotor activity and foraging behaviour. Persistently high corticosterone concentrations can also inhibit the immune function and reproductive system of birds (Cockrem, 2004). These changes usually help the bird to adjust to a stressful situation or event and respond appropriately for the benefit of the bird. However if basal stress is higher than usual or the magnitude and/or duration of the stress response is greater than usual then this can cause negative effects (Cockrem, 2004).

The behavioural and physiological changes that are caused by the stress response are part of what is known as an ‘emergency life history stage’. In an emergency life history stage the animal switches out of its normal ‘life history stage’ and into survival mode (Wingfield, 2003). The emergency life history stage includes both behavioural changes such as increased foraging or escape behaviour or the suppression of reproductive behaviour, and physiological changes such as the regulation of the immune system and increased gluconeogenesis (Wingfield & Ramenofsky 1999; Sapolsky et al. 2000; Wingfield & Romero 2001 as cited by Wingfield, 2003). This may be beneficial to wild animals as behaviours or physiological functions that are unnecessary or even deleterious in the current conditions are supressed and other alternative behaviours and physiological functions which enhance survival are promoted. Gluconeogenesis is also increased to avoid any long term deleterious effects which

are caused by sustained high levels of glucosteriods (Wingfield, 2003). While the immediate effects of a stressor may be very short, the recovery from them is not well studied in wildlife (eg. movement rates following the stress of capture) (Wingfield, 2003).

Capturing wildlife for conservation management is not a natural event and as such the physiological or behavioural changes which may be beneficial to survival during events such as predation, may have negative impacts. In conservation the health and fitness of the animal is of paramount importance, therefore those protocols used should be investigated and if possible changed or refined to minimise the stress impacts these protocols may be having.

1.2.2.2 Capture and Handling of Birds and the Avian Stress Response

Behavioural observations are a poor indicator of the stress a bird is experiencing (Lemaho *et al.*, 1992), while being critical in some situations. Therefore CORT is often used as measure stress for birds (Cockrem, 2004). Plasma concentrations of CORT have been shown to rise in a range of wild bird species following capture and handling including: European starlings, (*Sturnus vulgaris*), (Dawson & Howe, 1983); semipalmated sandpipers (*Calidris pusilla*), (Gratto-Trevor *et al.*, 1991); harlequin ducks (*Histrionicus histrionicus*), (Perfito *et al.*, 2002); Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), house sparrows (*Passer domesticus*), and Lapland longspurs (*Calcarius lapponicus*), (Romero & Romero, 2002); emperor penguin (*Aptenodytes forsteri*) and Adelie penguins (*Pygoscelis adeliae*), (Cockrem, 2008); and saddlebacks (*Philesturnus rufusater*), (Adams *et al.*, 2010). The plasma CORT response has been used to compare how stressful different capture types are in wild birds and whether different durations of birds in the net/trap influence the stress response (Romero & Romero, 2002). It has also been used to compare whether different handling protocols of wild birds affect the stress response differently (Cockrem, 2008).

1.3 AVIAN CAPTURE AND HANDLING TECHNIQUES

Discussing all the techniques that are and have been used to capture and handle birds is beyond the scope of this research but a broad description of the methods used is possible. There are pros and cons to each method and no one method is best for all situations.

Therefore the method of capture chosen will depend on the species, capture environment and purpose of capture.

1.3.1 Avian Capture Techniques

Most if not all species of birds are able to be captured (Whitworth *et al.*, 2007). Many methods use some sort of attractants such as live lure birds, artificial decoy birds, taped bird calls, food or water to encourage birds to the trap or capture site (Bub, 1991). Other methods require knowledge of areas frequented by the target species and yet other methods require some sort of pursuit of the target bird. The types of capture techniques used for birds can be loosely grouped into passive, reactive and active methods of capture.

Passive methods of capture involve techniques such as baited traps, snares or set nets. They involve setting up traps, snares or nets in a suitable area and either waiting at the location for an animal to be captured or leaving the area and checking the trap over a set period of time. Trapping methods such as funnel traps involve the placement of a wire (or similar) enclosure which has one or more funnel shaped entrances. The trap is baited and birds are able to enter the enclosure through the funnels but have difficulty escaping back through the funnels (Whitworth *et al.*, 2007). Funnel traps are an efficient method of capturing species such as Rio Grande turkeys (*Meleagris gallopavo intermedia*), (Davis, 1994). Leg snares are another capture method; they can be used for when the birds travel paths along the ground are known. They consist of loops of nylon tied with a slip knot, which are secured to the ground by a stake. The birds are captured when their leg passes though the loop and closes, securing them to the stake. They have been used to catch species such as Asiatic houbara bustard (*Chlamydotis undulata macqueenii*), (Seddon *et al.*, 1999). Mist nets are a popular method for capturing small birds and have been used in many studies to capture species such as Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) and house sparrows (*Passer domesticus*), (Romero & Romero, 2002). The technique uses a fine mesh net that is set between two upright poles across a highly frequented flight path. The mesh is inconspicuous and birds that fly into the net become easily entangled and then retrieved (Whitworth *et al.*, 2007).

Reactive methods of capture involve techniques such as clap traps or cannon nets. They involve setting up the clap trap or cannon net in a suitable area and waiting at the location for an animal to enter the desired area at which point the trap can be activated or the net fired. Clap traps are made of one or two rectangular or semicircular frames which are covered by a mesh net. The frame(s) are set so it lies flat on the ground and when activated the frame snap over, either trapping the bird between the two netted frames or between the netted frame and the ground. The trap is operated remotely when a bird is observed entering the appropriate position. Clap traps have been used to capture houbara bustards (Seddon *et al.*, 1999). Cannon nets are useful for capturing large numbers of birds which congregate in groups at predictable feeding or roosting areas. Cannon nets are set up in advance at a site where birds are known to congregate or they are attracted too. When the birds have arrive the cannon net uses a number of projectiles fired by explosive charges to launch a large net across the designated area thus ensnaring any birds under the net (Whitworth *et al.*, 2007).

Active methods of capture involve techniques such hand nets, capture by hand, corral traps and net-guns. They use more proactive methods of locating and capturing birds and are different from the other methods of capture mentioned because they often involve the pursuit of the target animal. Hand-nets such are common method for capturing birds that can be approached or pursued to within a close distance (Williams, 1995; Robertson & Colbourne, 2003; Youl, 2008). Some species are even able to be approached close enough that they can be captured by hand (Williams, 1995; Robertson & Colbourne, 2003; Youl, 2008). Corral traps are used to capture birds which are flightless due to either species morphology (Youl, 2008) or during the stage of moult when birds such as waterfowl lose their flight feathers (Whitworth *et al.*, 2007). The method involves herding the birds into capture pen or 'corral.' Two wings extend out from the corral in a funnel shape and act as barriers to draw the driven birds in to the corral (Whitworth *et al.*, 2007). Net-guns are a useful method of capture when the target species or population does not gather together in large groups or in situations where individual birds need to be targeted (Edwards & Gilchrist, 2011). Net-guns are discussed below in more detail.

Some passive capture methods don't require constant attention and therefore may allow for a greater trapping effort. These methods may also cause less acute stress to birds as they are not chased or subjected to loud noises. However these methods may not be appropriate if it is desirable to target specific individuals and if human intervention is required due to unforeseen circumstances (eg. bird is injured or snare is disturbed) then they are often not

alerted to the fact for some time. Also they are usually not suitable if prompt sampling is required. While more passive then other methods of capture, these methods may still cause birds stress (Romero & Romero, 2002), muscle damage (Mueller, 1999) and/or altered behaviour (Colwell *et al.*, 1988).

Reactive capture methods by contrast have a greater degree of control over which animals are captured. Certain species or individuals can be avoided if they are particularly threatened or not desirable. Unlike some of the passive methods they require a greater work effort as someone has to be present to fire/set off the technique. The animal is not pursued which may reduce stress however the method may be accompanied by a loud noise and the method may cause injury to the animal as the trap/net moves to ensnare the animal. This method may allow prompt sampling, unless a large number of individuals need processing. While no pursuit is involved, these methods may still cause the birds stress (Landys-Ciannelli *et al.*, 2002), muscle damage (Dabbert & Powell, 1993), and/or altered behaviour (Southern & Southern, 1983).

Active methods of capture may have the greatest potential to injure or stress the animal. These methods may often be accompanied by a pursuit which may increase the stress and exertion experienced by the animal (Williams & Thorne, 1996). Sometimes these methods even intentionally aim to exhaust the animal to allow it to be captured (Ellis, 1975; O'Gara & Getz, 1986; Ellis *et al.*, 1998). These methods are often very useful as they allow for individuals of a specific species, sex or age etc. to be targeted and so there is minimal if any by-catch. These methods often allow for rapid sampling. There is also the ability to call off the capture operation if signs of distress are observed in the target bird.

1.3.1.1 Net-Guns

Hand held net-guns were first developed from modified firearms for the live capture of deer in New Zealand (Yerex, 2001; Drew, 2012). Subsequently they became a useful method of capture for large mammals in North America (Barrett *et al.*, 1982) and Africa (Drager & Allan, 1988). They are also now used to capture a variety of avian species with mixed results on capture success and animal health. The design of the net-gun has progressed and diversified since its inception but the basic principle remains a portable unit that is fired handheld and launches a net attached to weights at the target animal in order to trap or ensnare the animal so that it can be captured alive.

Net-guns have been used to capture a range of birds for a range of research purposes using varying methods dependent on target species and sampling environment. Species captured include: mallard ducks (*Anas platyrhynchos*), (Mechlin & Shaiffer, 1980); golden eagles (*Aquila chrysaetos*), (O'Gara & Getz, 1986); marbled murrelets (*Brachyramphus marmoratus*), (Quinlan & Hughes, 1992); snail kites (*Rostrhamus sociabilis*), (Bennetts *et al.*, 1999); Asiatic houbara bustard (*Chlamydotis undulata macqueenii*), (Seddon *et al.*, 1999); black guillemots (*Cepphus grylle*), (Mehlum *et al.*, 1993); American bittern (*Botaurus lentiginosus*), (Huschle *et al.*, 2002); great egret (*Ardea albus*), white ibis (*Eudocimus alba*), glossy ibises (*Plegadis falcinellus*), roseate spoonbill (*Platalea ajaja*), and snowy egret (*Egretta thula*), (Herring *et al.*, 2008). This includes some species in New Zealand, for example research projects on Australasian bitterns (*Botaurus poiciloptilus*) (Teal, 1989), storm petrel (*Pealeornis maoriana*) (Stephenson *et al.*, 2008) and kea (*Nestor notabilis*) (van Klink, 2008 as cited by Melville 2011) have all used net-guns to some extent.

The net-guns available range from homemade designs to several commercial units and are either powered by compressed gas or blank rifle cartridges which fire between three and eight projectiles attached to a net. They have proved an effective means of capture however there has been one major health concern associated with using net-guns to capture birds which is projectile strikes. In several documented cases the projectile attached to the net hit the bird, breaking the birds wing (Huschle *et al.*, 2002) or even killing the bird outright (Mechlin & Shaiffer, 1980; Herring *et al.*, 2008). Due to this the New Zealand Bird Banding Manual (Melville, 2011) considers them only suitable for the capture of large robust species. This risk of injury or mortality is recognised as limiting the potential uses of net-guns on threatened species (Herring *et al.*, 2008).

The Super Talon net-gun manufactured by Advanced Weapons Technology (http://www.humanecapture.com) is one of the newer models of net-gun. It is distinctive in its use of compressed carbon dioxide as the propellant rather than gunpowder. It fires a 7.5 m^2 net secured to eight 24 g projectiles at 6.7 m/s out to a maximum cited range of 15m. It is considered difficult to achieve the maximum range in most conditions and the effective capture range of this model is as little as three to five metres (Edwards & Gilchrist, 2011). The nets supplied by the manufacturer come in either five or ten cm mesh sizes and the single use compressed CO_2 cartridges are also supplied by the manufacturer. It has been used successfully in the capture of grey phalarope (*Phalaropus fulicarius*) and white-rumped sandpiper (*Calidris fuscicollis*), (Edwards & Gilchrist, 2011); and also Pacific golden-plovers

(*Pluvialis fulva*), (Johnson *et al.*, 2011). This style of net-gun compared to others such as the Mechlin and Shaiffer (1980) design or the net-gun from Coda Enterprises (www.codaenterprises.com) may be advantageous because its lower power and the lower mass of the projectiles may mean it has a lower impact on the bird. However the lower power of this model of net-gun may mean it is less effective in the field and while the mass of the projectiles is lower, the increased number of them may result in an increased chance of them striking a bird. No injuries have been documented when using this net-gun (Edwards & Gilchrist, 2011; Johnson *et al.*, 2011). Net-guns are not regarded as a routine capture method by (Melville, 2011) and special permission is need for their use. As part of my research I aim to investigate the practicality and health impacts of net-guns for capturing threatened birds in New Zealand.

1.3.2 Avian Handling Techniques

The large number of bird species and wide ranging differences in anatomy and temperament means that no one handling technique is suitable for all species and birds should be handled according to the specific requirements of that species (Bub, 1991; Whitworth *et al.*, 2007). General handling rules include holding the bird firmly enough to stop it struggling but not so much that it is not able to breathe easily. Holding a bird too firmly can restrict breathing but holding a bird too softly may result in the bird struggling more which may increase the chances of it being injured (Whitworth *et al.*, 2007). Some species may require a more secure hold then others as they may struggle strongly to escape if they are held loosely (Bub, 1991).

One of the most used techniques for small birds is known as the ringer's hold where the bird is held in one hand. In this technique the bird is held with its wings closed and its back to the palm of hand. The head is positioned between the index and middle finger while the remaining fingers encircle the body (Whitworth *et al.*, 2007).

Medium sized birds such as ducks, small geese or larger shorebirds should be held using both hands. The usual technique is called the two-handed grip involves the handler placing their hands on either side of the bird so that the palms face the bird and the wings are contained. The thumbs of each hand should be on the back of the bird at the shoulder and the fingers of each hand should curly around the bird's chest and abdomen. The bird may be held in this manner either horizontally or vertically with the head facing up. The legs of the bird should

be tucked up against the bird's underside (Whitworth *et al.*, 2007). Other medium-sized birds such as raptors should be held by the handler with one hand around the abdomen while the other hand stretches the bird's legs out towards the back (Bub, 1991). In these species it may be particularly important to secure the beak and/or talons to prevent injury to the handler (Whitworth *et al.*, 2007).

Larger species of bird such as swans or geese may need to be restrained by two handlers. In this case one person should control the head and legs of the bird while the other holds the body and wings. In situations where one person is required to hold a larger bird then they may use the underarm hold. In this case the handler holds the bird's body under one arm so that the body is supported by the hand of the same arm and the wings are also restrained. The handlers other hand may then restrain the bird's head or be placed across the bird's back (Whitworth *et al.*, 2007). Some species require special handling techniques. Once captured birds that await processing may be held in holding cages or in fabric bags made of porous fabric depending the species. The specific handling techniques of the species studied will be described within each chapter.

1.4 USING SURROGATE SPECIES IN CONSERVATION

Research on conservation management techniques sometimes uses a surrogate or substitute species in place of the target species in order to simulate what conservation problems the target species or ecological community may face (Caro *et al.*, 2005). It may be used for threatened species management, when due to logistic or political reasons the target species cannot be sampled directly in order to determine what impact an anthropogenic force may be having, or to determine why an endangered population of animals is doing badly (Caro *et al.*, 2005; Githiru *et al.*, 2007). The use of surrogate species to model the other species is controversial and largely dismissed as inappropriate in conservation unless it meets certain criteria (Caro *et al.*, 2005; Murphy *et al.*, 2011).

The current use of substitute species in conservation seem to largely be at a population level, where the response of the surrogate species population demographics are assumed to be the same as the response of the endangered species demographics (Caro & O'Doherty, 1999;

Caro *et al.*, 2005; Murphy *et al.*, 2011). There is little information on using a surrogate species in conservation to assess the likely physiological health impacts on an endangered species. Caro *et al.* (2005) do note that in medicine mice are used as test subject for human procedures, with the assumption that their biology is similar enough to give insight into the impacts of the procedure, and that testing the procedure is not feasible on human subjects.

The use of a surrogate species to model the response of endangered species should be used with caution, and only when actual data on the endangered species are not obtainable (Caro *et al.*, 2005; Murphy *et al.*, 2011). There must also be some sort of validation of the data acquired through the surrogate before the information is applied to management decisions (Murphy *et al.*, 2011). The use of a surrogate species to assess the physiological impacts of capture will make similar assumptions as the conservation surrogacy literature and the choice of the surrogate will need validation. The reasons a particular surrogate species is chosen to model another must be clearly stated, as must any assumptions that are made (Caro & O'Doherty, 1999; Caro *et al.*, 2005).

1.5 SELECTED MEASURES OF THE PHYSIOLOGICAL EFFECTS OF CAPTURE AND HANDLING

1.5.1 Creatine Kinase (CK)

Creatine kinase is an enzyme that is abundant in all types of muscle tissue (Fudge, 1999), and is involved in the process of delivering energy to muscles (Tortora & Grabowski, 2003). When muscle tissue is damaged, CK is released from the damaged cells and leaks into the blood stream (Cardinet, 1989; Williams & Thorne, 1996). Elevations in the plasma CK concentration are associated almost exclusively to some form of muscle damage and it is considered the best indicator of muscle damage in birds (Franson *et al.*, 1985; Lumeij *et al.*, 1988). Creatine kinase is cleared from the blood in a relatively short time compared with enzymes such as AST, with a mean half-life of 3.07 hours and standard deviation of 0.59 hours in racing pigeons (*Columba livia domestica*) (Lumeij *et al.*, 1988). Therefore any elevations in plasma CK can be assumed to be related to relatively recent events causing muscle damage. Peak plasma CK concentrations following muscle damage may occur around 24 hours following the event (Bailey *et al.*, 1997).

1.5.2 Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) is present in muscle and liver tissue (Fudge, 1999). When either muscle tissue or liver tissue is damaged AST is leaked into the bloodstream (Williams & Thorne, 1996).The concentration of AST in the plasma of a bird is therefore an indication that damage to either or both of these tissue types has occurred. While not as specific to muscle damage as CK, plasma AST concentrations often used in conjunction with plasma CK to assess the occurrence of muscle damage and capture myopathy in birds (Williams & Thorne, 1996). While AST rises slower following muscle damage compared to CK (Williams & Thorne, 1996), it has a longer half-life (Lumeij *et al.*, 1988) and persists in the animal longer after the initial damage compared to CK.

1.5.3 Glutamate dehydrogenase (GLDH)

Glutamate dehydrogenase (GLDH) is a mitochondrially bound enzyme (Fudge, 1999) that catalyses the reversible reaction of L-glutamate to 2-ketoglutarate and ammonia (Hudson & Daniel, 1993). GLDH therefore plays a major role in ammonia metabolism, which occurs mainly in the liver and kidney (Nissim, 1999). In the mammalian brain GLDH also has a key role in oxidative metabolism of the major excitatory neurotransmitter glutamate (Anderson & Swanson, 2000). GLDH is also recognized as a key enzyme in the regulation of insulin (Sener *et al.*, 1982).Clinically elevated concentrations of plasma increase in GLDH concentration are usually associated with certain types of acute liver damage (Lumeij *et al.*, 1988; Fudge, 1999). However it is found in significant concentrations in brain and kidney tissue also. Due to its role in binding ammonia, short term changes in plasma GLDH are expected to be associated with nitrogen (protein) metabolism but this has yet to be validated in birds. It is eliminated in a comparatively very short time from the blood, with a mean half-life of 0.68 hours and standard deviation of 0.17 hours in racing pigeons (Lumeij *et al.*, 1988).

Because GLDH is usually only present in trace amounts and because it is a mitochondrially bound enzyme, it means that elevations in plasma concentrations are likely due to recent tissue necrosis not degeneration, in the brain, liver or kidneys (Ritchie *et al.*, 1994).

1.5.4 Uric acid (UA)

Uric acid (UA) is the major nitrogenous waste in birds. It mainly produced in the liver and is eliminated from the body in the urine via the kidneys (Fudge, 1999). Plasma concentrations of UA may rise due to severe dehydration, extensive renal damage or because the birds that have recently eaten high-protein meal (Fudge, 1999). The half-life of plasma UA and rate at which it is eliminated from the blood in birds is not readily available. One aspect of pathophysiology of capture myopathy is that it may lead to renal damage, as myoglobin released from the damaged muscles may cause renal tubular necrosis (Bartsch *et al.*, 1977; Spraker, 1993; Williams & Thorne, 1996). Bar-tailed godwits (*Limosa lapponica baueri*) suffering from capture myopathy showed elevated plasma UA in the days following capture, likely due to renal damage, and plasma UA was suggested as a potential prognostic indicator of death (Ward *et al.*, 2011). The effects of capture on plasma UA within hours of capture are not well documented but this analyte may provide some insight into the effect of capture on renal physiology. However a large amount of natural variation in plasma UA can occur due to feeding and this may complicate the use of this analyte to assess renal function (Lumeij & Remple, 1991).While it may take a loss of 70% or more of kidney function to result in pathological increases in plasma UA (Lierz, 2003), smaller changes in plasma UA may be observable due to capture.

1.5.5 Corticosterone (CORT)

Following a stressful event the hypothalamic pituitary adrenocortical (HPA) axis, which controls CORT release, is activated resulting in the secretion of CORT from the adrenal gland (Siegel, 1980). The stress hormone corticosterone (CORT) can therefore be used as a measure of the degree of stress the bird is experiencing (Cockrem *et al.*, 2004). However seasonal variations in baseline concentrations of plasma CORT and the response of plasma CORT to acute stress have been observed (Dawson & Howe, 1983; Romero & Wingfield, 1998; Perfito *et al.*, 2002; Reneerkens *et al.*, 2002). There may also be variation in baseline concentrations of CORT or the response of plasma CORT due to factors such as gender (Perfito *et al.*, 2002) or age (Romero *et al.*, 1998). Hormones such as CORT are largely removed from circulation when they bind at target sites and are metabolised by enzymes in the tissues. This happens particularly in the kidneys or liver. The by-products of the metabolism and small amounts of the unchanged hormone will then be excreted in the urine or bile (Bentley, 1998). The half-life of hormones such as CORT varies considerably between

species (Bentley, 1998) and even but was found to be 22 minutes in male broiler chickens (*Gallus domesticus*) (Birrenkott & Wiggins, 1984).

1.6 THESIS AIMS AND ORGANISATION

The overall aim of this thesis is to investigate the physiological impact that routine capture and handling can have on the muscles and stress response of surrogates of threatened species of birds. This is undertaken by using a captive or wild surrogate species with which to model the physiologically impacts of general capture and the specific threatened handling protocol on the threatened species.

Chapter Two investigates the physiological impacts capture and handling has on layer hens (*Gallus domesticus*). This is done to model the potential effects that kiwi (*Apteryx spp.*) best practice capture and handling protocol may be having on the birds muscle biochemistry and stress response. Chapter Three investigates the physiological impacts of capture and handling on wild pūkeko (*Porphyrio porphyrio melanotus*). This is done to model the potential effects that takahē (*Porphyrio hochstetteri*) best practice handling protocols may be having on the birds muscle biochemistry and stress response. Chapter Four investigates the physiological impacts of capture and handling on wild mallard ducks (*Anas platyrhynchos*). This is done to model the potential effects that capture and handling of threatened New Zealand waterfowl may be having on the birds muscle biochemistry and stress response. Pateke (*Anas chlorotis*) best practice handling protocol are used.

The impacts of the capture and handling process on the surrogate species are assessed by serially blood sampling the birds and determining the changes of plasma CK, AST, CORT, GLDH and in some cases UA. These biochemical analytes allow the impact of the procedures on the muscles, stress response and select organ function of the surrogate to be assessed. Therefore the procedures used can be evaluated and suitability of the protocols used on threatened species may be considered adequate, need refinement or require further research.

References

Abbott, C. W., Dabbert, C. B., Lucia, D. R., & Mitchell, R. B. (2005). Does muscular damage during capture and handling handicap radiomarked northern bobwhites? *Journal of Wildlife Management, 69*(2), 664-670.

Adams, N. J., Parker, K. A., Cockrem, J. F., Brunton, D. H., & Candy, E. J. (2010). Corticosterone responses and post-release survival in translocated North Island Saddlebacks (*Philesturnus rufusater*) in New Zealand. *Emu, 110*(4), 296-301.

Anderson, C. M., & Swanson, R. A. (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia, 32*(1), 1-14.

Anonymous (1987). *IUCN position statement on the translocation of living organisms: introductions, re-introductions, and restocking.* http://intranet.iucn.org/webfiles/doc/SSC/SSCwebsite/Policy_statements/IUCN_Position_Statement_on_Translocation_of_Living_Organisms.pdf (accessed 20 May 2013). International Union for Conservation of Nature (IUCN), Gland, Switzerland.

Armstrong, D. P., & McLean, I. G. (1995). New Zealand translocations: Theory and practice. *Pacific Conservation Biology, 2*(1), 39-54.

Bailey, T. A., Wernery, U., Naldo, J., & Samour, J. H. (1997). Plasma concentration of creatine kinase and lactate dehydrogenase in houbara bustards (*Chlamydotis undulata macqueenii*) immediately following capture. *Comparative Haematology International, 7*(2), 113-116.

Barrett, M. W., Nolan, J. W., & Roy, L. D. (1982). Evaluation of a hand held net gun to capture large mammals. *Wildlife Society Bulletin, 10*(2), 108-114.

Bartsch, R. C., McConnell, E. E., Imes, G. D., & Schmidt, J. M. (1977). Review of exertional rhabdomyolysis in wild and domestic animals and man. *Veterinary Pathology, 14*(4), 314-324.

Bassett, S. (2012). Operation Nest Egg Incubation and Chick Rearing Best Practice Protocols. Wellington: Oralis Limited.

Basson, P. A., & Hofmeyr, J. M. (1973). Mortalities associated with wildlife capture operations. In E. Young (Ed.), *The Capture and Care of Wild Animals; The Work of Eighteen Veterinary, Medical, and Wildlife Experts* (pp. 151-160). Cape Town and Pretoria: Human and Rousseau.

Bennetts, R. E., Dreitz, V. J., Kitchens, W. M., Hines, J. E., & Nichols, J. D. (1999). Annual survival of snail kites in Florida: radio telemetry versus capture-resighting data. *Auk, 116*(2), 435-447.

Bentley, P. J. (1998). *Comparative Vertebrate Endocrinology* (Third edition ed.): Cambridge University Press.

Birrenkott, G. P., & Wiggins, M. E. (1984). Determination of dexamethasone and corticosterone half-lives in male broilers. *Poultry Science, 63*(5), 1064-1068.

Bollinger, T., Wobeser, G., Clark, R. G., Nieman, D. J., & Smith, J. R. (1989). Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by 3 methods for banding. *Journal of Wildlife Diseases, 25*(2), 225-231.

Bub, H. (1991). *Bird trapping and bird banding: a handbook for trapping methods all over the world* Ithaca, New York: Cornell University Press.

Businga, N. K., Langenberg, J., & Carlson, L. (2007). Successful treatment of capture myopathy in three wild greater sandhill cranes (*Grus canadensis tabida*). *Journal of Avian Medicine and Surgery, 21*(4), 294-298.

Cardinet III, G. H. (1989). Skeletal muscle function. In J. J. Kaneko (Ed.), *Clinical Biochemistry of Domestic Animals* (4th ed., pp. 463-495). New York: Academic Press, Inc.

Caro, T., Eadie, J., & Sih, A. (2005). Use of substitute species in conservation biology. *Conservation Biology, 19*(6), 1821-1826.

Caro, T. M., & O'Doherty, G. (1999). On the use of surrogate species in conservation biology. *Conservation Biology, 13*(4), 805-814.

Carsia, R. V., & Harvey, S. (2000). Adrenals. In G. C. Whittow (Ed.), *Sturkie's Avian Physiology* (pp. 489-537). San Diego: Academic Press.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A., & Reynolds-Hogland, M. L. (2008). An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy, 89*(4), 973-990.

Cockrem, J. F., Adams, D. C., Bennett, E. J., Candy, E. J., Hawke, E. J., Henare, S. J., & Potter, M. A. (2004). Endocrinology and the conservation of New Zealand birds. In M. S. Gordon & S. M. Bartol (Eds.), *Experiemental Approaches to Conservation Biology* (pp. 101-121). Berkeley and Los Angeles: University of California Press.

Cockrem, J. F., Potter, M. A., Barrett, D. P., & Candy, E. J. (2008). Corticosterone responses to capture and restraint in emperor and Adelie penguins in Antarctica. *Zoological Science, 25*(3), 291-298.

Cockrem, J. F., & Silverin, B. (2002). Sight of a predator can stimulate a corticosterone response in the great tit (Parus major). *General and Comparative Endocrinology, 125*(2), 248-255.

Colwell, M. A., Gratto, C. L., Oring, L. W., & Fivizzani, A. J. (1988). Effects of blood-sampling on shorebirds: injuries, return rates, and clutch desertions. *Condor, 90*(4), 942-945.

Cox, R. R., & Afton, A. D. (1998). Effects of capture and handling on survival of female northern pintails. *Journal of Field Ornithology, 69*(2), 276-287.

Czujkowska, A., Malicka, E., & Kruszewicz, A. (2009). Capture myopathy in seven razorbills (*Alca torda*) following capture in a fishing net. *Proceedings of the International Conference on Diseases of Zoo and Wild Animals, Beekse Bergen, The Netherlands, 20th-24th May 2009*, 209-215.

Dabbert, C. B., & Powell, K. C. (1993). Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases, 29*(2), 304-309.

Davis, B. D. (1994). A funnel trap for Rio Grande turkey. *Proceedings of the Forty-Eighth Annual Conference - Southeastern Association of Fish and Wildlife Agencies*, 109-116.

Dawson, A., & Howe, P. D. (1983). Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *General and Comparative Endocrinology, 51*(2), 303-308.

Drager, N., & Allan, N. (1988). The use of a netgun to capture African ungulates. *Zeitschrift Fur Jagdwissenschaft, 34*(4), 256-260.

Drew, K. (2012). *Deer and deer farming - From deer stalking to helicopters.* http://www.TeAra.govt.nz/en/photograph/15804/live-capture-by-net-gun (accessed 25 April 2013). Te Ara - the Encyclopedia of New Zealand.

Edwards, D. B., & Gilchrist, H. G. (2011). A new means of catching shorebirds: the Super Talon Net Gun. *Wader Study Group Bulletin, 118*(2), 134-136.

Ellis, D. H. (1975). 1st experiments with capturing golden eagles by helicopter. *Bird Banding, 46*(3), 217-219.

Ellis, D. H., Hjertaas, D., Johns, B. W., & Urbanek, R. P. (1998). Use of a helicopter to capture flighted cranes. *Wildlife Society Bulletin, 26*(1), 103-107.

Franson, J. C., Murray, H. C., & Bunck, C. (1985). Enzyme activities in plasma, kidney, liver, and muscle of 5 avian species. *Journal of Wildlife Diseases, 21*(1), 33-39.

Fudge, A. M. (1999). *Laboratory Medicine: Avian and Exotic pets*: Saunders.

Githiru, M., Lens, L., Bennun, L. A., & Matthysen, E. (2007). Can a common bird species be used as a surrogate to draw insights for the conservation of a rare species? A case study from the fragmented Taita Hills, Kenya. *Oryx, 41*(2), 239-246.

Glaser, A., van Klink, P., Elliott, G., & Edge, K. (2010). Whio/blue duck (*Hymenolaimus malacorhynchos*) Recovery Plan 2009–2019. Wellington: Department of Conservation.

Gratto-Trevor, C. L., Oring, L. W., & Fivizzani, A. J. (1991). Effects of blood sampling stress on hormone levels in the semipalmated sandpiper. *Journal of Field Ornithology, 62*(1), 19-27.

Green, G. H. (1980). Capture Myopathy ('Cramp') in Waders. *Wader Study Group Bulletin, 28*, 15.

Gummer, H., & Evans, K. (2003). Brown teal/pateke (*Anas chlorotis*) Husbandry Manual. Ruawai: Brown Teal Recovery Group.

Hanley, C. S., Thomas, N. J., Paul-Murphy, J., & Hartup, B. K. (2005). Exertional myopathy in whooping cranes (*Grus americana*) with prognostic guidelines. *Journal of Zoo and Wildlife Medicine, 36*(3), 489-497.

Herraez, P., Sierra, E., Arbelo, M., Jaber, J. R., de los Monteros, A. E., & Fernandez, A. (2007). Rhabdomyolysis and myoglobinuric nephrosis (capture myopathy) in a striped dolphin. *Journal of Wildlife Diseases, 43*(4), 770-774.

Herring, G., Gawlik, D. E., & Beerens, J. M. (2008). Evaluating two new methods for capturing large wetland birds. *Journal of Field Ornithology, 79*(1), 102-110.

Hofle, U., Millan, J., Gortazar, C., Buenestado, F. J., Marco, I., & Villafuerte, R. (2004). Self-injury and capture myopathy in net-captured juvenile red-legged partridge with necklace radiotags. *Wildlife Society Bulletin, 32*(2), 344-350.

Holmes, T., & Caskey, D. (2001). Translocation of wild-caught and captive-reared blue duck *Hymenolaimus malacorhynchos*. Wellington: Department of Conservation.

Holzapfel, S., Robertson, H. A., McLennan, J. A., Sporle, W., Hackwell, K., & Impey, M. (2008). Kiwi (*Apteryx spp.*) Recovery Plan 2008-2018. Wellington: Department of Conservation.

Hudson, R. C., & Daniel, R. M. (1993). L-glutamate dehydrogenases distribution: properties and mechanism. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 106*(4), 767-792.

Huschle, G., Toepfer, J. E., Brininger, W. L., & Azure, D. A. (2002). Capturing adult American bitterns. *Waterbirds, 25*(4), 505-508.

Jacques, C. N., Jenks, J. A., Deperno, C. S., Sievers, J. D., Grovenburg, T. W., Brinkman, T. J., Swanson, C. C., & Stillings, B. A. (2009). Evaluating ungulate mortality associated with helicopter net-gun captures in the Northern Great Plains. *Journal of Wildlife Management, 73*(8), 1282-1291.

Johnson, O. W., Fielding, L., Fox, J. W., Gold, R. S., Goodwill, R. H., & Johnson, P. M. (2011). Tracking the migrations of Pacific golden-plovers (*Pluvialis fulva*) between Hawaii and Alaska: New insight on flight performance, breeding ground destinations, and nesting from birds carrying light level geolocators. *Wader Study Group Bulletin, 118*(1), 26-31.

Kreeger, T. J., White, P. J., Seal, U. S., & Tester, J. R. (1990). Pathological responses of red foxes to foothold traps. *Journal of Wildlife Management, 54*(1), 147-160.

Landys-Ciannelli, M. M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J. C., & Castr Ring, G. (2002). Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica*. *Physiological and Biochemical Zoology, 75*(1), 101-110.

Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology, 148*(2), 132-149.

Lemaho, Y., Karmann, H., Briot, D., Handrich, Y., Robin, J. P., Mioskowski, E., Cherel, Y., & Farni, J. (1992). Stress in birds due to routine handling and a technique to avoid it. *American Journal of Physiology, 263*(4), R775-R781.

Lierz, M. (2003). Avian renal disease: pathogenesis, diagnosis, and therapy. *The veterinary clinics of North America. Exotic animal practice, 6*(1), 29-55, v.

Lumeij, J. T., Debruijne, J. J., Slob, A., Wolfswinkel, J., & Rothuizen, J. (1988). Enzyme activities in tissues and elimination half-lives of homologous muscle and liver enzymes in the racing pigeon (*Columba livia domestica*). *Avian Pathology, 17*(4), 851-864.

Lumeij, J. T., & Remple, J. D. (1991). Plasma urea, creatinine and uric-acid concentrations in relation to feeding in peregrine falcons (*Falco peregrinus*). *Avian Pathology, 20*(1), 79-83.

Marco, I., Mentaberre, G., Ponjoan, A., Bota, G., Manosa, S., & Lavin, S. (2006). Capture myopathy in little bustards after trapping and marking. *Journal of Wildlife Diseases, 42*(4), 889-891.

Massey, J. G. (2006). Summary of an oiled bird response. *Journal of Exotic Pet Medicine, 15*(1), 33-39.

Mechlin, L. M., & Shaiffer, C. W. (1980). Net firing gun for capturing breeding waterfowl. *Journal of Wildlife Management, 44*(4), 895-896.

Mehlum, F., Gabrielsen, G. W., & Nagy, K. A. (1993). Energy expenditure by black guillemots (*Cepphus grylle*) during chick-rearing. *Colonial Waterbirds, 16*(1), 45-52.

Melville, D. S. (2011). New Zealand Bird Banding Manual. Wellington: Department of Conservation.

Minton, C. D. T. (1993). Stress myopathy in captured waders. *Wader Study Group Bulletin, 70*, 49-50.

Mueller, J. M. (1999). *Effect of red imported fire ants on reproduction, health, and survival of northern bobwhites.* Texas Tech University, Lubbock.

Murphy, D. D., Weiland, P. S., & Cummins, K. W. (2011). A critical assessment of the use of surrogate species in conservation planning in the Sacramento-San Joaquin Delta, California (USA). *Conservation Biology, 25*(5), 873-878.

Nissim, I. (1999). Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *American Journal of Physiology-Renal Physiology, 277*(4), F493-F497.

O'Gara, B. W., & Getz, D. C. (1986). Capturing golden eagles using a helicopter and net gun. *Wildlife Society Bulletin, 14*(4), 400-402.

O'Connor, S. M., Maloney, R. F., & Pierce, R. J. (2007). Pateke (*Anas chlorotis*) Recovery Plan, 2005–10. Wellington: Department of Conservation.

Perfito, N., Schirato, G., Brown, M., & Wingfield, J. C. (2002). Response to acute stress in the harlequin duck (*Histrionicus histrionicus*) during the breeding season and moult: relationships to gender, condition, and life-history stage. *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 80*(8), 1334-1343.

Powell, R. A., & Proulx, G. (2003). Trapping and marking terrestrial mammals for research: Integrating ethics, performance criteria, techniques, and common sense. *Ilar Journal, 44*(4), 259-276.

Quinlan, S. E., & Hughes, J. H. (1992). Techniques for capture and radio tagging of marbled murrelets. *Proceedings of the Western Foundation of Vertebrate Zoology, 5*(1), 117-121.

Reneerkens, J., Morrison, R. I. G., Ramenofsky, M., Piersma, T., & Wingfield, J. C. (2002). Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. *Physiological and Biochemical Zoology, 75*(2), 200-208.

Ritchie, B.W., Harrison, G.J., & Harrison, L.R. (1994). *Avian Medicine: Principles and Applications*. http://avianmedicine.net/publication_cat/avian-medicine/ (accessed 25 November 2013). Wingers Publishing Inc. Lake Worth, Florida.

Robertson, H. A., & Colbourne, R. M. (2003). Kiwi (*Apteryx spp*.) Best Practice Manual Wellington: Department of Conservation.

Romero, L. M., & Romero, R. C. (2002). Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor, 104*(1), 129-135.

Romero, L. M., Soma, K. K., & Wingfield, J. C. (1998). The hypothalamus and adrenal regulate modulation of corticosterone release in redpolls (*Carduelis flammea* - An arctic-breeding song bird). *General and Comparative Endocrinology, 109*(3), 347-355.

Romero, L. M., & Wingfield, J. C. (1998). Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology, 119*(1), 31-36.

Ruder, M. G., Noel, B. L., Bednarz, J. C., & Keel, M. K. (2012). Exertional myopathy in pileated woodpeckers (*Dryocopus pileatus*) subsequent to capture. *Journal of Wildlife Diseases, 48*(2), 514-516.

Seddon, P. J., Launay, F., Van Heezik, Y., & Al Bowardi, M. (1999). Methods for live trapping houbara bustards. *Journal of Field Ornithology, 70*(2), 169-181.

Sener, A., Owen, A., Malaisselagae, F., & Malaisse, W. J. (1982). The stimulus secretion coupling of amino acid-induced insulin release .11. kinetics of deamination and transamination reactions. *Hormone and Metabolic Research, 14*(8), 405-409.

Sharma, A., & Murthy, K. S. (2007). Effect of work on sub clinical exertional myopathy in working equines. *Fifth International Colloquium on Working Equines. The future for working equines. Addis Ababa, Ethiopia, 30 October-2 November, 2006.*, 107-109.

Siegel, H. S. (1980). Physiological stress in birds. *Bioscience, 30*(8), 529-534.

Southern, L. K., & Southern, W. E. (1983). Responses of ring-billed gulls to cannon-netting and wing-tagging. *Journal of Wildlife Management, 47*(1), 234-237.

Spraker, T. R. (1993). Stress and capture myopathy in artiodactylids. In M. E. Fowler (Ed.), *Zoo and Wild Animal Medicine: Current Therapy* (Vol. 3, pp. 481-488). Philadelphia: W.B. Saunders Company.

Spraker, T. R., Adrian, W. J., & Lance, W. R. (1987). Capture myopathy in wild turkeys (*meleagris gallopavo*) following trapping, handling and transportation in Colorado. *Journal of Wildlife Diseases, 23*(3), 447-453.

Stephenson, B. M., Gaskin, C. P., Griffiths, R., Jamieson, H., Baird, K. A., Palma, R. L., & Imber, M. J. (2008). The New Zealand storm-petrel (*Pealeornis maoriana* Mathews, 1932): first live capture and species assessment of an enigmatic seabird. *Notornis, 55*(Part 4), 191-206.

Teal, P. J. (1989). *Movement, habitat use and behaviour of Australasian bittern (Botaurus poiciloptilus) in the lower Waikato wetlands.* University of Waikato Hamilton.

Tortora, G. J., & Grabowski, S. R. (2003). *Principles of Anatomy and physiology: tenth edition*. New York: John Wiley & Sons, Inc.

Ward, J. M., Gartrell, B. D., Conklin, J. R., & Battley, P. F. (2011). Midazolam as an adjunctive therapy for capture myopathy in bar-tailed godwits (*Limosa lapponica baueri*) with prognostic indicators. *Journal of Wildlife Diseases, 47*(4), 925–935.

Whitworth, D., Newman, S., Mundkur, T., & Harris, P. (2007). *Wild Birds and Avian Influenza: an Introduction to Applied Field Research and Disease Sampling Techniques.* http://www.fao.org/docrep/010/a1521e/a1521e00.htm (accessed 25 April 2013). Food and Agriculture Organisation of the United Nations (FAO).

Wickes, C., Crouchley, D., & Maxwell, J. (2009). Takahe (*Porphyrio hochstetteri*) Recovery Plan 2007–2012. Wellington: Department of Conservation.

Williams, E. S., & Thorne, E. T. (1996). Exertional Myopathy (Capture Myopathy). In A. Fairbrother, L. N. Locke & G. L. Hoff (Eds.), *Noninfectious Diseases of Wildlife* (Second ed., pp. 181-193). London: Manson Publishing Ltd.

Williams, M. (1995). Social structure, dispersion and breeding of the Auckland Island teal. *Notornis, 42*(4), 219-262.

Wingfield, J. C. (2003). Control of behavioural strategies for capricious environments. *Animal Behaviour, 66*, 807-815.

Wobeser, G. A. (1997). *Diseases of Wild Waterfowl* (Second Edition ed.). New York: Plenum Press.

Wolfaardt, A. C., Williams, A. J., Underhill, L. G., Crawford, R. J. M., & Whittington, P. A. (2009). Review of the rescue, rehabilitation and restoration of oiled seabirds in South Africa, especially African penguins *Spheniscus demersus* and Cape gannets *Morus capensis*, 1983-2005. *African Journal of Marine Science, 31*(1), 31-54.

Yerex, D. (2001). *Deer: the New Zealand Story*. Christchurch: Canterbury University Press.

Youl, J. M. (2008). *An investigation into lead exposure in free-ranging kea (Nestor notabilis), takahe (Porphyria hochstetteri) and Australasian harriers (Circus approximans) in New Zealand.* Masters of Veterinary Science, Massey University, Palmerston North.

CHAPTER TWO

A STUDY ON LAYER HENS (*Gallus domesticus*) AS A SURROGATE FOR ASSESSING THE PHYSIOLOGICAL IMPACTS OF CAPTURE AND HANDLING ON KIWI (*Apteryx spp*.)

Abstract

The management of many populations of highly threatened kiwi taxa (*Apteryx spp*.) requires the capture and handling of some individuals for monitoring, translocations and research. While this management intervention is justified and highly useful to kiwi conservation it does pose potential physiological and health impacts to the birds. In this study layer hens (*Gallus domesticus*) were used as surrogates to model theses physiological impacts.

Hens in a treatment group went through a simulated field kiwi capture event entailing a chase, capture and handling scenario compared to a control group that were contained in box or small room. Both groups were serially blood sampled over 72 hours after which all birds were euthanized and their muscles examined histopathologically.

To assess the physiological impact of capture, biochemical analytes measured included plasma concentrations of the enzymes creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and the stress hormone corticosterone (CORT).

The chase event in the treatment hens elicited a stronger stress response compared to normally sampling protocol in the control hens . There was some indication of increased muscle damage occurring in the control group but this was confounded by sample week. The hens were found to have altered physiology at the commencement of the study, probably due to the high metabolic demands of egg production. The significant variation in their 'normal' physiology and consequent physiological response to the trail led to the conclusion that layer hens are inappropriate surrogates to use in models for kiwi or other wild birds.

2.1 INTRODUCTION

The capture and handling of wildlife is an indispensable component of intensive conservation management in New Zealand and elsewhere. While least disturbance management is preferred there are occasions in intensive conservation management or research that require species to be captured in order to perform certain procedures or obtain certain information (Powell & Proulx, 2003; Holzapfel *et al.*, 2008). However with every capture and handling event the animal being sampled or tagged has the potential to be injured or have its normal physiology or behaviour altered (Southern & Southern, 1983; Kreeger et al., 1990; Williams & Thorne, 1996; Cattet et al., 2008; Jacques et al., 2009). For this reason each conservation/research programme that uses these capture techniques must not only have a justifiable reason for doing so (Ponjoan *et al.*, 2008), but should also actively seek out ways to minimise the impacts upon the animal, within the constraints of personnel safety and practicality (Cattet *et al.*, 2008). As part of threatened species management wild kiwi (*Apteryx spp.*) are captured for monitoring, sampling or translocations. The aim of this study is to investigate the physiological health impacts of routine capture and handling on kiwi using layer hens (*Gallus domesticus*) as a surrogate with which to model the impacts of these protocols.

2.1.1 Status, Ecology and Management of Kiwi (*Apteryx spp.*)

Kiwi are endemic to New Zealand and are one of the most nationally valued and intensively managed birds in the country. Within the genus *Apteryx* there are five formal species: brown kiwi (*Apteryx mantelli*), tokoeka (*A. australis*), rowi (*A. rowi*), great spotted kiwi (*A. haastii*) and little spotted kiwi (*A. owenii*), (Holzapfel *et al.*, 2008). Within both brown kiwi and tokoeka there exist four geographically seperated and genetically distinct forms (Baker *et al.* 1995; Burbidge *et al.* 2003; Shepherd & Lambert 2008 as cited in Holzapfel *et al.*, 2008). The final decision on whether these forms are to be considered subspecies or species has not been made and each of the 11 taxonomic units mentioned is managed separately (Holzapfel *et al.*, 2008).

All kiwi taxa are threatened to some degree and under the New Zealand Threat Classification System range from nationally critical (rowi) through to range restricted (little spotted kiwi),

(Holzapfel *et al.*, 2008; Miskelly *et al.*, 2008). Although the decline of some managed populations of kiwi has been halted or reversed over the last 15-20 years, the overall population of some of the most abundant species (brown kiwi, great spotted kiwi, tokoeka) is still in decline (Robertson & Colbourne, 2003; Holzapfel *et al.*, 2008). In addition even though the decline has been halted in the most vulnerable taxa, rowi and Haast tokoeka (a form of *A. australis*), the small number of populations and small overall population size of these taxa make them the most vulnerable to extinction (Holzapfel *et al.*, 2008).

Kiwi populations have declined since the arrival of humans in New Zealand (Holzapfel *et al.*, 2008). Introduced predators such as cats and stoats pose a major threat to kiwi and in particular are responsible for high levels of mortality in juvenile and young kiwi (McLennan *et al.*, 1996). Reducing the rate of predation of young kiwi was identified as a key management component to halt the decline of brown kiwi (McLennan *et al.*, 1996).

In order to halt the decline of kiwi and combat the problem of high predation rates in young kiwi, BNZ Operation Nest Egg (ONE) was developed in 1994 (Bassett, 2012). ONE is a management technique that involves removing kiwi eggs and chicks from the wild and rearing them in captive institutions until they are large enough (~1000g) that they are considered to be out of the high risk period where they are more prone to predation, at which point they are released back into the wild (Bassett, 2012). Using this technique has increased the chances of a chick surviving to adulthood from 5% to 65% (Bassett, 2012). It has been used on all species of kiwi except little spotted kiwi and has proved extremely effective at quickly growing and establishing new populations particularly with the most vulnerable kiwi populations (Bassett, 2012). ONE has now become a highly valued conservation tool and has been used every breeding season since 1995 (Bassett, 2012) and has been instrumental in increasing the population of rowi and in helping boost productivity in Haast tokoeka (Holzapfel *et al.*, 2008). ONE has proved to be much more effective as a management tool in reversing population decline in brown kiwi compared to predator trapping or poisoning (Robertson *et al.*, 2011). While not all wild kiwi are handled, capture and handling is an essential part of ONE as the birds used are routinely caught for transmitter changes, translocations and monitoring (Colbourne *et al.*, 2005; Bassett, 2012). For kiwi health and welfare reasons and because it is often used for the vulnerable taxa, the impact of routine capture and handling kiwi should be assessed.

2.1.2 Capture and Handling Protocols Used for Kiwi (*Apteryx spp.*)

2.1.2.1 Capture impacts

The capture and handling of an animal can result in capture myopathy (muscle damage) due to the stressful nature of the event and exertion and struggling of animals during the process (Williams & Thorne, 1996). This muscle damage can be found in either the skeletal or cardiac muscle and is thought to be a result of the lactate generated by exerted muscle, causing local and metabolic acidosis. In the muscle tissue, the low pH causes an increased permeability of cell membranes and cellular damage (Bollinger *et al.*, 1989). Clinical signs of muscle damage may include weakness, muscle stiffness, ataxia, an increased respiratory and pulse rate, hyperthermia and depression. In extreme cases this can progress to disinterest in the animal's surroundings, recumbency, and potentially death (Williams & Thorne, 1996). In addition to the acute form of the disease, clinical signs of capture myopathy may take hours, days or even weeks to develop (Basson & Hofmeyr, 1973). Even a case of subclinical capture myopathy may potentially result in a loss of fitness through altered behaviours such as reduced foraging (Cattet *et al.*, 2008) or inability to escape or fight off predators. This is especially relevant given the major factor in kiwi decline is predation by introduced mammalian species (McLennan *et al*., 1996).

The occurrence of muscle damage can be determined by evaluating the serum levels of enzymes released from damaged muscle (Williams & Thorne, 1996). Creatine kinase (CK) and aspartate aminotranferase (AST) are considered the two most useful enzymes for this purpose (Williams & Thorne, 1996). Consequently a correlation between elevated concentrations of these two enzymes and the clinical signs of capture myopathy has been observed (Ward *et al.*, 2011). In particular, serum CK levels are considered the most appropriate indication of muscle damage in birds (Franson *et al.*, 1985), as this enzyme is much more specific to damaged muscle and responds more quickly compared to AST which is also released by other organs when damaged, such as the liver (Cardinet, 1989; Williams & Thorne, 1996).

In addition to potentially causing muscle damage, capture and handling also has the potential to cause a bird distress. Following a stressful event birds respond to the stimulus with both specific and nonspecific regulatory processes which govern a range of behavioural and

physiological changes that allow the bird to better meet the new challenge (Siegel, 1980). A characteristic of the avian nonspecific stress response is the secretion of the avian stress hormone corticosterone (CORT) from the adrenal glands (Siegel, 1980; Carsia & Harvey, 2000). Measurement of changes in the plasma concentration of CORT in birds can therefore be used to assess degree of stress a bird is experiencing (Cockrem *et al*., 2004). Plasma corticosterone has been used to assess the stress response of a range of species including kiwi (Adams, 2000) and layer hens (Beuving & Vonder, 1978). It is therefore an appropriate measure of the stress response for this study.

2.1.2.2 Kiwi Capture and Handling protocol

Many of the current management strategies of require routine capture and handling kiwi for a variety of reasons (Jolly & Colbourne, 1991; Robertson & Colbourne, 2003; McLennan *et al.*, 2004; Gasson, 2005; Robertson *et al.*, 2011; Ha *et al.*, 2013). The capture process used in a particular instance varies however the handling protocol used is similar and is based on best practice guidelines that have not been fully evaluated with regards to muscle damage.

2.1.2.2.1 Capture

Kiwi are captured during both the day and night and the techniques used for capture depend on this, the operators experience, the terrain and population densities. Kiwi may be located by radio transmitter, trained dogs or by plotting the calls of male birds. Night time captures include capturing active kiwi in light forest understory, either by hand or using a hand net. Alternatively a set net (mist or fishing net) can be positioned during the day outside the birds burrow. The net and burrow are observed and the bird can be captured when it emerges after dark. Daytime captures involve partially digging out and/or extracting the bird from its daytime burrow using bare hands. Birds are removed from the burrow by the handler grasping both the birds' legs with one hand at the unfeathered part of the tibiotarsus and slowly pulling the bird out (Robertson & Colbourne, 2003). Chicks are removed from burrows similarly (Bassett, 2012) but female birds are not captured in the breeding season (Robertson & Colbourne, 2003). Some kiwi are flighty and will desert their burrows when people approach (Robertson & Colbourne, 2003). In particular Haast Tokoeka are described as very active flighty birds at capture which often require pursuits to capture them even

during day captures (*pers. comm.* G. Hopkins 16th April 2010). Captures of kiwi which involve pursuits probably have the most impact on the bird due to increased stress and exertion in the bird. One method uses a trained kiwi dog to capture kiwi in the first few hours of the night. Kiwi are located and consequently called in by a handler who mimics kiwi calls. When the kiwi are close by the dog is released by the handler to indicate and locate the kiwi which is then caught by the handler (Robertson & Colbourne, 2003). A great spotted kiwi was found dead in its burrow the day after it had been pursued unsuccessfully by a kiwi ranger and their kiwi dog. The bird was submitted for post mortem but no cause of death was determined.

2.1.2.2.2 Handling

Once removed from the burrow or upon capture of the kiwi, the handler uses one hand to take hold of the bird at the bare part of both legs. The bird is then inverted while the handler supports the kiwi's body by resting it on their free arm or across their knees. The legs of the kiwi are then taped together with electrical tape around the tarsus while its body continues to be supported. Special training and care is needed when handling kiwi as their lack of functional sternum, weak pectoral muscles and ribcage means they can be easily injured. If the kiwi needs to be held for an extended period of time (for example because the dug out burrow needs fixing) then protocol dictates the bird with legs still taped, is placed within a bird bag and hung from a nearby tree until the bird can be processed and released. Held and restrained in this manner a kiwi can have blood samples taken, be measured or weighed, and have a transmitter or band attached (Robertson & Colbourne, 2003). Chicks are handled similarly (Bassett, 2012). Field weights of kiwi are taken by suspending the kiwi upside down, supported by the Pesola spring balance hooked through the tape binding their legs. Once the bird has stopped struggling a weight can be taken (Robertson & Colbourne, 2003). The total handling times are to be kept as short as possible to minimise stress on the bird, however no maximum handling time is currently set out in the kiwi best practice manual (Robertson & Colbourne, 2003). The duration of the handling event is variable between handlers and will change depending on the purpose of the capture event and even among species but is usually between 15-40 minutes (*pers. comm.* G. Hopkins 16th April 2010; *pers. comm.* D. Kay 16th April 2010; *pers. comm.* S. Yong 16th April 2010). Kiwi are often released with little post-capture monitoring, for unusual behaviour/signs of distress, often being placed straight back in to burrows (Robertson & Colbourne, 2003).

There is some evidence that prolonged handling of kiwi may result in both subclinical and clinical capture myopathy in the field, with three of the four great spotted kiwi sampled during a translocation exhibiting elevated CK values, one of which was extremely high (Gasson, 2005). In this operation the kiwi were transported in bags by helicopter with their legs taped together which may have contributed to the degree of muscular damage seen.

Any factors which are a health risk and may be potentially hindering kiwi survival or reproductive success should be investigated further so that they can be ruled out or refinements can be made. Another subtler consequence is that if capture and handling is affecting animal fitness and survival, even indirectly it can cause bias and confound the data (Williams & Thorne, 1996). This may have implications in some research for example Robertson *et al.* (2011) captured kiwi and used the survival data generated by these individuals to assess management techniques. If capture was negatively affecting kiwi then this could bias their findings and therefore the assessment of certain management techniques.

Ideally this study would have been carried out on kiwi in the field; however permission for sampling was denied by the Kiwi Recovery Group, citing the rarity of some of these taxa, the variable conditions of sampling in the field and the need to keep handling times to a minimum. I was therefore compelled to consider the use of layer hens as a surrogate species with which to model the impacts of capture and handling on kiwi.

2.1.3 Suitability of Layer Hens (*Gallus domesticus*) as a Surrogate for Assessing Handling Protocols used for Kiwi (*Apteryx spp.*)

Layer hens were chosen as a surrogate for kiwi because they are an accessible and morphologically similar species with which to model the physiological impacts of capture and handling on kiwi. Kiwi are flightless birds with only vestigial wings, however they have strong legs and are good runners. Depending on the species adult birds weigh between 1000-4000g (Reid & Williams, 1975; Heather & Robertson, 2005 as cited in Holzapfel *et al.*, 2008). The closest living relatives of kiwi are emus and cassowaries (Cooper et al., 1992) and while these species and other ratites share morphological similarities with kiwi, the vast difference in size and weight, and accessibility for research means these birds were not suitable surrogates. Therefore layer hens selected to be in a similar weight range to kiwi were

chosen as suitable surrogates. While they are not totally flightless they spend most of their time upon the ground. Along with other domesticated chicken breeds, layer hens are descended from red jungle fowl (*Gallus gallus*) (Fumihito *et al.*, 1994).

2.1.4 Specific Aims of the Study

The main objective of the study was to assess current capture and handling practices used for kiwi and if possible suggest refinements to the process based on the results found using chickens as surrogates. To this end, this study investigated the physiological changes in layer hens in response to capture and handling.

The specific aims of the study were to:

1) use layer hens to simulate capture (chase vs. burrow capture) and handling protocol (blood sampled and handled treatment vs. blood sampled and boxed control) of wild kiwi;
2) determine plasma biochemical changes indicative of muscle damage, selected organ functions and the stress response to capture and handling;
3) assess the potential health impacts of the procedures on the layer hens; and
4) extrapolate from this study conclusions on the current capture and handling process of wild kiwi and, if relevant, suggest refinements or further research.

2.1.5 Hypothesis

It is expected that capture and handling will cause layer hens to experience subclinical muscle damage and exhibit a stress response as shown by an increase in circulating plasma concentrations of muscle enzymes and stress hormones.

2.1.6 Approval for Study

All procedures were carried out with permission from the Massey University Animal Ethics Committee (MUAEC 10/110).

2.2 MATERIALS AND METHODS

The experiment took place over two weeks at the Massey University Poultry Research and Feed Processing Unit (Poultry Farm Road, Palmerston North, New Zealand) with a separate group of control and treatment chickens in each week.

2.2.1 Experimental Methodology

Due to the logistics of repeated blood sampling birds at regular intervals it was necessary to separate the trial in to two separate study weeks, with a different group of birds in each week. In experimental week one (17-23 January 2011) 16 layer hens were obtained from a poultry egg-laying farm. These chickens were briefly put under a general anaesthetic at Massey University in order to place a catheter in the medial metatarsal vein and in one case the brachial vein. After catheter placement these birds were moved 5 minutes by road to the Massey Poultry Research Unit. Half of the birds became control birds and half became treatment birds. In week two (24-31 January 2011) 18 layer hens were obtained from the poultry farm; 10 of these were put in the control group and 8 were put in the treatment group. These birds were transported directly to the Massey Poultry Research Unit and did not undergo anaesthetic or catheter placement.

The birds used were HyLine Brown hens sourced from Turks Poultry farm (108 Purcell Street, Foxton, New Zealand) and were taken at 47 weeks of age and selected by the staff to be in good physical condition. The birds were selected and removed from the cage by a member of staff and carried upside down with between one to three birds in each hand. The birds were then given a quick physical examination to see if there were any obvious broken bones or lameness. One bird was rejected and replaced as she was considered significantly lower in body condition then the other birds. The birds were placed in chicken crates for transport with either eight or nine birds in a crate. The chickens were transported in the crates for approximately 40 minutes by road to the Poultry Research and Feed Processing Unit. At the poultry unit each bird was given a band so that it could be individually identified and randomly placed in one of two separate but identical rooms. The birds in one of these rooms

became treatment birds while the birds in the other became control birds. The trial commenced the following day.

Sampling started each day around 8am and alternated between treatment groups where possible. When it was time for sampling, the handler would enter the treatment or control room, identify and retrieve the specific bird by cornering it and picking it up by hand. A blood sample was performed using heparinised 25 gauge needles and 1ml syringes and between 0.5 ml and 1 ml of blood was taken from either a placed catheter, the medial metatarsal vein, the brachial vein or occasionally from the jugular vein. If a sample was taken from a catheter then the catheter was first flushed with 0.5ml of heparinised saline solution and then the first 0.6 ml of fluid withdrawn from the catheter was discarded. All birds with catheters had them flushed at the end of each day also with 0.5 ml of heparinised saline solution.

Control birds were handled and blood sampled at 0, 30, 60, 120 minutes and 24, 48, 72 hours after capture. Between samples at 0, 30, 60, 120 minutes, the control chickens were kept individually in a ventilated box near the sampling area. At 120 minutes they were returned to their rooms and for the 24, 48, 72 hour samples the chickens were picked up from their room and taken to the sampling area, blood sampled and then returned to their room.

All birds in treatment and control groups were handled by necessity for blood sampling at each time point. During this process the birds were carefully removed from either the box (30, 60, 120 minute samples) or from the housing room (0 minute, 24, 48, 72 hour samples) and held either on their backs with their bodies supported with one wing extended or on their side with their body supported and one leg outstretched. Following the blood sample, the bird was held for a short period to ensure haemostasis of the needle site had occurred and then returned to the box or room.

Treatment birds were also handled and sampled at 0, 30, 60, 120 minutes and 24, 48, 72 hours. However in order to simulate the chase that can often happen in wild kiwi capture, treatment birds were first taken from the pens and blood sampled (time = -10 minutes) and then given a simulated chase event where the chickens were walked around an open room by the handler who kept them moving. The chicken was ‘chased’ in this manner until the sample at time 0 needed to be taken. Once the time 0 sample had been taken the chicken was given a ‘handling event.’ This involved additional handling of the chicken in accordance with the handling protocol from the kiwi best practice guide (Robertson & Colbourne, 2003). Firstly

the bird had its legs taped together at the tarsus just below the hock joint, using insulation tape. The bird was then weighed by suspending it from the tape used to bind its legs. All treatment birds were then held by a single handler in the same manner. As prescribed by the kiwi best practice guide, the bird was then held supported on its back with the handler using one hand firmly holding the bound legs and the other hand (or person's knees) to support the birds back. The insulation tape binding the legs was removed immediately prior to the blood sample at 30 minutes. Between samples at 30, 60, 120 minutes, the treatment chickens were kept individually in a ventilated box near the sampling area. At 120 minutes they were returned to their rooms and for the 24, 48, 72 hour samples the chickens were picked up from their room and taken to the sampling area, blood sampled and then returned to their room.

2.2.2 Laboratory Methodology

The heparinised blood samples were kept on ice and within 10 hours of being taken the samples were centrifuged at 5000 rpm for five minutes. The plasma was then pipetted off into separate microcentrifuge tubes before being frozen and stored at -20°C until biochemical analysis could be performed.

The plasma was submitted to a commercial lab and batch-analysed at New Zealand Veterinary Pathology (Palmerston North, New Zealand). The biochemical parameters measured for all samples were CK, AST and glutamate dehydrogenase (GLDH). The plasma CORT concentration was analysed at the Institute of Veterinary, Animal & Biomedical Sciences, Massey University, Palmerston North, New Zealand. The concentration of CORT was measured by radioimmunoassay using a method that is well established for the measurement of CORT in birds (Cockrem *et al.*, 2009; Adams *et al.*, 2010; Cockrem *et al.*, 2012).

2.2.2.1 Determining the concentration of CK in the plasma

The method used to determine the concentration of CK in the samples is derived from the formulation recommended by the International Federation of Clinical Chemistry (IFCC) (Schumann *et al.*, 2002a). The CK assay used is an automated photometric assay using a Roche/Hitachi P800 analyser. The test principle is as follows: substrates (creatine phosphate

and ADP) and buffer are added to the sample, and the CK in the sample transfers the phosphate group from creatine phosphate to ADP, giving creatine and ATP as products. A second enzyme (hexokinase) is added which converts glucose to glucose-6-phosphate (G6P) using the ATP produced in the first step. A third enzyme (glucose-6-phosphate dehydrogenase) is added which then oxidises the G6P produced in the second step to gluconate-6-phosphate while reducing NADP to NADPH. The rate of formation of NADPH is measured photometrically by the increase in absorbance and is directly proportional to the CK activity in the sample. Extra reagents are also added to activate the CK enzyme if it has been oxidised during sample collection, and to inhibit other enzymes which might otherwise interfere with the test.

2.2.2.2 Determining the concentration of AST in the plasma

The method used to determine the concentration of AST in the samples is an optimised method derived from the formulation recommended by the International Federation of Clinical Chemistry (IFCC) (Schumann *et al.*, 2002b). The method used is an automated photometric assay using a Roche/Hitachi P800 analyser. The test principle is as follows: substrates (aspartic acid, α-ketoglutarate) and buffer are added to the sample. The AST present in the sample catalyses the reaction of the substrates into glutamic acid and oxaloacetate. The rate of oxaloacetate formation gives the AST activity in the sample so the increase in oxaloacetate is determined by adding a second enzyme (malate dehydrogenase) which converts the glutamic acid to malic acid while oxidising NADH to NAD. The rate of decrease of NADH is directly proportional to the rate of formation of oxaloacetate, thus photometrically measuring the rate of decrease in NADH gives the AST activity in the sample.

2.2.2.3 Determining the concentration of GLDH in the plasma

The method used to determine the concentration of GLDH in the samples is derived from the formulation recommended by the German Society for Clinical Chemistry (DGKC) (Anonymous, 1972). The GLDH assay used is an automated photometric assay using a Roche/Hitachi P800 analyser. The test principle is as follows: substrates (α-ketoglutarate, NADH, ammonia) and buffer are added to the sample and the GLDH in the sample acts a catalyst and reduces the α-ketoglutarate and adds an amino group to yield glutamic acid and

NAD as products. The amount of NADH consumed is measured photometrically by the decrease in absorbance and is directly proportional to the GLDH activity in the sample.

2.2.2.4 Determining the concentration of CORT in blood samples

Corticosterone concentrations in plasma diluted in phosphate buffered saline with gelatine (PBSG) were measured by radioimmunoassay by the method used by Cockrem *et al.* (2008). Plasma samples were initially spun for 10 minutes at 18, 000×g to separate lipid from the plasma. The clear plasma from below the lipid layer was transferred to another tube and diluted in PBSG for assay. Samples were assayed in duplicate. Ten microlitres of diluted plasma were incubated for 2 hours at room temperature (22–25°C) with iodinated corticosterone and antiserum from a Corticosterone Radioimmunoassay Kit (MP Biomedicals, USA). Precipitant solution (MP Biomedicals, USA) was added and each sample vortexed thoroughly, then 50 µl egg white (10 g/l dried egg white [Sigma] in PBSG) was added to increase adhesion of the pellet to the tube after centrifugation. The samples were incubated for 15 minutes at room temperature to separate bound and free corticosterone, and then centrifuged for 15 minutes, and the supernatant aspirated, and the pellets were counted on a LKB Wallac 1261 Multigamma gamma counter.

The sensitivity of the corticosterone assay was the minimum hormone level that could be consistently distinguished from zero. It was determined as the hormone concentration at the mean - 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity was 0.52 ng corticosterone/ml of plasma.

Solutions of corticosterone in PBSG at concentrations that gave approximately 80, 50 and 20% binding on the standard curve were used as low, medium and high quality controls in every assay. The mean concentrations of corticosterone in these solutions were 234.9$\pm$19.8, 574.2 $\pm$ 47.2 and 1769.5 $\pm$ 127.7 pg/ml respectively. The intra-assay coefficient of variation for each solution was determined by conducting an assay with twenty duplicates of each solution. The intra-assay coefficients of variation for corticosterone were 8.4%, 6.0% and 7.2% for low, medium and high solutions respectively. Inter-assay coefficients of variation were calculated from duplicates of the solutions included at the beginning and end of each assay. The inter-assay coefficients of variation for ten assays were 7.9%, 8.4% and 11.5% for low, medium and high solutions respectively.

2.2.3 Statistical Methodology

Statistical analyses on the data were performed using PASW Statistics 18 for Windows 7.0 (SPSS Inc., Chicago, Illinois, USA). All results are presented as means (±S.E.M), unless otherwise stated.

The data were compared between sampling weeks one and two to see if they could be pooled for further analysis between the control and treatment groups. To do this a one-way analysis of variance (ANOVA) was used to compare the starting concentration of CK, AST and CORT in the first sample. For this analysis the first sample of the control group (0 min) and the treatment group (-10 minutes) were pooled within each week. The CORT needed a natural log transformation to achieve homogeneity of variances. The GLDH data were not normally distributed and normality could not be achieved by transforming the data. A Mann-Whitney test was therefore used to compare the starting concentrations of GLDH between the sampling weeks

All repeated measures of CK, AST, and GLDH were assessed for significant differences over time and between the treatment and control groups during week one and separately during week two. A multivariate repeated-measures ANOVA was used to compare the samples from 0 minutes through 72 hours. This method of analysis does not allow for missing values and therefore only birds that had all seven blood samples taken were included in the repeated measures analysis. When comparing control and treatment groups during sampling week one a natural log transformation was performed on the data sets for CK and CORT data to achieve normality and homogeneity of variances. The GLDH data were not normally distributed and it could not be achieved by transforming the data. A Mann-Whitney test was therefore used to compare the concentrations of GLDH between control and treatment groups at each sampling point and a Friedmans Test was used to assess for significant differences over time in week one and separately in week two.

In order to determine the effect of the ‘chase event’ on the physiology of the treatment birds a repeated-measures ANOVA was performed birds on just the treatment group using the -10 minutes and 0 minutes. This was performed separately in week one and week two.

In order to determine whether the delay between picking a chicken up from its pen and taking the first blood sample (either time 0 for control birds or -10 min for treatment birds) had an

effect on baseline levels of the analytes measured, the starting plasma concentrations for each of the parameters was plotted against the pre-sample time and a linear regression carried out. This was done separately within week one and week two. The pre-sample time data were not available for every bird. To determine whether the delay between picking a chicken up from its pen and taking the first blood sample) had an effect on further analysis between control and treatment birds a one-way ANOVA was used to compare the first blood sample of control and treatment birds in week one and again separately in week two.

2.3 RESULTS

In week one, one of the birds was behaving abnormally and on closer inspection it was found to have a broken wing; this bird was excluded from sampling and received veterinary attention. Upon post mortem one bird from week one and one bird from week two were also found to have a broken wings; the samples from both these bird were excluded from analysis. In week two I was unable to obtain a blood sample from two further birds so they were also excluded from analysis. Out of the 34 chickens this left a sample size of 29 birds including 14 control birds and 15 treatment birds.

2.3.1 Confounding Factors in the Analysis

2.3.1.1 Bird with liver damage

One of the birds exhibited extremely high plasma GLDH concentrations in all samples but particularly in the samples taken on the first day which were 50-100 times higher than most of the other birds. The plasma AST concentrations of this bird were also around twice that of other birds. This is a strong indication of acute liver disease in this bird and it was decided to exclude this bird from further analysis resulting in 14 control birds and 14 treatment birds.

2.3.1.2 Sampling Week Differences

Chickens sampled in week one were found to have significantly different initial concentrations of the biochemical analytes at the first blood sample compared to chickens sampled in week two. At the first sampling point (0 minutes for control birds and -10 minutes for treatment birds pooled together) the concentration of plasma CORT in chickens sampled in week one was approximately twice that of those sampled in week two (P=0.001). Starting plasma concentrations of CK, AST and GLDH were not significantly different between chickens in week one and week two (P =0.275; P =0.546; P=228 respectively). The difference in plasma CORT between sampling weeks could have a confounding effect on the treatment versus control analysis and therefore the analysis between control and treatment birds was carried out separately for week one and week two.

2.3.2 The Effect of Pre-sample Time on the Starting Concentration of the Analytes

The plasma biochemistry parameters analysed from the first sample taken from each bird were affected by the pre-sample time (length of time between picking a bird up and the sample being taken). The average pre-sample time for chickens in week one was 182±27 seconds (mean±SE) and in week two was 245±34 seconds (mean±SE).

In order to determine whether the starting concentrations of the analytes were affected by the pre-sample time I plotted the concentration of each analyte in the first sample (0 minutes for control birds, -10 minutes for treatment birds) against the length of time it took to achieve the sample. An anlaysis of the correlation between the two variables was then performed.

While a there appeared to be a slight linear relationship between the pre-sample time and the concentration of plasma CORT (Figure 2.1) it was not significant either week (P=0.0816 and P=0.113). The other analytes also did not exhibit significantly higher plasma concentrations in the first sample as pre-sampling time increased in either week one (CK P=0.422, AST P=0.427, GLDH P=0.162) or week two (CK P=0.384, AST P=0.452, GLDH P=0.673).The concentration of the analytes was also not significantly different in the first sample between control and treatment groups in either week one (P=0.598) or week two (P=0.985). Therefore even control and treatment birds were considered affected similarly up to this point and further analysis comparing the treatment groups within each week was considered valid.

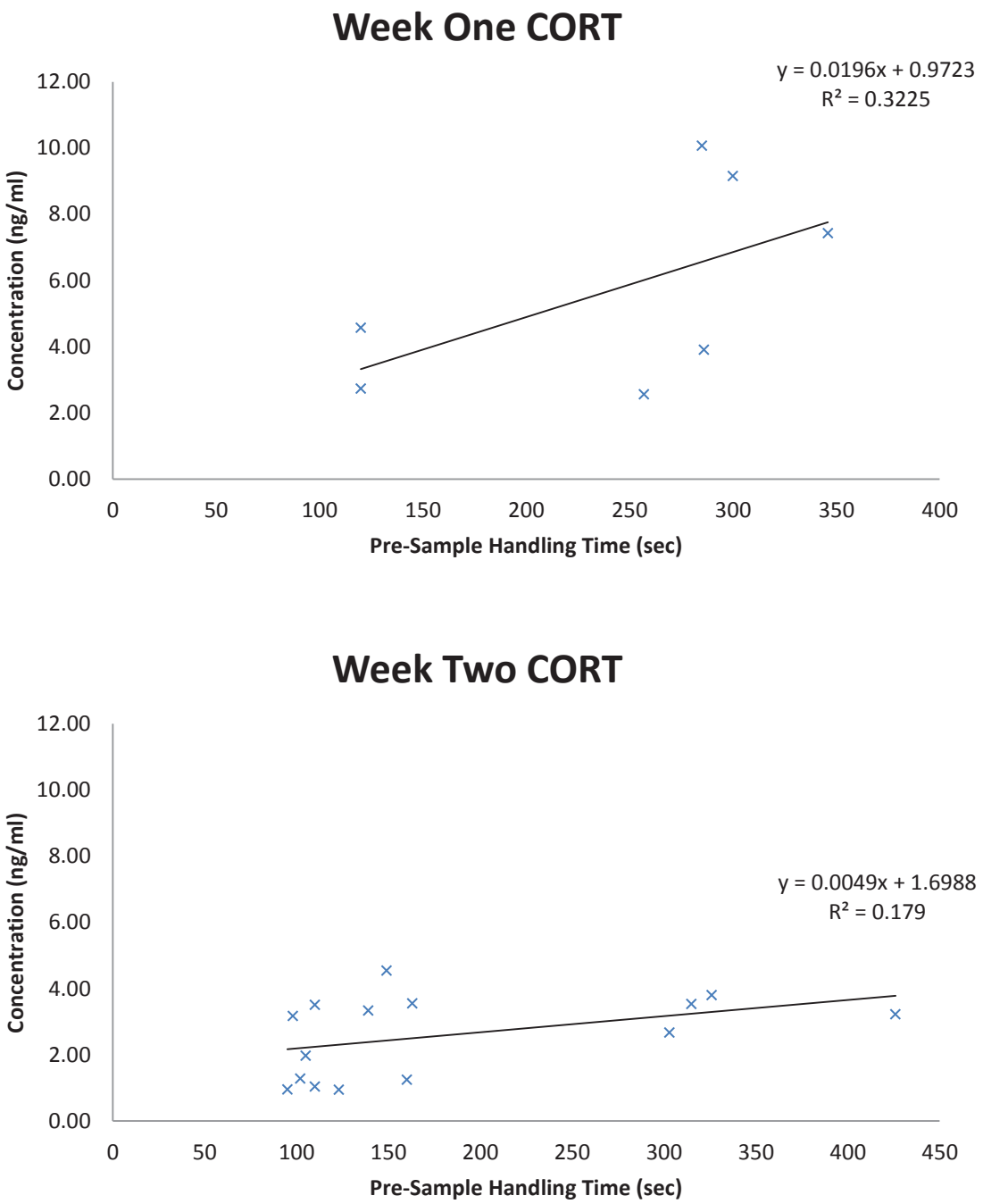


Figure 2.1 The effect of the length of pre-sampling time, between picking up a chicken and blood sampling (at the 0 minutes sampling point) on the starting plasma concentration of corticosterone (CORT) in layer hens (*Gallus domesticus*) in New Zealand, January 2011. Control and treatment groups are pooled as the protocol for both groups at this point is identical.

2.3.3 Physiological Responses of Chickens to Capture and Handling

Chickens showed a measurable physiological response to 'capture and handling' and exhibited significant differences between treatment and control groups, however the effect of sampling week confounded the analysis.

2.3.3.1 Chase Effects

The chase period (-10min to 0min) had a measurable physiological impact on chickens in the treatment group although there was some variation between weeks. Plasma CORT increased over the chase period. This increase was not significant during week one (P=0.194) but was significant during week two (P=0.011). In contrast the slight increase in plasma CK for chased birds was found to be significant in week one (P=0.033) but no difference was observed in week two (P=0.862). Plasma AST and GLDH were not found to change significantly in chased birds in either week one (P =0.340 and P =0.627 respectively) or week two (P =0.260 and P =0.417 respectively).

2.3.3.2 Effect of Capture and Handling on Muscle Physiology

The plasma concentration of CK peaked at 24 hour in both week one and week two before decreasing to approximately equal to or below the starting concentration. Despite the most dramatic peak occurring in the control group in week one the changes in CK concentration over time were not significant in week one (P=0.123), but were significant in week two (P=0.013). In week two *post hoc* analysis did not reveal any significant changes between specific sampling points. Similarly, the changes in concentration of AST were not significant in week one (P=0.123), but were significant in week two (P=0.023). In week two, *post hoc* analysis did not reveal any significant changes between sampling points and there appears to just be a general trend for AST concentrations to be higher around the 24, 48 and 72 hour sampling points.

In week one, chickens in the control group showed increased concentrations of CK (P=0.030) and AST (P=0.034) compared to chickens in the treatment group (Figure 2.2, Figure 2.3).

Plasma CK was consistently higher in the control group over all the sampling points and *post hoc* analysis revealed it to be significantly higher in control birds at 30 minutes (P=0.024) and 120 minutes (P=0.044). Also in week one, plasma AST was also consistently higher in the control group over all the sampling points and *post hoc* analysis revealed it to be significantly higher in control birds at 0 minutes (P=0.018), 30 minutes (P=0.022), 60 minutes (P=0.020), and 120 minutes (P=0.021). In contrast to this in week two both groups exhibited similar responses in the changing concentrations of CK (P=0.230) and AST (P=0.832) after capture and handling.

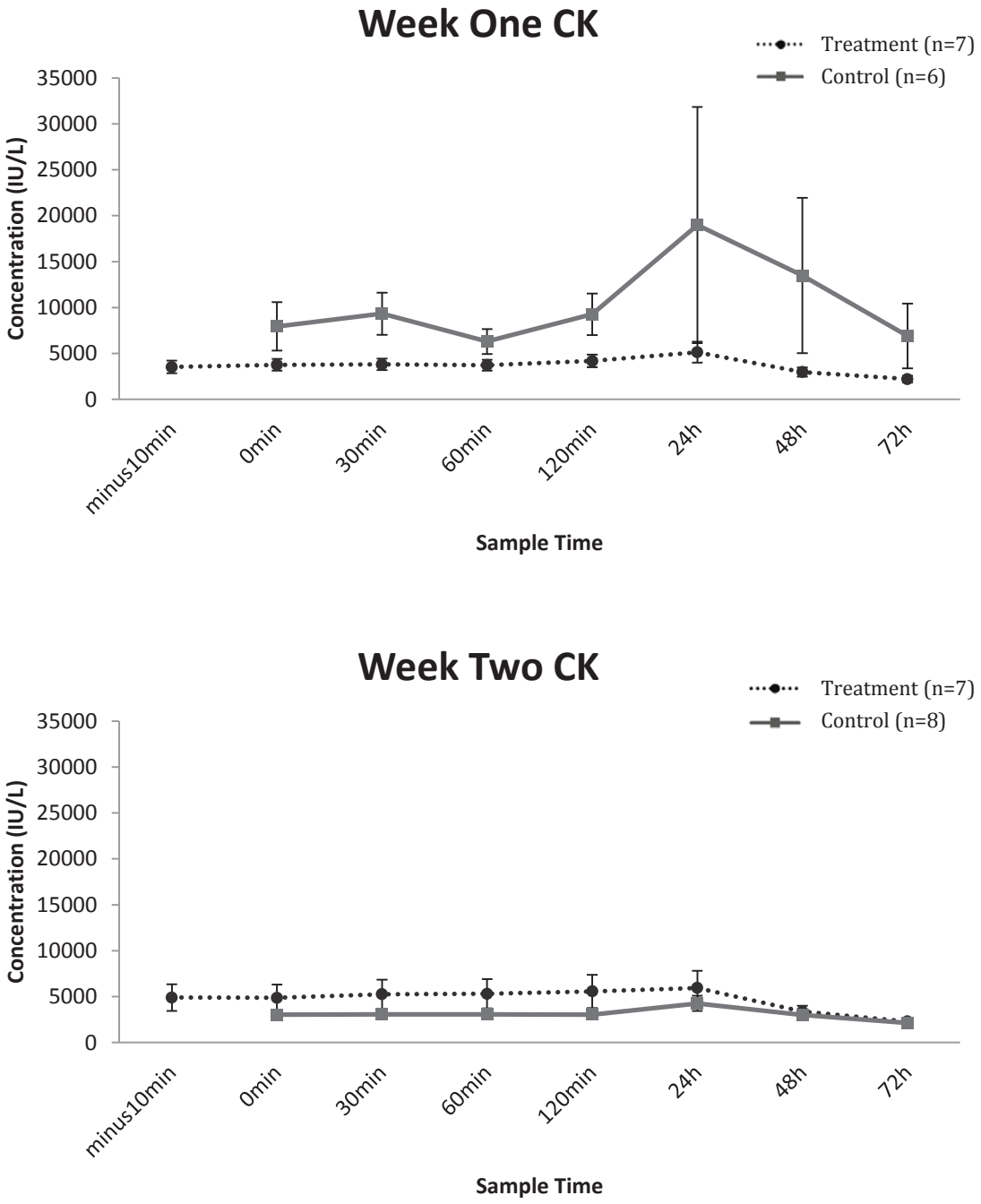


Figure 2.2 The effect of handling on plasma concentrations of creatine kinase (CK) in layer hens (*Gallus domesticus*) in New Zealand, January 2011. Values shown are means ± S.E.M. Individuals in the control group were immediately captured and blood sampled at 0 minutes, 30 minutes, 60 minutes and 120 minutes, and were placed in control between samples. They were again sampled at 24hr, 48hr and 72hr. The treatment group were subjected to a simulated kiwi capture event where after their first sample, they were chased for up to 10min then sampled at capture (0min), the birds were then handled for 30 minutes before blood sampling. Samples at 60 minutes to 72 hour followed the same protocol as control birds.

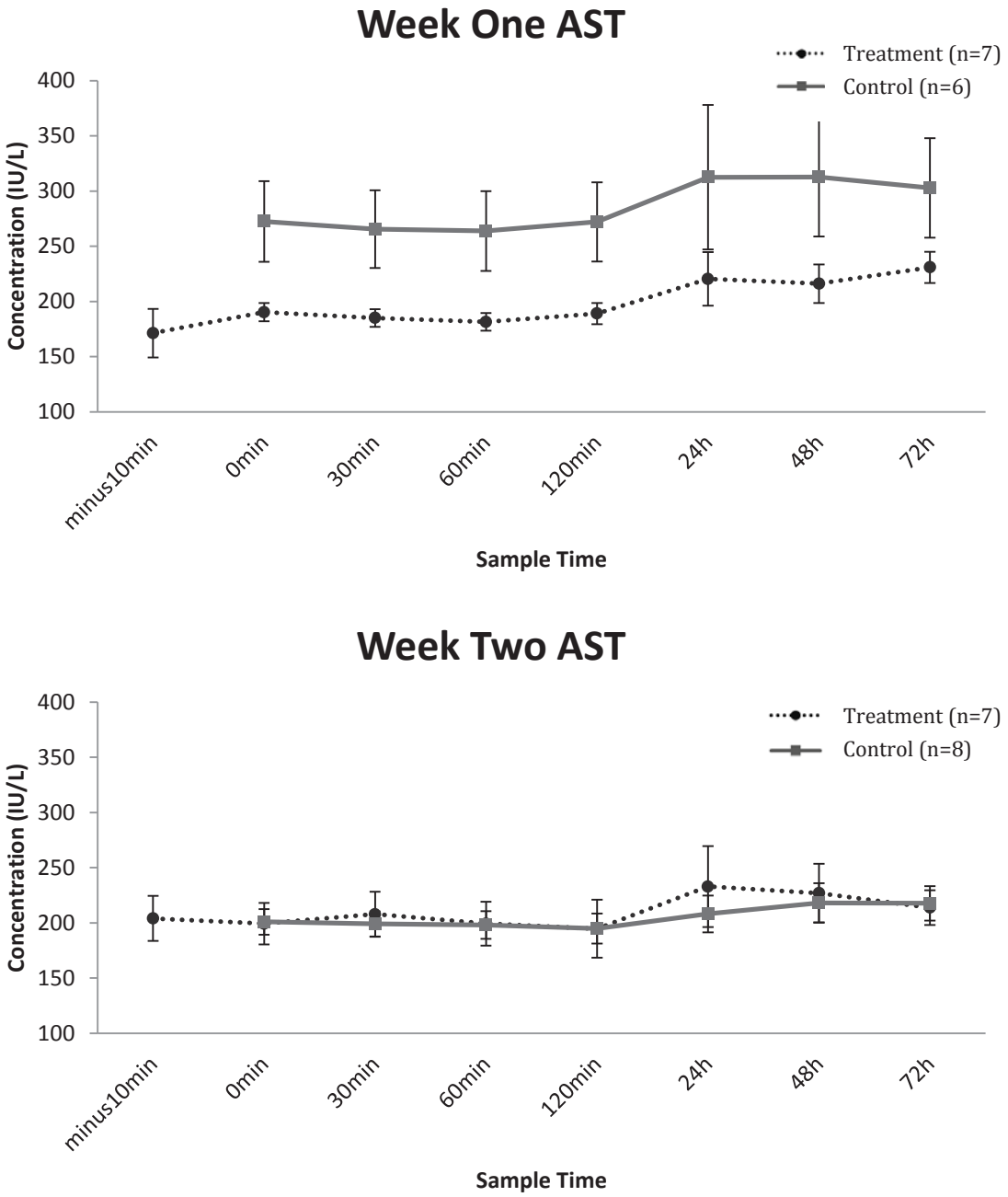


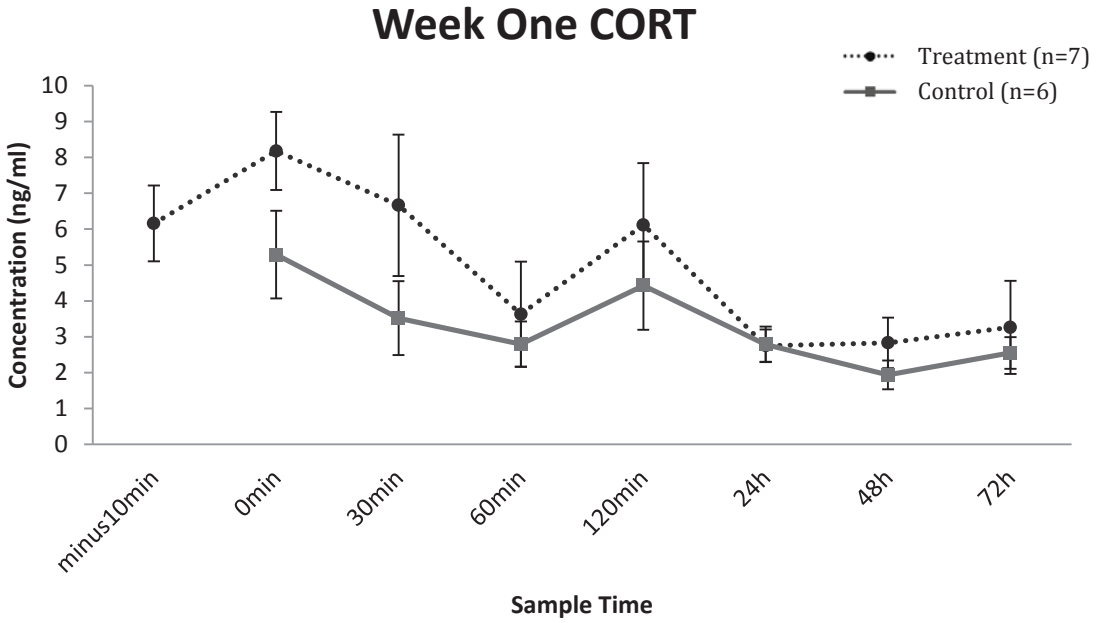
Figure 2.3 The effect of handling on plasma concentrations of aspartate aminotransferase (AST) in layer hens (*Gallus domesticus*) in New Zealand, January 2011. Values shown are means ± S.E.M. Individuals in the control group were immediately captured and blood sampled at 0 minutes, 30 minutes, 60 minutes and 120 minutes, and were placed in boxes between samples. They were again sampled at 24hr, 48hr and 72hr. The treatment group were subjected to a simulated kiwi capture event where after their first sample, they were chased for up to 10min then sampled at capture (0min), the birds were then handled for 30 minutes before blood sampling. Samples at 60 minutes to 72 hour followed the same protocol as control birds.

2.3.3.3 Effect of Capture and Handling on Stress Physiology

In both weeks birds in the treatment group showed higher plasma CORT concentrations at 0 minutes and 30 minutes compared to the control group (Figure 2.4), however the difference in CORT response between treatment groups was not significant in either week one (P=0.211) or week two (P=0.390).

Plasma CORT concentrations changed significantly over time in both week one (*P*=0.002)**,** and week two (*P*=<0.001). In week one, plasma CORT appeared to start at an elevated level for both groups before generally decreasing in concentration to below this initial concentration at the 24hr, 48, 72hr sampling points. *Post hoc* analysis of the data in week one revealed that the CORT concentrations at the 0 minutes sampling point were significantly higher than those at the 60 minutes (*P*=0.002), 24hr (*P*=0.015), 48hr (*P*=0.016) and 72 hour (*P*=0.015) sampling points.

In week two for both control and treatment groups, the initial concentration of plasma CORT in the birds was significantly lower then week one (*P*=0.001). The CORT response of the control group in week two remained relatively constant over time, however the treatment group showed a marked elevation of plasma CORT at the 0 minutes and 30 minutes sampling points due to the chase event (see above), before returning to similar concentrations to the first sample at -10 minutes. *Post hoc* analysis of the data in week two revealed that the CORT concentrations at the 0 minutes sampling point were significantly higher than at the 120 minutes (*P*=0.039), 24hr (*P*=0.040), 48hr (*P*=0.001) and 72 hour (*P*=0.002) sampling points. Also, the CORT concentrations at the 30 minutes sampling point were significantly higher than at the 48hr (*P*=0.047) and 72 hour (*P*=0.032) sampling points.



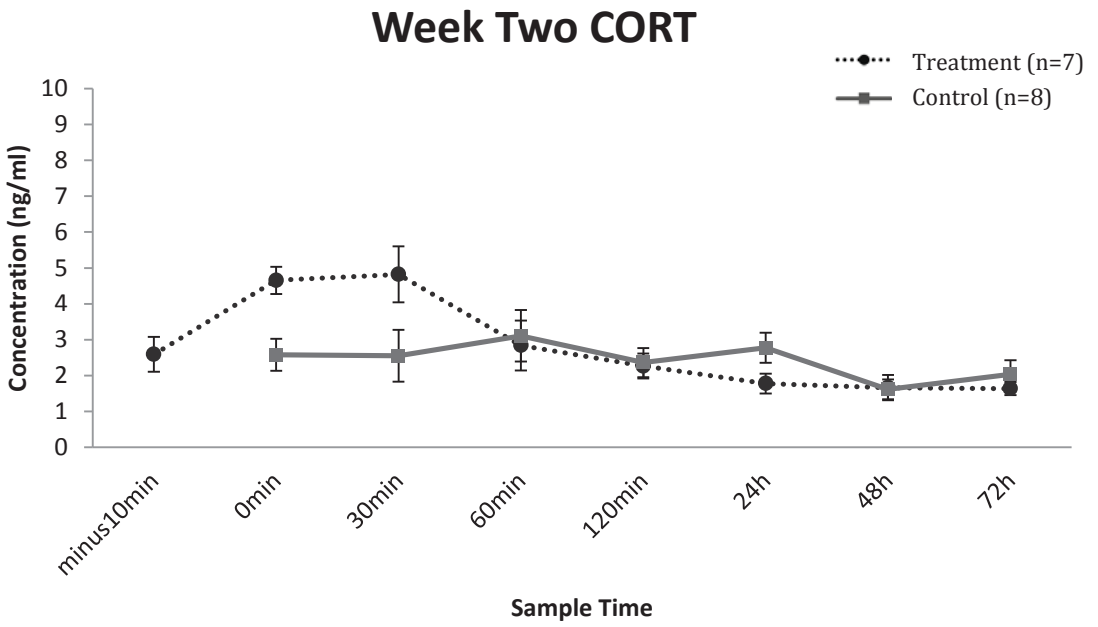


Figure 2.4 The effect of handling on plasma concentrations corticosterone (CORT) in layer hens (*Gallus domesticus*) in New Zealand, January 2011. Values shown are means ± S.E.M. Individuals in the control group were immediately captured and blood sampled at 0 minutes, 30 minutes, 60 minutes and 120 minutes, and were placed in boxes between samples. They were again sampled at 24hr, 48hr and 72hr. The treatment group were subjected to a simulated kiwi capture event where after their first sample, they were chased for up to 10min then sampled at capture (0min), the birds were then handled for 30 minutes before blood sampling. Samples at 60 minutes to 72 hour followed the same protocol as control birds.

2.3.3.4 Effect of Capture and Handling on GLDH

The hens showed low concentrations of plasma GLDH which did not change significantly over time or between control and treatment groups, in either week ($P>0.05$ for all).

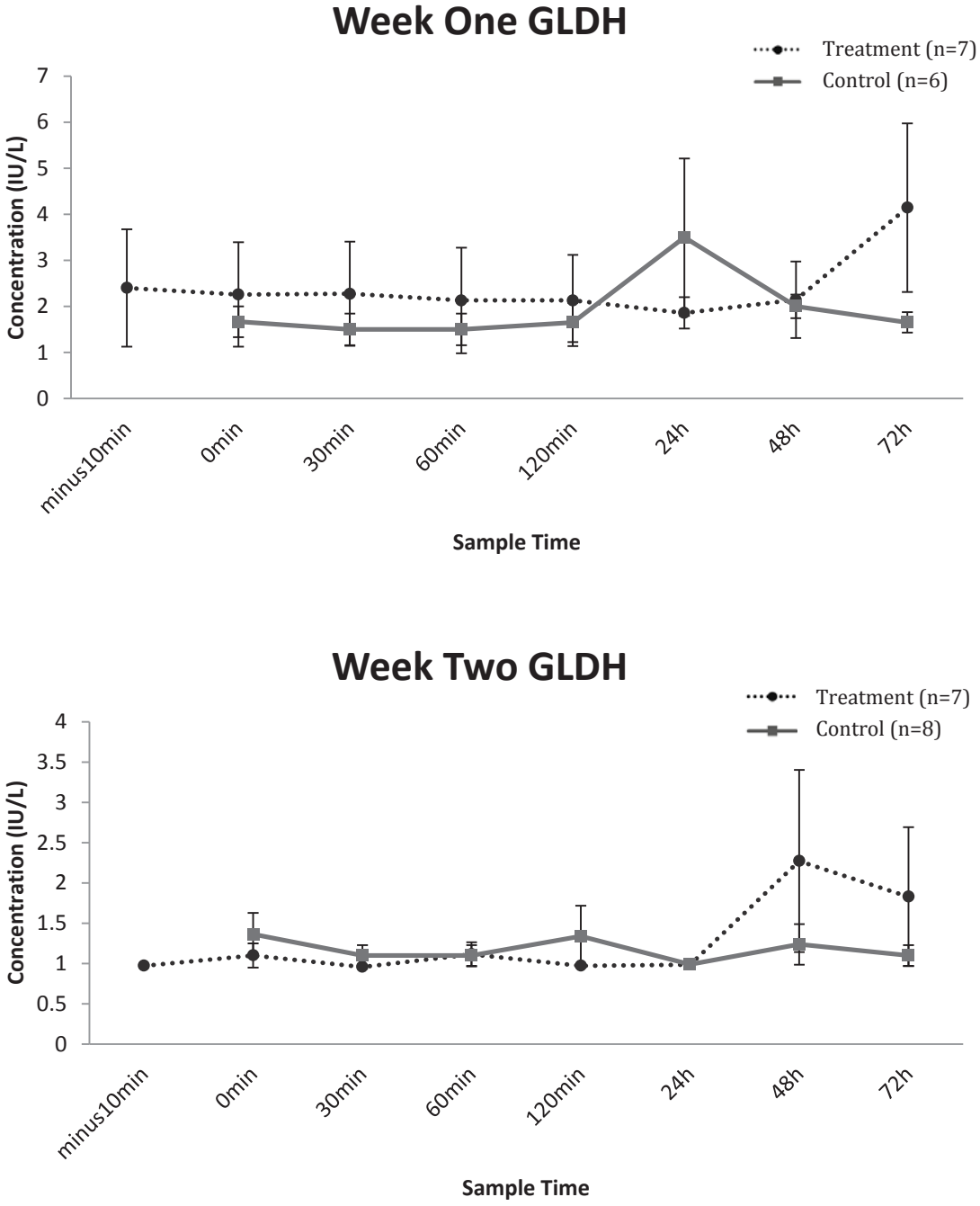


Figure 2.5 The effect of handling on glutamate dehydrogenase (GLDH) in layer hens (*Gallus domesticus*) in New Zealand, January 2011. Values shown are means ± S.E.M. Individuals in the control group were immediately captured and blood sampled at 0 minutes, 30 minutes, 60 minutes and 120 minutes, and were placed in boxes between samples. They were again sampled at 24hr, 48hr and 72hr. The treatment group were subjected to a simulated kiwi capture event where after their first sample, they were chased for up to 10min then sampled at capture (0min), the birds were then handled for 30 minutes before blood sampling. Samples at 60 minutes to 72 hour followed the same protocol as control birds.

2.3.4 Pathology

At post mortem one treatment bird in week one and one control bird in week two had small haemorrhages in the skeletal muscle. Histological examination of the birds revealed acute changes in skeletal and cardiac muscle in some of the birds sampled (Table 2.1). Five of the birds had neoplastic cells infiltrating the myocardium and in one bird also the skeletal muscle. One other bird also had neoplastic lymphocytes in fascia near skeletal muscle. The neoplasia is suspected to be due to retroviral disease. No major trends were observed between control and treatment birds in the incidence of these changes.

Table 2.1 Summary of the frequency of histological findings during post mortem on layer hens (*Gallus domesticus*) following experimental procedures on capture and handling techniques in New Zealand, January 2011. Individuals in the control group were immediately captured and blood sampled at 0 minutes, 30 minutes, 60 minutes and 120 minutes, and were placed in boxes between samples. They were again sampled at 24hr, 48hr and 72hr. The treatment group were subjected to a simulated kiwi capture event where after their first sample, they were chased for up to 10min then sampled at capture (0min), the birds were then handled for 30 minutes before blood sampling. Samples at 60 minutes to 72 hour followed the same protocol as control birds. Birds were euthanized 24-72 hours following the final sample. The experiment was performed twice over two separate study weeks.

	neoplasia in skeletal muscle	neoplasia in cardiac muscle	acute degenerative changes in skeletal muscle	acute degenerative changes in cardiac muscle
Control	0	2	7	2
Treatment	2	3	8	2
Week one	1	3	5	3
Week two	1	2	10	1
Total number of birds with the condition (n=28)	2	5	15	4

2.4 DISCUSSION

2.4.1 Impact of capture and handling on layer hen muscle physiology

The plasma concentrations of muscle enzymes in the chickens did not change as expected. In other studies with wild birds, capture and handling has resulted in the rapid elevation of these enzymes in plasma associated with leakage of the enzymes from damaged myocytes (Bollinger *et al.*, 1989; Dabbert & Powell, 1993; Bailey *et al.*, 1997). Within week one, chickens did not respond to capture with significant elevations in plasma CK or AST, although the CK concentrations in the control group were highly variable especially at 24 hour. This suggests at least one bird responded to the event with a CK response indicative of muscular damage. The reason for the lack of muscular response in both groups was not determined but a possible explanation for the difference between the chickens in my study and the previously published studies on other species (Bollinger *et al.*, 1989; Dabbert & Powell, 1993; Bailey *et al.*, 1997) could include the low overall muscle mass of these birds which were sourced as end-of-lay caged hens and therefore had lower enzyme activities. Alternatively the handling treatment may not have been severe enough to cause muscle damage compared to other studies. It is unlikely that the catheters used in week one were the cause of the observed elevated plasma CK and AST concentrations as then the treatment group in week one would be expected to exhibit the same trend.

In week one the differences in plasma CK and AST concentrations between control and treatment groups were significant and since these enzymes did not change significantly over time it suggests that the control group started with higher concentrations of these enzymes which they maintained throughout. The most likely hypothesis to explain this difference is that the some or all birds in the control group experienced some type of muscle damage prior to the sampling that the treatment birds did not. The results of week two would lend support to this hypothesis. The treatment group from both week one and two and the control group from week two respond much more similarly to each other compared to the control group in week one.

The cause of the elevation of plasma CK concentrations in the control group in only week one is unclear but may have been due to fighting between individuals. When layer hens are kept in small groups they form a dominance hierarchy (Schjelderup-Ebbe, 1922;Wood-Gush,

1971; Rushen, 1982; Syme et al., 1983 as cited in D'Eath & Keeling, 2003) and show aggression towards unfamiliar birds (Guhl and Allee, 1944; Maier, 1964; Craig et al., 1969; Bradshaw, 1992; Dawkins, 1995; D'Eath and Stone, 1999 as cited in D'Eath & Keeling, 2003). When the membership of a group is changed aggression between birds is high (Guhl and Allee, 1944; Craig et al., 1969 as cited in D'Eath & Keeling, 2003). Since the birds in my study were taken from a range of cages at the poultry farm, they would have had to establish a new hierarchy and there was likely fighting between individuals that could have resulted in muscle damage in the day between obtaining the birds and sampling. By chance the control group of chickens in week one could have been made up of hens with similar competitive ability resulting in more fighting between individuals in this group to establish dominance and therefore a higher level of muscle damage in this group prior to sampling. However, it should also be noted that I observed no fighting in the group and there were no injuries present that would suggest such intra-specific aggression.

While not observable in week one the changes in plasma CK and AST over the sampling period were significant in week two. There was a gradual increase in plasma CK concentration up to a low peak at 24 hours after which it decreased. This aspect of the chickens' muscular physiology response to capture and handling is similar to other studies on birds which have shown plasma CK to peak at 24 hours following capture (Bailey *et al.*, 1997; Ward *et al.*, 2011). Plasma AST responded similarly but peaked around 24-48 hour and was still elevated at 72 hours. This would agree with the studies that have found circulating AST concentrations persist longer then CK in the bloodstream (Lumeij *et al.*, 1988). However, as previously mentioned, the physiological changes observed in the chickens are significantly attenuated compared to other species. The cause of the peak in CK and AST at 24-48 hours may also have been a result of the cumulative effect of repeated blood sampling in a relatively short period of time. Although if this is a potential cause of the increase the samples at 24, 48 and 72 hours do not appear to cause additional increases in the responses.

What is apparent from these results is that the layer hens in this study in both weeks had baseline concentrations of plasma CK and AST that were much higher than is considered normal for other avian species (Franson *et al.*, 1985) and were at levels which could be considered to be indicative of clinical myopathy (Bollinger *et al.*, 1989). The starting values in my study were also much higher than has been reported in chickens in another study which showed baseline concentrations of plasma CK at 143±71 IU/L and AST 49±31 IU/L (mean±SD) in 25 week old layer hens (Macrae *et al.*, 2006).

These high values of baseline CK and AST and attenuated response to capture and handling could be due to a number of factors such as the physical condition of the birds, including the reduction of total muscle mass associated with their confined caging, metabolic conditions associated with extended periods of egg-laying, the transportation process or fighting to establish dominance. The rate of egg production required of layer hens puts a level of demand on their bodies that is not seen in wild birds, and is detrimental to their health as shown by the high rate of fractures in layer hens, particularly at the end of lay (Nicol *et al.*, 2006; Sherwin *et al.*, 2010). Some evidence of this poor health was observed in the hens in my research with three of the hens in my study having broken wings and one showing elevations of GLDH in plasma indicating acute liver disease. Post mortem examination was not extensive and focussed on obtaining muscle samples, a higher incidence of current or historic injury may have been observed on closer inspection. This may have affected the physiology of the birds which were included in the results and thus affected my findings.

There are a number of metabolic diseases in commercial chickens that are associated with high egg production or rapid growth causing a body system to fail because of the demand placed on the organ or system (Julian, 2005). Some of these diseases such as 'deep pectoral myopathy,' 'sudden death syndrome' and nutritional myopathy have the potential to cause muscle damage in the birds (Julian, 2005). However these conditions are largely due to the highly developed muscles in birds bred for meat rather than layer hens (Soike & Bergmann, 1998). The high metabolic demands placed on the birds used in my study when they were commercial layer hens may have caused the abnormal physiology and therefore affected the results (Nicol *et al.*, 2006).

Another factor that may have caused muscle damage in the birds prior to sampling and thus elevated starting CK and AST concentrations could have been the handling of the birds at the poultry farm and subsequent transport to the study site. The birds may have experienced physiological muscle damage or capture myopathy during their removal from the cages at the poultry farm and/or the potentially altered health of the layer hens used in this experiment may have led to the birds being physical injured during this process. The starting concentrations of plasma CK and AST observed during sampling may have been elevated due to these events a day prior to sampling. The effect of transport may have been compounded by the previously mentioned health condition of the layer hens used.

2.4.1.1 Pathology Findings

The pathological findings indicate similar results to the muscle enzymes; some of the hens in this study were experiencing muscle damage. Unlike biochemistry which cannot indicate the location of muscular damage the histology shows the damage occurred in both cardiac and skeletal muscle. The pathology reflects recent muscle damage but it cannot show whether this was caused during the sampling protocol or just before it started. The pathology also did not find any differences in the frequency of occurrence of muscle lesions between control and treatment groups. The common finding of cardiac and skeletal muscle neoplasia in these birds suggests a high prevalence of retroviral infection in the poultry sheds, adding further evidence to the conclusion that these birds are not suitable as physiological models for wild species.

2.4.2 Impact of capture and handling on layer hen stress physiology

There was a difference in CORT response between sampling weeks that appears to be largely due to high initial CORT concentrations in week one. Therefore, there was likely some stressor in week one that caused high initial CORT concentrations in both groups of birds in week one prior to sampling. Interestingly high CORT was seen in both groups and was not solely associated with the increased level of muscle damage apparent in the control group. In contrast the starting CORT concentrations in week two was around half that of week one and the CORT response appeared less variable. This increase in stress may have been the result of the catheters that were placed in the week one birds the previous day. The presence of the catheters may have acted as a stressor partially elevating the plasma CORT concentration in all the week one birds.

The concentration of CORT in the hens in week two was still above and more variable then the baseline CORT concentrations of 2 ng/ml found in layer hens in other studies (Beuving & Vonder, 1978). This difference to other studies is probably due to the longer pre-sampling time in my study resulting in partially elevated CORT at the 0 minutes sample. Chloupek *et al.* (2011) recommends a pre-sampling time not exceeding two minutes to ensure that plasma levels of CORT are not influenced by handling stress. Of the two weeks the CORT response observed in week two is probably more reflective of normal birds.

While there was not a significant difference between the CORT response of the control and treatment groups in either week there is obvious increased elevation in CORT concentration in the treatment group over the chase period. Over the handling period the plasma CORT remains at similar concentrations but becomes more variable by 30 minutes, perhaps starting to decline in week two. This pattern was observable in both week one and two even though birds in week two already showed elevated plasma CORT. The CORT response has begun to resolve by the 60 minutes in both weeks. This short term pattern is similar to that documented in poultry by Fraisse and Cockrem (2006). This highlights the importance of the chase period in provoking a stress response and suggests that the handling protocol has a minimal impact on the stress response after the stressful nature of the chase event. Interestingly the control group in week one which started at already elevated plasma CORT at 0 minutes follows a similar pattern decreasing in concentration until 60 minutes. In both groups in both weeks there is minimal elevation of CORT in the 24, 48 and 72 hour samples showing basal level of CORT had returned to normal. With the exception of the elevations of plasma CORT at 120 minutes in both treatment groups in week one (but not week two), the results suggest that the chase period rather than capture, handling or sampling is the major stressor for these birds.

These results are seemingly in contrast with another study which found CORT concentrations in layer hens to rapidly become elevated over a short period of handling and sampling (Beuving & Vonder, 1978). Studies on broilers have also shown CORT elevations within a few minutes following even brief handling (Voslarova *et al.*, 2011), although the handling protocol is different compared to that used in my study and the birds are crated after handling. The act of picking up the bird and handling it to sample it, even for a short time, should have been enough to initiate a CORT response according to these other studies.

Chloupek *et al.* (2011) did find that rough and inverted handling and carrying of broilers resulted in higher plasma CORT compared to upright carrying and gentle handling, however their sampling and handling was carried out within the time it took to take the first blood sample in my study and the CORT concentrations observed in their study were below those observed at 0 minutes in my study. The birds in my study were carried upright and handled gently; this, combined with the relatively long pre-sample time may have meant that the stress response of control birds in my study was minimal compared to birds handled roughly and took place before the first sample was taken.

Some the findings of my study are comparable with previous work on the stress response of brown kiwi. Similar to the treatment group in my study, captive and wild brown kiwi have been shown to respond to capture and handling with elevations in plasma CORT (Adams, 2000). In contrast with my study on layer hens plasma CORT was still elevated 60 minutes post capture in wild and captive kiwi. Captive brown kiwi in nocturnal enclosures took until 180 minutes following capture to return to normal levels and brown kiwi in outdoor pens still showed significantly elevated plasma CORT at 420 minutes after capture (Adams, 2000). In a similar finding to my study the duration of handling (4min vs. 15min) in wild kiwi immediately following capture did not affect the plasma CORT (Adams, 2000), suggesting that in kiwi as with my layer hens it is the capture rather than handling that is the more significant stressor.

2.4.3 Impact of capture and handling on layer hen liver physiology

The liver of layer hens in high production systems is fragile and conditions such as hepatic lipidosis and hepatic steatosis have been observed in these types of birds (Julian, 2005). However plasma GLDH concentrations remained very low for the majority of birds and the elevated AST concentrations probably reflected muscle damage when looked at in association with CK concentrations.

2.4.4 Assessment of layer hens as a surrogate for kiwi

The starting concentrations of CK in the hens in this study are much higher than reference ranges for either brown kiwi (521-971 IU/L) or rowi (447-1071 IU/L). The starting concentrations of AST are also higher than for brown kiwi (64-138 IU/L) but do fall within the reference range of rowi (132-276 IU/L), (Morgan, 2008). The patterns of change in these plasma enzymes are also very different to published studies in wild birds following capture and handling (Bollinger *et al.*, 1989; Dabbert & Powell, 1993; Bailey *et al.*, 1997).

The hens also appeared to have a reduced stress response to the sampling process and are therefore likely not indicative of stress response observed in wild birds. Therefore, I conclude that the hens in this study are not likely to approximate the normal physiology of kiwi and as a result are not suitable as surrogates to assess the impact of capture and handling on kiwi or other wild birds.

The results of this study therefore have limited use for management practices of kiwi or other wild species and I do not recommend the use of layer hens as a surrogate species in further research. Further research could use free range chickens to help eliminate some of the possible reasons that may have caused layer hens to be an inappropriate surrogate for kiwi. However I would advise the use of a less domesticated species that is reared in a free ranging environment, for example game birds such as pheasants which are reared for release. This may provide a better surrogate then chickens and would still allow for more controlled conditions then sampling wild birds.

In addition, if sampling captive birds, I advise sampling the birds on site or giving them at least a week to settle into the new surroundings and recover from the handling and transport process. This would minimise any muscle damage caused by handling and transport affecting the results of the experiment, and would also allow any fighting within the groups to settle before commencing the experiment. Alternately, the first blood sample could be taken at the farm when the bird is removed from its cage and the transportation could become part of the experiment following the kiwi transportation protocol.

Alternatively a wild species that is common and accessible may better approximate the physiology of kiwi and also give a more accurate representation of the stress and behaviour involved with capturing birds in the wild. This would potentially providing a better surrogate to act as a model for kiwi, but there is an inevitable trade-off of which would result in lower frequency and duration of sampling and less control over biotic and abiotic factors. Ideally, these experiments would best be carried out on kiwi to eliminate the inter-species variance that confounds studies using surrogate species.

2.4.5 Conclusions & Recommendations

These findings are of limited use due to confounding factors such as existing muscle damage. The results do indicate that when looking at the stress impacts of capture and handling further research should perhaps emphasise the importance of the capture/chase period rather than handling. As mentioned I also recommend a different choice of surrogate species with which to model the impacts of capture and handling on kiwi and if a captive species is used then the birds should be sampled on location or after they have recovered from transport.

References

Adams, D. C. (2000). *Corticosterone responses of captive and wild Northern brown kiwi (Apteryx mantelli)* Unpublished masters thesis, Massey University, Palmerston North.

Adams, N. J., Parker, K. A., Cockrem, J. F., Brunton, D. H., & Candy, E. J. (2010). Corticosterone responses and post-release survival in translocated North Island Saddlebacks (*Philesturnus rufusater*) in New Zealand. *Emu, 110*(4), 296-301.

Anonymous. (1972). (Deutsche Gesellschaft für Klinische Chemie). Empfehlungen der Deutschen Gesellschaft für Klinische Chemie (DGKC). *Journal of Clinical Chemistry & Clinical Biochemistry, 10,* 182–193.

Bailey, T. A., Wernery, U., Naldo, J., & Samour, J. H. (1997). Plasma concentration of creatine kinase and lactate dehydrogenase in houbara bustards (*Chlamydotis undulata macqueenii*) immediately following capture. *Comparative Haematology International, 7*(2), 113-116.

Bassett, S. (2012). Operation Nest Egg Incubation and Chick Rearing Best Practice Protocols. Wellington: Oralis Limited.

Basson, P. A., & Hofmeyr, J. M. (1973). Mortalities associated with wildlife capture operations. In E. Young (Ed.), *The Capture and Care of Wild Animals; The Work of Eighteen Veterinary, Medical, and Wildlife Experts* (pp. 151-160). Cape Town and Pretoria: Human and Rousseau.

Beuving, G., & Vonder, G. M. A. (1978). Effect of stressing factors on corticosterone levels in plasma of laying hens. *General and Comparative Endocrinology, 35*(2), 153-159.

Bollinger, T., Wobeser, G., Clark, R. G., Nieman, D. J., & Smith, J. R. (1989). Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by 3 methods for banding. *Journal of Wildlife Diseases, 25*(2), 225-231.

Cardinet III, G. H. (1989). Skeletal muscle function. In J. J. Kaneko (Ed.), *Clinical Biochemistry of Domestic Animals* (4th ed., pp. 463-495). New York: Academic Press, Inc.

Carsia, R. V., & Harvey, S. (2000). Adrenals. In G. C. Whittow (Ed.), *Sturkie's Avian Physiology* (pp. 489-537). San Diego: Academic Press.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A., & Reynolds-Hogland, M. L. (2008). An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy, 89*(4), 973-990.

Chloupek, P., Bedanova, I., Chloupek, J., & Vecerek, V. (2011). Changes in selected biochemical indices resulting from various pre-sampling handling techniques in broilers. *Acta Veterinaria Scandinavica, 53*.

Cockrem, J. F., Adams, D. C., Bennett, E. J., Candy, E. J., Hawke, E. J., Henare, S. J., & Potter, M. A. (2004). Endocrinology and the conservation of New Zealand birds. In M. S. Gordon & S. M. Bartol (Eds.), *Experiemental Approaches to Conservation Biology* (pp. 101-121). Berkeley and Los Angeles: University of California Press.

Cockrem, J. F., Barrett, D. P., Candy, E. J., & Potter, M. A. (2009). Corticosterone responses in birds: Individual variation and repeatability in Adelie penguins (*Pygoscelis adeliae*) and other species, and the use of power analysis to determine sample sizes. *General and Comparative Endocrinology, 163*(1-2), 158-168.

Cockrem, J. F., Potter, M. A., Barrett, D. P., & Candy, E. J. (2008). Corticosterone responses to capture and restraint in emperor and Adelie penguins in Antarctica. *Zoological Science, 25*(3), 291-298.

Cockrem, J. F., Satterlee, D. G., Candy, E. J., & Castille, S. A. (2012). Fecal corticosterone metabolites and plasma corticosterone in Japanese quail selected for low or high plasma corticosterone responses to brief restraint. *Domestic Animal Endocrinology, 42*(4), 249-255.

Colbourne, R., Bassett, S., Billing, T., McCormick, H., McLennan, J., Nelson, A., & Robertson, H. (2005). The development of Operation Nest Egg as a tool in the conservation management of kiwi. Wellington: Department of Conservation.

Cooper, A., Mourerchauvire, C., Chambers, G. K., Vonhaeseler, A., Wilson, A. C., & Paabo, S. (1992). Independent Origins of New Zealand Moas and Kiwi *Proceedings of the National Academy of Sciences of the United States of America, 89*(18), 8741-8744.

D'Eath, R. B., & Keeling, L. J. (2003). Social discrimination and aggression by laying hens in large groups: from peck orders to social tolerance. *Applied Animal Behaviour Science, 84*(3), 197-212.

Dabbert, C. B., & Powell, K. C. (1993). Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases, 29*(2), 304-309.

Fraisse, F., & Cockrem, J. F. (2006). Corticosterone and fear behaviour in white and brown caged laying hens. *British Poultry Science, 47*(2), 110-119.

Franson, J. C., Murray, H. C., & Bunck, C. (1985). Enzyme activities in plasma, kidney, liver and muscle of five avian species. *Journal of Wildlife Diseases, 21*(1), 33-39.

Fudge, A. M. (1999). *Laboratory Medicine: Avian and Exotic pets*: Saunders.

Fumihito, A., Miyake, T., Sumi, S. I., Takada, M., Ohno, S., & Kondo, N. (1994). One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proceedings of the National Academy of Sciences of the United States of America, 91*(26).

Gasson, P. A. (2005). Translocation of great spotted kiwi/roa (*Apteryx haasti*) to Rotoiti Nature Recovery Project. Nelson: Department of Conservation.

Ha, H. J., Alley, M. R., Howe, L., Castro, I., & Gartrell, B. (2013). Avipoxvirus infections in brown kiwi (*Apteryx mantelli*). *New Zealand Veterinary Journal, 61*(1), 49-52.

Holzapfel, S., Robertson, H. A., McLennan, J. A., Sporle, W., Hackwell, K., & Impey, M. (2008). Kiwi (*Apteryx spp.*) Recovery Plan 2008-2018. Wellington: Department of Conservation.

Jacques, C. N., Jenks, J. A., Deperno, C. S., Sievers, J. D., Grovenburg, T. W., Brinkman, T. J., Swanson, C. C., & Stillings, B. A. (2009). Evaluating ungulate mortality associated with helicopter net-gun captures in the Northern Great Plains. *Journal of Wildlife Management, 73*(8), 1282-1291.

Jolly, J. N., & Colbourne, R. M. (1991). Translocations of the little spotted kiwi (*Apteryx owenii*) between offshore islands of New Zealand. *Journal of the Royal Society of New Zealand, 21*(2), 143-149.

Julian, R. J. (2005). Production and growth related disorders and other metabolic diseases of poultry. *Veterinary Journal, 169*(3), 350-369.

Kreeger, T. J., White, P. J., Seal, U. S., & Tester, J. R. (1990). Pathological responses of red foxes to foothold traps. *Journal of Wildlife Management, 54*(1), 147-160.

Lumeij, J. T., Debruijne, J. J., Slob, A., Wolfswinkel, J., & Rothuizen, J. (1988). Enzyme activities in tissues and elimination half-lives of homologous muscle and liver enzymes in the racing pigeon (*Columba livia domestica*). *Avian Pathology, 17*(4), 851-864.

Macrae, V. E., Mahon, M., Gilpin, S., Sandercock, D. A., & Mitchell, M. A. (2006). Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (*Gallus domesticus*). *British Poultry Science, 47*(3), 264-272.

McLennan, J. A., Dew, L., Miles, J., Gillingham, N., & Waiwai, R. (2004). Size matters: predation risk and juvenile growth in North Island brown kiwi (*Apteryx mantelli*). *New Zealand Journal of Ecology, 28*(2), 241-250.

McLennan, J. A., Potter, M. A., Robertson, H. A., Wake, G. C., Colbourne, R., Dew, L., Joyce, L., McCann, A. J., Miles, J., Miller, P. J., & Reid, J. (1996). Role of predation in the decline of kiwi, Apteryx spp, in New Zealand. *New Zealand Journal of Ecology, 20*(1), 27-35.

Miskelly, C. M., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Powlesland, R. G., Robertson, H. A., Sagar, P. M., Scofield, R. P., & Taylor, G. A. (2008). Conservation status of New Zealand birds. *Notornis 55*(3), 117-135.

Morgan, K. J. (2008). Kiwi first aid and veterinary care. Wellington: Department of Conservation.

Nicol, C. J., Brown, S. N., Glen, E., Pope, S. J., Short, F. J., Warriss, P. D., Zimmerman, P. H., & Wilkins, L. J. (2006). Effects of stocking density, flock size and management

on the welfare of laying hens in single-tier aviaries. *British Poultry Science, 47*(2), 135-146.

Ponjoan, A., Bota, G., De La Morena, E. L. G., Morales, M. B., Wolff, A., Marco, I., & Manosa, S. (2008). Adverse effects of capture and handling little bustard. *Journal of Wildlife Management, 72*(1), 315-319.

Powell, R. A., & Proulx, G. (2003). Trapping and marking terrestrial mammals for research: Integrating ethics, performance criteria, techniques, and common sense. *Ilar Journal, 44*(4), 259-276.

Robertson, H. A., & Colbourne, R. M. (2003). Kiwi (*Apteryx spp*.) Best Practice Manual Wellington: Department of Conservation.

Robertson, H. A., Colbourne, R. M., Graham, P. J., Miller, P. J., & Pierce, R. J. (2011). Experimental management of brown kiwi *Apteryx mantelli* in central Northland, New Zealand. *Bird Conservation International, 21*(2), 207-220.

Schumann, G., Bonora, R., Ceriotti, F., Clerc-Renaud, P., Ferrero, C. A., Ferard, G., Franck, P. F. H., Gella, F. J., Hoelzel, W., Jorgensen, P. J., Kanno, T., Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J. M., Linsinger, T. P. J., Misaki, H., Panteghini, M., Pauwels, J., Schimmel, H. G., Vialle, A., Weidemann, G., & Siekmann, L. (2002a). IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. Part 2. Reference procedure for the measurement of catalytic concentration of creatine kinase. *Clinical Chemistry and Laboratory Medicine, 40*(6), 635-642.

Schumann, G., Bonora, R., Ceriotti, F., Ferard, G., Ferrero, C. A., Franck, P. F. H., Gella, F. J., Hoelzel, W., Jorgensen, P. J., Kanno, T., Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J. M., Linsinger, T. P. J., Misaki, H., Panteghini, M., Pauwels, J., Schiele, F., Schimmel, H. G., Weidemann, G., & Siekmann, L. (2002b). IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. *Clinical Chemistry and Laboratory Medicine, 40*(7), 725-733.

Sherwin, C. M., Richards, G. J., & Nicol, C. J. (2010). Comparison of the welfare of layer hens in 4 housing systems in the UK *British Poultry Science, 51*(4), 488-499.

Siegel, H. S. (1980). Physiological stress in birds. *Bioscience, 30*(8), 529-534.

Soike, D., & Bergmann, V. (1998). Comparison of skeletal muscle characteristics in chicken bred for meat or egg production. I. Histopathological and electron microscopic examination. *Journal of Veterinary Medicine. Series A: Physiology, Pathology, Clinical Medicine, 45*(3), 161-167.

Southern, L. K., & Southern, W. E. (1983). Responses of ring-billed gulls to cannon-netting and wing-tagging. *Journal of Wildlife Management, 47*(1), 234-237.

Voslarova, E., Chloupek, P., Vosmerova, P., Chloupek, J., Bedanova, I., & Vecerek, V. (2011). Time course changes in selected biochemical indices of broilers in response to pretransport handling. *Poultry Science, 90*(10), 2144-2152.

Ward, J. M., Gartrell, B. D., Conklin, J. R., & Battley, P. F. (2011). Midazolam as an adjunctive therapy for capture myopathy in bar-tailed godwits (*Limosa lapponica baueri*) with prognostic indicators. *Journal of Wildlife Diseases*, *47*(4), 925–935.

Williams, E. S., & Thorne, E. T. (1996). Exertional myopathy (capture myopathy). In A. Fairbrother, L. N. Locke & G. L. Hoff (Eds.), *Noninfectious Diseases of Wildlife* (2nd ed., pp. 181-193). London: Manson Publishing Ltd.

CHAPTER THREE

WILD PŪKEKO (*Porphyrio porphyrio melanotus*) AS A SURROGATE FOR ASSESSING THE PHYSIOLOGICAL IMPACTS OF CAPTURE AND HANDLING ON TAKAHĒ (*Porphyrio hochstetteri*)

Abstract

The management of the endangered takahē (*Porphyrio hochstetteri*) requires some birds to be captured and handled for monitoring, translocations and research. These procedures are justified and highly useful, however they may have a detrimental impact of the physiology and health of these birds. This study used pūkeko (*Porphyrio porphyrio melanotus*) as a surrogate species to model the physiological impact of capture and handling on takahē.

Thirty wild pūkeko were captured using a net-gun at the Awapuni Sustainable Development Centre in Palmerston North from July, 2011 until February, 2012. Ten pūkeko were also shot on farmland in the Manawatu. Captured pūkeko were held and blood sampled immediately once captured and twice more 30 and 120 minutes following capture. Birds in the control group were placed in a box between blood samples and birds in the treatment group were placed in a clean pillowcase and held up simulating a weighing event. The birds were then cradled on their backs with both legs held firmly and the birds back supported from underneath. After the 30 minute sample treatment birds were placed in the box also.

To assess the physiological impact of capture, biochemical analytes measured included plasma concentrations of uric acid (UA), creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and the stress hormone corticosterone (CORT).

Capture caused significant elevations in plasma CK, AST and CORT showing subclinical muscle damage and an acute stress response were occurring in the pūkeko following capture. The muscle damage and the stress response the bird's experienced were greater when the pūkeko were captured in flight. Capture also had a significant if less clearly defined impact on renal and gastro-intestinal physiology. Handling protocols of treatment birds did not cause any measurable effects on the biochemical analytes. Baseline levels of the biochemical analytes of pūkeko that were captured using a net-gun and those that were shot were similar. The time of day the pūkeko were captured caused significant variation in the concentration of plasma GLDH and UA.

3.1 INTRODUCTION

The conservation management of many of New Zealand's endangered species requires the capture of wild animals for monitoring, translocation or research purposes. In every capture and handling event animals may be injured or have their normal physiology or behaviour altered (Kreeger *et al.*, 1990; Williams & Thorne, 1996). My study, set out in this chapter, investigated the physiological effects of capture using net-guns on pūkeko (*Porphyrio porphyrio melanotus*) as a surrogate species to assess current handling protocols used for the critically endangered takahē (*Porphyrio hochstetteri*). My aim was to investigate how capture and handling affects the physiology of the birds.. This is important for the capture of any wild animal, but critical when capturing endangered animals such as takahē because the health of each individual has significant conservation value.

3.1.1 Status, Ecology and Management of Takahē (*Porphyrio hochstetteri*)

Takahē (*Porphyrio hochstetteri*), previously known as *P. mantelli*, is a species of flightless rail endemic to New Zealand. They are the largest extant member of the *Rallidae* and stand 50 cm high by 63 cm long, weighing 4 kg on average (Lee & Jamieson, 2001). Takahē were once widespread in both the North and South islands and occurred in forest and grassland habitat. However they suffered massive population declines and were thought to be extinct until a population restricted to alpine tussock habitat in the Murchison Mountains was rediscovered in 1948 (Wickes *et al.*, 2009). Since 1948 the main agent of decline has been habitat deterioration caused by introduced red deer (*Cervus elaphus*) (Wickes *et al.*, 2009). More recently stoat predation on takahē has been shown to be a major factor in population decline, particularly in seasons where beech and tussock masting events cause stoat plagues (Wickes *et al.*, 2009). Strategies have been put in place to manage both habitat deterioration and predation including the translocation of individuals to several offshore islands and fenced mainland reserves (Wickes *et al.*, 2009). They are listed as 'endangered' on the International Union for Conservation of Nature (IUCN) Red List, with a current estimated population of 227 adults, or 340-350 birds in total (Anonymous, 2012a). Under the New Zealand Threat Classification System, takahē are classified as 'nationally critical' (Miskelly *et al.*, 2008).

Following their rediscovery, a large amount of research was carried out on the species' ecological requirements, breeding biology and population size (Wickes *et al.*, 2009) but there has been very little study of takahē physiology (Lee & Jamieson, 2001).

3.1.2 Status, Ecology and Management of Pūkeko (*Porphyrio porphyrio melanotus*)

Pūkeko are one of 13 subspecies of swamphen (*Porphyrio porphyrio*) (Ripley *et al.*, 1977). These subspecies are distributed globally over a wide geographical range inhabiting countries such as South Africa, Turkey, the Philippines, Australia and New Zealand (Trewick, 1997). The pūkeko subspecies is found in Australia and New Zealand. Recent genetic research indicates that there may be a greater genetic distance between the subspecies of *Porphyrio porphyrio* than there is between species of some other rail genera such as *Rallus*, and that each subspecies could potentially be redefined as separate species (Trewick, 1997). Pūkeko are currently listed as of 'Least Concern' on the IUCN Red List (Anonymous, 2012a) and are classified as a game bird in New Zealand (Anonymous, 1953, 2013).

3.1.3 Capture and Handling Protocols used for Takahē (*Porphyrio hochstetteri*)

3.1.3.1 Takahē Capture protocol

As part of the conservation management of takahē, birds are only captured for necessary research purposes, health screening or to be moved between reserves. The capture and handling of takahē is otherwise kept to a minimum (Kilduff *et al.*, 2011). Takahē can have significant weight loss following a stressful capture (Kilduff *et al.*, 2011). Captive and wild takahē are captured using several methods: by hand, hand-net, capture pens, using food lures, or by using loose nylon nets (Rose, 2000; Youl, 2008; Kilduff *et al.*, 2011). There are times when capturing birds requires a chase. Chases occur more often during operations to capture wild birds in the Murchison Mountains (*pers. comm.* P. Marsh 17th September 2012). Usually after a short chase of around two minutes the team of two or three people surround the bird and it is captured by hand. Sometimes captures will take longer than anticipated; there is

anecdotal evidence of a capture related death of a takahē in the 1980's following a long chase (*pers. comm.* P. Marsh 17th September 2012). Captures involving chases are kept as short as possible but there is no established cut-off period on chase lengths and the decision to continue is made by the rangers on location and their observations of the birds. Birds have been chased around for a period of up to 20 minutes before but this is often not sustained and may involve stopping between a couple of capture efforts (*pers. comm.* P. Marsh 12th December 2012). Set nylon nets are used as a common method in takahē captures on offshore islands. The nets and birds are under constant observation during the process and takahē do not often get badly entangled in the net. Once a bird is captured it is usually untangled from the net in less than one minutes (*pers. comm.* P. Marsh 17th September 2012). Birds are observed for signs of stress upon release such as panting, unsteadiness on their legs or a lack of inclination to escape. However, most birds run off upon release and this combined with the often densely vegetated environment means the birds' behaviour after the immediate release is often not observable (*pers. comm.* P. Marsh 12th December 2012).

3.1.3.2 Takahē Handling Protocol

The current handling protocol (Kilduff *et al.*, 2011) dictates that when a takahē is first approached it is first seized by the legs followed by the head. The handler holds the bird around the legs with one hand putting a finger between the legs to control them. The other hand holds the takahē's head around the skull using their thumb on top of the birds head and fingers underneath the beak and around the neck (Figure 3.1). Care is taken to not hold the neck too tightly or to cover the nostrils (Kilduff *et al.*, 2011). Takahē may be handled upright or on their sides or back depending on the procedure being performed. Handling times will vary depending on the samples needed, experience of the team and co-operation of the bird. Rose (2000) recorded handling times ranging from 14 to 26 minutes on the 32 birds in her study.

Figure 3.1 A takahē being held by a Department of Conservation staff member.

3.1.3.3 Capture Impacts

There has been little research into the effect of the current capture and handling protocol on takahē regarding muscle damage and stress, although during one study, two takahē captured in the Murchison Mountains displayed biochemical profiles that were suggestive of an acute myopathy (Rose, 2000). In this study, the plasma creatine kinase (CK) concentration of each bird was 73,720 and 118,480 IU/L, while the plasma aspartate aminotransferase (AST) concentration was 1640 and 2600 IU/L respectively. In comparison, the other birds in the area had a mean CK of 1230 IU/L and mean AST of 296 IU/L (Rose, 2000). While the value

of CK and AST that reflect debilitating muscle damage are unknown (Bollinger *et al.*, 1989; Dabbert & Powell, 1993), a correlation between elevated concentrations of plasma CK and AST and clinical signs of capture myopathy is well established (Ward *et al.*, 2011). Rose (2000) postulates exertion as the cause of these extremely high plasma concentrations of muscle enzymes, however no clinical signs of capture myopathy in the two Murchison Mountain takahē were recorded, although the period of post capture monitoring is not recorded. As only one blood sample was taken from each of these birds (Rose, 1990), it cannot be ruled out that the elevated concentrations of CK and AST were not due to capture but instead due to an underlying disease or recent injury. However, these enzyme elevations do reflect significant muscle injury and the possibility that they are induced by capture and handling warrants further investigation. The Takahē Recovery Plan (Crouchley, 1994) states that management practices that visibly impact takahē, such as capture attempts leading to nest desertion or hatching failure, will be halted. However if the impacts are subclinical or not obviously attributed to capture, they may not be recognised, let alone addressed and mitigated.

In other species capture has been found to cause signifiacant detrimental effects to the birds being studied. Cox and Afton (1998) found that survival of Northern Pintails (*Anas acuta*) in the days following release after capture and radio tagging was positively related to the birds' flight quality on release. Deaths following release were largely attributed to increased predation on birds that flew poorly on release. However there are capture events of many species including takahē where the state of the bird's mobility on release is not observable due to the environment or may develop some time after the capture event. In these circumstances delayed onset of clinical signs of capture myopathy or even subclinical capture myopathy may still reduce the birds' ability to escape or fight off predators. Bailey *et al.* (1997) found elevated levels of plasma CK in houbara bustards (*Chlamydotis undulata macqueeni*) that exhibited no clinical signs of capture myopathy. These values were similar to or higher then one of the authors had found in bustards of the same species which exhibited clinical signs (weakness, paralysis, abnormal movement). Spraker *et al.* (1987) post mortemed wild turkeys (*Meleagris gallopavo*) following trapping, transporting and handling and found that even though clinical signs of capture myopathy were rare the incidence of capture myopathy at the cellular level was relatively high. They thought this may have been due to quick and incomplete physical examination of the bird before euthanasia. This is relevant as wild birds are often aimed to be released as quickly as possible, they too may not

show physical signs and may potentially have experience cellular damage consistent with capture myopathy.

In the absence of clinical signs or subsequent animal mortality, further impacts of capture may go undetected but have wider reaching effects. Capture impacts may result in reduced movement rates in an animal in the weeks following capture and repeat captures of the same animal can result in lower body condition (Cattet *et al.*, 2008). This may result in a reduction of an animal's chances of survival and reproductive success (Cattet *et al.*, 2008). Because some animals may exhibit no clinical signs of capture myopathy and because the behavioural and physiological impacts of capture may not be readily or immediately apparent they may go undetected.

3.1.4 Suitability of Pūkeko as a Surrogate for Assessing Handling Protocols Used for Takahē (*Porphyrio hochstetteri*)

Takahē (*Porphyrio hochstetteri*) are only caught for necessary management and research purposes (Crouchley, 1994; Kilduff *et al.*, 2011)**.** Due to their endangered and protected status it was not feasible to capture wild takahē for this research. Pūkeko were selected as the surrogate species because they are the closest extant genetic relative of the takahē (Bunin & Jamieson, 1996). There are also many morphological and behavioural similarities between the two species (Figure 3.2). Two notable differences between the species include the flightlessness and heavier, stockier build of takahē. A study by Suttie and Fennessy (1992) comparing the internal anatomy of the two species found takahē to be about three times larger in body mass and exhibited larger leg muscles (M. flexor cruris lateralis) relative to the breast muscles (M. pectoralis) compared with pūkeko. This is likely linked to the flightlessness in takahē.

Figure 3.2 A visual comparison between pūkeko (left) and takahē (right). Note that the photographs are not to scale.

Takahē have significantly deeper beaks then pūkeko, as well as having shorter intestines and larger recta (Suttie & Fennessy, 1992). In areas where takahē and pūkeko co-exist they share similar diets. The main exception is that pūkeko feed on a much greater proportion of animal matter although some invertebrate predation has been known in takahē (Trewick, 1996). Pūkeko have also been used to cross foster takahē chicks with some success (Bunin & Jamieson, 1996).

In this research it is assumed that the minimal differences in the normal physiology between the species will mean that the responses to capture in the two species will be similar. Pūkeko provide a suitable and accessible species to indicate whether the capture process could be causing subclinical capture myopathy in takahē and whether further investigation is warranted.

3.1.5 Specific Aims of the Study

As stated, the main aim of the study is to assess current capture and handling practices used for takahē and if possible suggest refinements to the process based on the results in pūkeko. To this end, this study investigated the physiological changes in wild pūkeko in response to capture and handling.

The specific aims of the study were to:

1) establish baseline plasma biochemical values for pūkeko using a group of shot birds in our study area;
2) capture wild pūkeko and obtain serial blood samples following capture and treat each bird according to either the control or treatment methodology;
3) determine plasma biochemical changes indicative of muscle damage, selected organ functions and the stress response to capture and handling;
4) assess the field practicality and potential health impacts of using a net-gun to capture wild pūkeko; and
5) extrapolate from the pūkeko study conclusions on the current capture and handling process for takahē and, if relevant, suggest refinements.

3.1.6 Approval for Study

All procedures were carried out with permission from the Massey University Animal Ethics Committee (MUAEC 11/33). An *Authority to Disturb and/or Kill Game Birds* was granted by Fish & Game New Zealand (File No: 1181). The local indigenous people Rangitaane O Manawatu were also consulted regarding the use of the native species pūkeko in this research.

3.2 MATERIALS AND METHODS

To establish normal plasma biochemical values for pūkeko, ten pūkeko killed using a .22 calibre rifle with a single shot to the head in June 2011. Blood samples were taken from the from the heart using a syringe and needle immediately after death in the field. To assess biochemical changes in pūkeko after capture and handling, 36 pūkeko were captured using a net-gun (Super Talon Net-Gun, Advanced Weapons Technology, La Quinta, California, USA), between July 2011 and February 2012. Blood samples were taken from the medial metatarsal vein on either leg or from the brachial vein on either wing, at intervals of 0 minutes (immediately after capture), at 30 minutes and at 120 minutes.

3.2.1 Study Site

For the baseline group, the pūkeko were shot in three locations (Figure 3.3). The first was a private property consisting of pasture based farmland and several ponds in the Ashhurst area (40°28’62’’S, 175°74’41’’E), while the second was in the Feilding area, again within pasture based farmland which contained several ponds (40°17’99’’S, 175°53’98’’E). The final location was the Awapuni Sustainable Development Centre in Palmerston North (40°38’93’’S, 175°57’90’’E) which consisted of an area of large compost stacks and unkempt grassland. One, three and six pūkeko were shot at these locations respectively.

All pūkeko captured were caught with the net-gun at the Awapuni Sustainable Development Centre in Palmerston North, New Zealand; during either daylight in the late afternoon, the twilight hours, or at night. The ‘daylight’ was defined as the period from 1600 hours to sunset, and the nautical definition of ‘twilight’ was used, which is the period between sunset and the time of day the centre of the sun is geometrically 12 degrees below the horizon (Anonymous, 2012b). This means twilight is roughly equal to one hour following sunset. Nautical twilight rather than civil twilight (which is shorter) was chosen as I felt pūkeko were still active and there was sufficient light for movement and foraging for up to an hour past sunset.

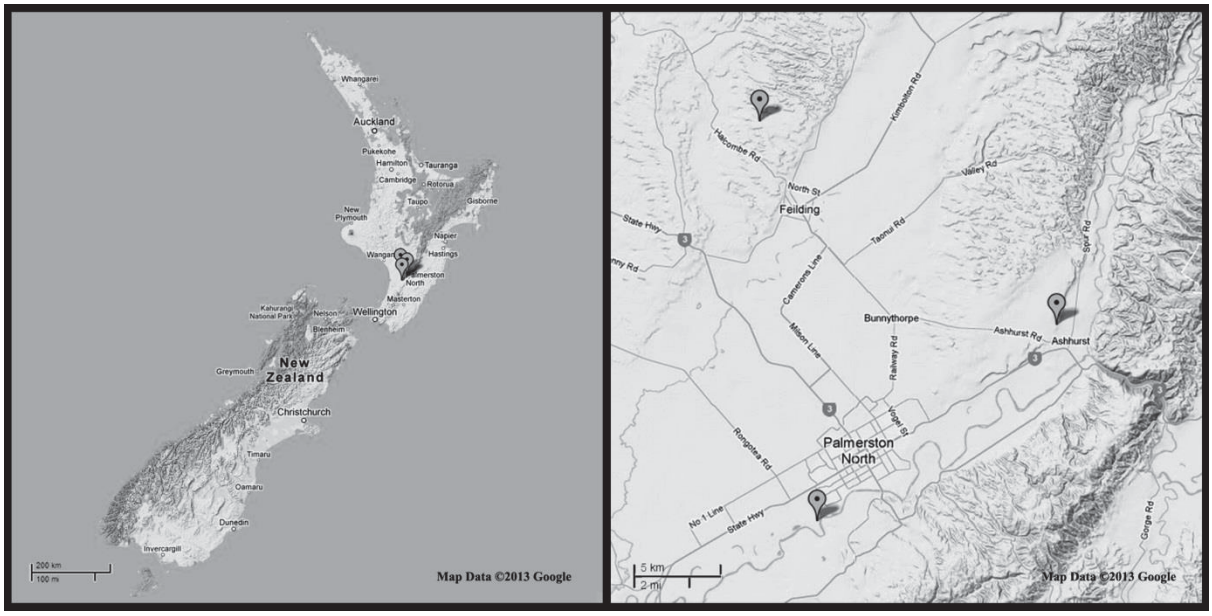


Figure 3.3 A map of New Zealand (left) and a close up of the study area within the Manawatu (right) showing the location of the study sites at Palmerston North, Fielding and Ashurst.

3.2.2 Field Methodology

A net-gun with 5 cm mesh nets was used for capture by a field team consisting of a net-gun operator and at least one assistant. Some captures were conducted at night in conjunction with a spotlight. Once the pūkeko was ensnared, the shooter moved to the bird and immediately restrained it by hand to minimise further movement and entanglement until the assistant arrived. If the bird was relatively free then it was removed immediately from the net and wrapped in a towel prior to sampling. In cases where the bird was considerably entangled in the net, the bird was wrapped in a towel and sampled while still in the net and then untangled after the first sample was taken.

All the pūkeko sampled in both the control and treatment groups were handled by necessity for removal from the net and again at each further blood sample. Handling during the blood sampling process involved holding a bird either on its back with its body supported and one wing extended in order to sample the brachial vein, or on its side with its body supported and one leg outstretched in order to sample the medial metatarsal vein. The samples were collected using heparinised 25 gauge needles and 1ml syringes. The aim was to take blood samples of 1ml, but due to the necessity to get quick samples sometimes no more than 0.5ml of blood was possible. The first sample is referred to as ‘0 minutes’ in this study. Following the blood sample, the bird was held for a short period to ensure haemostasis of the sampling

site was achieved using digital pressure and a swab. The pūkeko was then untangled from the net (unless it had been removed prior to sampling), and designated as either one of the control group or the treatment group. In order to achieve even sample sizes, birds were divided into these groups alternately.

The control group underwent minimal further handling excepting for blood sampling using the method already mentioned and the pūkeko were placed immediately in a ventilated cardboard box (35 cm wide by 50 cm long by 35 cm tall) in which they could move around in. They remained in the box until a further blood sample was taken at 30 minutes. The birds were then returned to the box until the final blood sample was taken at 120 minutes.

Pūkeko in the treatment group were placed in a clean pillowcase and held up simulating a weighing event. The bird was then cradled on its back with both legs held firmly in one hand with the handler's other arm supporting the bird from underneath. It was held in this manner until the 30 minutes blood sample was taken. The bird was then placed unrestrained inside a ventilated box (as above) until a final blood sample was taken at 120 minutes.

For both the control and treatment groups, once the final blood sample had been taken at 120 minutes, each pūkeko was given a short physical examination for any injuries and the bird was temporarily marked with white paint on its bill to ensure it wasn't captured and sampled on more than one occasion. The bird was then released at the same location as captured and observed for signs of distress. The behaviour at release was recorded. Other data recorded include whether the pūkeko was captured in flight or on the ground, whether the pūkeko had been a juvenile or adult, the behaviour of the pūkeko throughout the sampling process and the length of time between capture (or removal of the bird from the box) and a blood sample being taken. The time of day each bird was captured was also recorded and birds were allocated into 'daylight/twilight captures' or 'night captures' to distinguish whether they were captured during the hours of visible light (afternoon and twilight hours) or during the hours of darkness. Birds were classed as being captured in winter (July-September) or summer (January-February).

3.2.3 Laboratory Methodology

The heparinised blood samples were kept on ice and within 12 hours of being taken the samples were centrifuged at 5000 rpm for five minutes. The plasma was then pipetted off into a separate microcentrifuge tube before being frozen and stored at -20°C until biochemical analysis could be performed.

A sub-sample of plasma was submitted to a commercial diagnostic laboratory (New Zealand Veterinary Pathology, Palmerston North, New Zealand) and batch-analysed for plasma concentrations of CK, AST, glutamate dehydrogenase (GLDH) and uric acid (UA). Those pūkeko which were shot did not have the plasma concentrations of uric acid (UA) measured. The methods used to analyse these biochemical parameters has been previously described in Chapter Two except for UA which is described below.

The plasma CORT concentrations were measured at the Institute of Veterinary, Animal & Biomedical Sciences, Massey University, Palmerston North, New Zealand. The concentration of CORT was measured by radioimmunoassay as described in Chapter Two.

3.2.3.1 Determining the Concentration of Uric Acid in the plasma

The method used to determine the concentration of UA in the samples is a modification of the colorimetric method derived developed by Town *et al.* (1985). The UA assay used is an automated photometric assay using a Roche/Hitachi P800 analyser. The test principle is as follows: the assay measures the amount of uric acid by adding uricase enzyme. The uricase oxidises uric acid to allantoin and produces hydrogen peroxide as a by-product. A second enzyme (horseradish peroxidise) is added which uses the hydrogen peroxide produced in the first step to convert a colourless precursor to a red dye. The amount of red dye produced is measured photometrically and therefore gives the amount of UA in the sample.

3.2.4 Statistical methodology

Statistical analysis was performed using PASW Statistics 18 for Windows 7.0 (SPSS Inc., Chicago, Illinois, USA). All results are presented as means (± one standard error (S.E.M.), unless otherwise stated.

Comparison of mean plasma CK, AST and GLDH between pūkeko captured with the net-gun and those birds which were shot was conducted using a one-way analysis of variance (ANOVA). Where necessary the data were transformed using natural log or reciprocal transformations to achieve normality and homogeneity of variances.

Using a multivariate repeated-measures ANOVA, all repeated measures of CK, AST, GLDH and UA were assessed for significant differences over the 0, 30 and 120 minutes samples and between: control and treatment groups; adult and juvenile groups; flight or ground; season (winter vs. summer); time of day groups (daylight/twilight vs. night) and age.

This method of analysis does not allow for missing values and therefore only birds that had all three samples successfully taken were included in the repeated-measures analysis. This resulted in an effective sample size of 30 pūkeko with samples suitable for analysis, 15 birds in each of the treatment and control groups.

3.3 RESULTS

A sample size of 30 birds was suitable for analysis with 15 birds in each of the control and treatment groups. Of the netted pūkeko, 18 birds were captured while on the ground, 4 were captured while roosting in bushes and a further 8 birds were captured while in escaping in flight.

3.3.1 Field Observations

The average time between capture and taking of the first blood sample ('0 minutes') was 3 minutes and 13 seconds (±11 seconds), while the average time it took between capture and untangling of a bird was 6 minutes and 47 seconds (±1 minute 7 seconds). During the sampling process (from capture through to release), 30% (9/30) of birds captured were heard to vocalise; 50% (15/30) of birds were noted to have struggled during handling or in the net; and 43% (13/30) of birds were observed to pant at some point during the whole process. Birds typically ran off or flew away upon release. Two pūkeko, however, stood still on release for under a minute before walking away and a further two birds stood still on release for under a minute before taking flight. Among these four birds, none were noted as being unsteady on the legs or unable to stand. None of the pūkeko captured suffered any significant injury or exhibited any clinical signs of capture myopathy during the sampling or upon release. There were some minor feather abrasions on the flight feathers of three birds (9%) which may have been a result of the net filament scouring their surface during capture. The damage was extremely minor and all three of these birds were observed flying on release.

In this study, pūkeko captured using the net-gun were usually captured at a range of five to seven metres from the shooter, but the effective range was three to ten metres, depending on the wind direction and strength and also on whether the bird was stationary or in flight. Pūkeko were captured on foot or by using a car as a moving hide similar to that described by Mechlin and Shaiffer (1980). However, the pūkeko became more wary of capture attempts after several sampling days even when these days were widely separated due to bad weather. The increasing difficulty of capture by net-gun plus the restriction of sampling at the site until after working hours (after 1600 hours) meant a similar method to that used by Seddon *et al.* (1999) was adopted and birds were thereafter sampled at night using a spotlight to locate and distract the bird from the shooter's approach. The most successful method was for the spotter to hold the bird in the beam of the spotlight using LED spotlights (LED Lenser H14,

Zweibrueder Optoelectronics GmbH & Co.KG, Solingen, Germany). The net-gun was then primed and the shooter approached at around 45 degrees from the beam of light. Once the bird was in range, the net was released from the net-gun. While birds did not necessarily freeze when caught in the spotlight, they were much less prone to flight than during the day, and they often stayed still or crept under nearby cover.

3.3.2 Confounding Factors in the Analysis

Before the effects of capture and handling were assessed between the two groups, the data set *a priori* was examined for the effect of potential confounding factors, including time of sampling, seasonal variations, age class of the birds sampled, entanglement time and whether the bird was captured in flight.

3.3.2.1 Responses of Pūkeko to Capture During the Day and at Night

Due to the small sample size the birds captured in the afternoon and twilight hours were pooled for a total of six pūkeko captured during the daylight/twilight period and 24 birds captured at night.

Overall, pūkeko captured in this study showed significant differences in their response to capture (as indicated by plasma biochemistry) and the results suggest these differences were a consequence of the time of day they were captured. Samples from pūkeko captured during the daylight/twilight period had significantly higher concentrations of plasma GLDH (P=0.011) and UA (P=0.017), compared to those pūkeko captured at night (Figure 3.4). Further analysis of GLDH between the groups indicated that the mean concentration of plasma GLDH was significantly higher in daylight/twilight birds across all sampling points (0 minutes (P=0.006), 30 minutes (P=0.015) and 120 minutes (P=0.021)), when compared to night captured birds. Plasma UA was also found to be significantly higher the in the daylight/twilight birds at 0 minutes (P=0.001) and 30 minutes (P=0.022) sampling points, but not at the 120 minutes sampling point (P=0.371). There was no significant differences in the mean concentration of plasma CK (P=0.755), AST (P=0.565), and CORT (P=0.926) between the daytime/twilight and night groups.

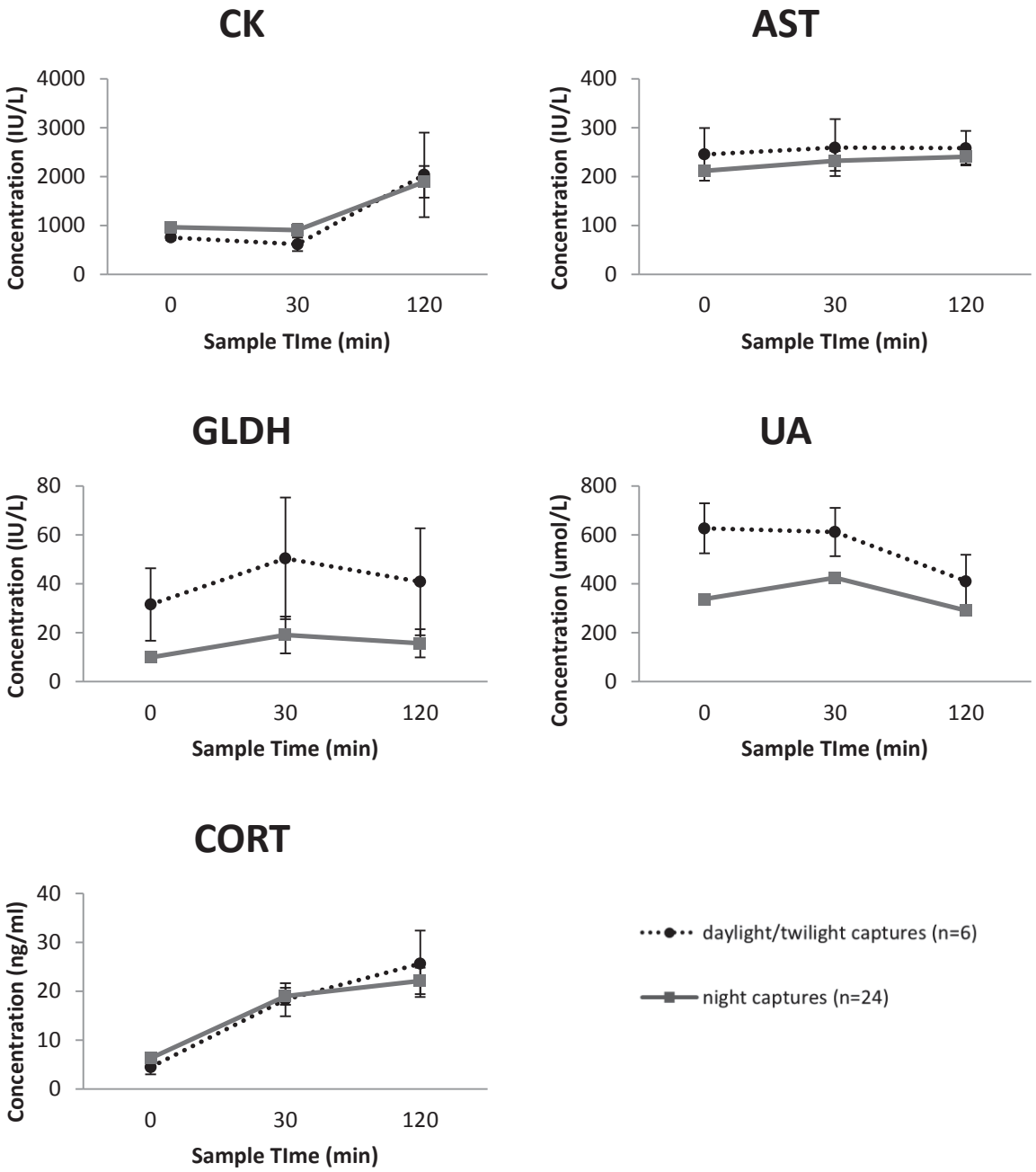


Figure 3.4 The night/day variation in the plasma levels of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in pūkeko (*Porphyrio porphyrio melanotus*) following capture in New Zealand, July 2011-February 2012. Values shown are means ± S.E.M. The birds were separated into daylight/twilight captures consisting of birds sampled between 1600 hours and the end of twilight (when the sun is 12 degrees below the horizon); and night captures consisting of birds sampled between in the hours of darkness following the end of twilight.

3.3.2.2 Effects of Seasonal Variation on the Biochemical Responses of Pūkeko to Capture

Overall, pūkeko captured in this study showed no significant seasonal differences in their response following capture for any of the biochemical parameters. Each of the biochemical parameters was compared between pūkeko captured in winter (n=15) and in summer (n=15) with no significant differences in the mean concentration of plasma CK (*P*=0.599), AST (*P*=0.652), GLDH (*P*=0.055) UA (*P*=0.073) and CORT (*P*=0.085).

However, within the pūkeko captured only at night there were significantly greater mean concentrations of plasma GLDH in 10 winter-caught birds compared to the 14 summer-caught birds (*P*=0.009) (Figure 3.5). None of the other biochemical parameters were significantly different between the seasons.

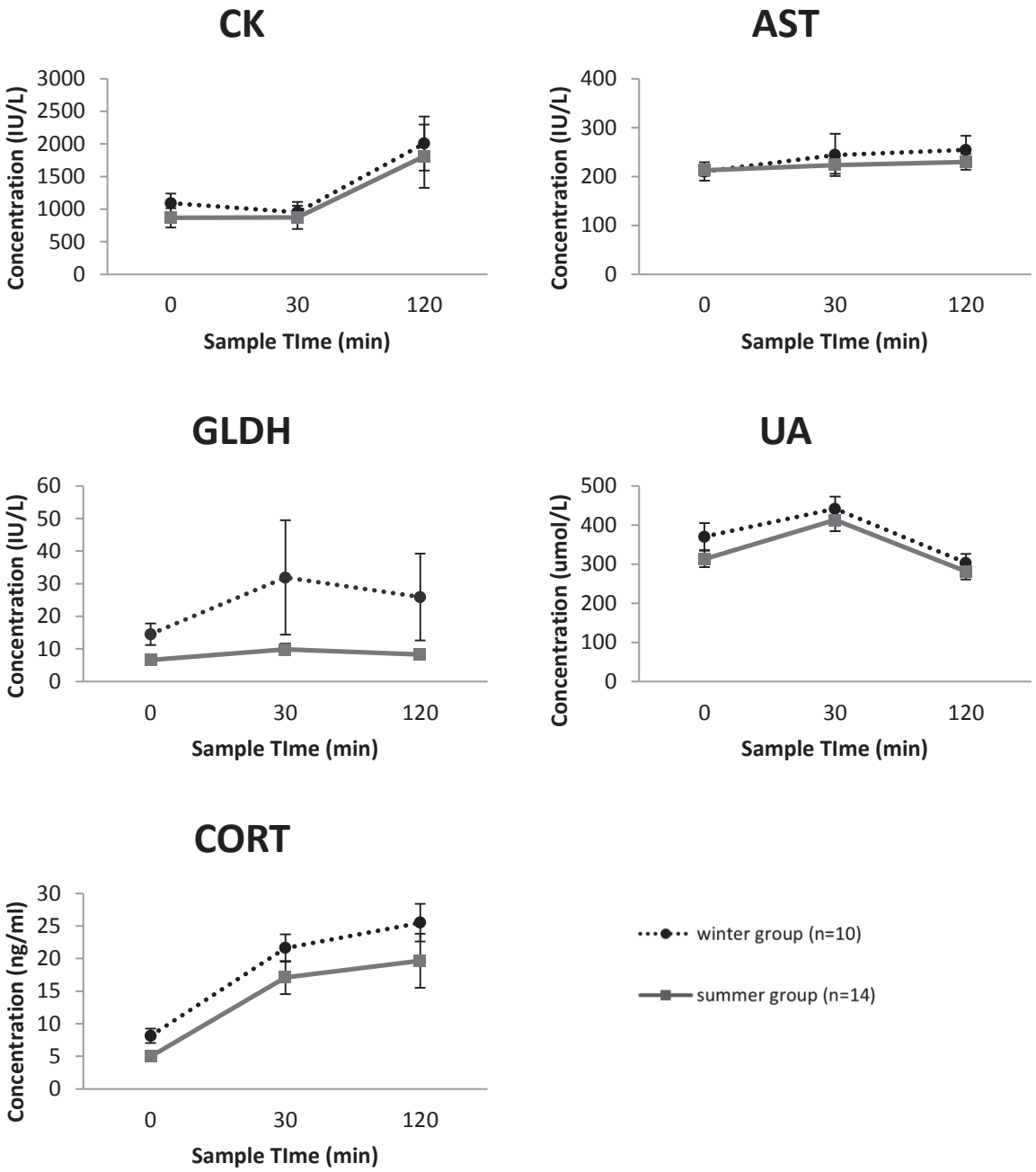


Figure 3.5 The seasonal variation of the plasma levels of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in pūkeko (*Porphyrio porphyrio melanotus*) following capture in New Zealand, July 2011-February 2012. Values shown are means ± S.E.M. The birds captured during the hours of night were separated into birds captured during winter and those captured during summer.

3.3.2.3 Effects of Age on the Biochemical Responses of Pūkeko to Capture

There were no significant difference in the response of adult and juvenile pūkeko in either the full data set (20 adult and 10 juvenile birds), or when looking at birds captured only during the night (15 adult and 9 juvenile birds).

3.3.2.4 Conclusion on Confounding Factors

Results suggest that the time of day the bird was captured is the only confounding factor in the analysis of control versus treatment birds. Therefore, birds captured during daylight/twilight hours were excluded and further analysis comparing control and treatment groups was performed solely on the data from the pūkeko captured at night (n=24).

3.3.3 Physiological Responses of Pūkeko to Capture and Handling

Within the pūkeko captured at night there were 12 birds in the control group and 12 birds in the treatment group. In addition, the 10 pūkeko that were shot dead were used to establish a baseline to compare the 0 minutes samples with. Although as these birds were shot and sampled during the day and the time of day the sample was taken was found to have an effect on netted birds, comparisons with this group have limited value although not for all biochemical analytes.

3.3.3.1 Establishing a Robust Baseline

The delay between capture and blood sampling of the control and treatment birds did not have a significant effect on the mean plasma concentration of any of the biochemical parameters tested. The 0 minutes samples of the netted birds (n=23, one bird excluded due to missing value) were plotted against the length of time it took to achieve the sample for all the biochemical parameters (Figure 3.6). There were no linear relationships between the pre-sample time and the concentration of plasma CK (P=0.233), AST (P=0.998), GLDH (P=0.195), UA (P=0.194) or CORT (P=0.347).

Despite the shot group being sampled during the daylight/twilight, the samples taken at 0 minutes of the control and treatment groups were compared to the group of shot pūkeko and there were no significant differences in the 0 minutes mean concentrations of plasma CK

(*P*=0.691), AST (*P*=0.993), GLDH (*P*=0.343) or CORT (*P*=0.137) between the three groups. Pooling the netted birds (control and treatment groups) and comparing the netted birds to the shot group did not reveal any significant differences in plasma concentrations of CK (*P*=0.390), AST (*P*=0.903), GLDH (*P*=0.175) or CORT (*P*=0.099). Plasma concentrations of UA were not available for the shot group. It was therefore concluded that that the 0 minutes samples of pūkeko captured with the net-gun in this study are valid baseline values to examine the effect of handling.

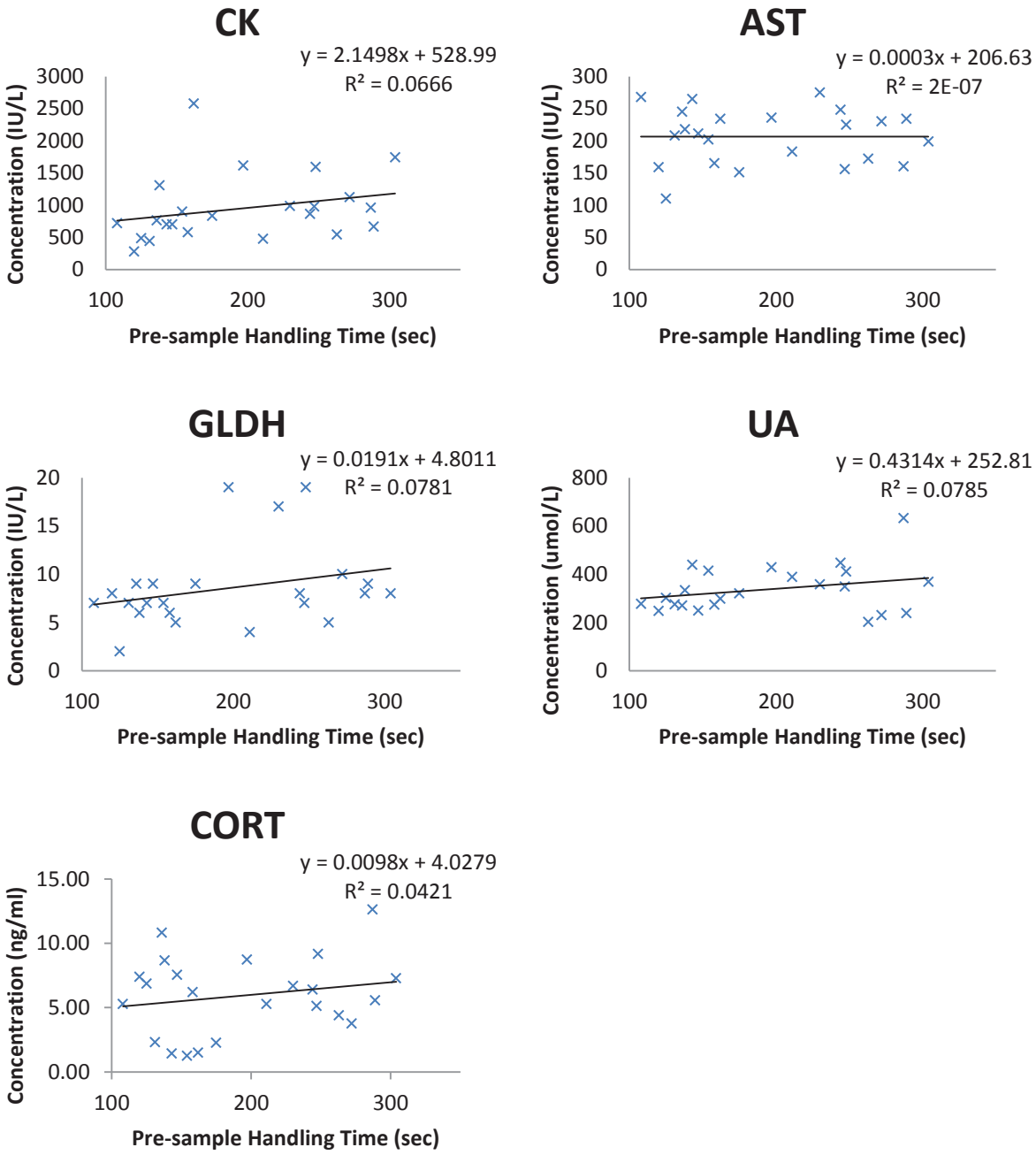


Figure 3.6 The effect of time between capture and blood sampling (at the 0 minutes sampling point) on changes in the plasma concentration of creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT) in pūkeko (*Porphyrio porphyrio melanotus*) following capture by net-gun at night in New Zealand, July 2011-February 2012.

3.3.3.2 Effect of Capture and Handling on Muscle Physiology

Capture had measurable physiological impact on the enzymes associated with muscle damage of pūkeko with significant elevations occurring over the sampling period in the mean concentration of CK and AST. However, no differences were observed between control and treatment group (Figure 3.7).

The mean concentration of plasma CK increased significantly over the times sampled (P<0.001) but was not significantly different between control and treatment groups (P=0.725). While no difference was found in the mean plasma CK concentration between 0 minutes and 30 minutes (P=1.000), the increase in the mean plasma CK concentration between 0 minutes and 120 minutes and especially between 30 minutes and 120 minutes was significant (P=0.005 and P=0.001 respectively). Furthermore extending the handling period of pūkeko rather than placing them in a box did not have an impact on concentrations of plasma CK.

The mean concentration of plasma AST was found to increase significantly over time (P=0.037) but was not significantly different between control and treatment groups (P=0.526). No difference was found in the mean plasma AST concentration between 0 minutes and 30 minutes (P=0.399), nor between 30 minutes and 120 minutes (P=0.818). However the overall increase in the mean plasma AST concentration between 0 minutes and 120 minutes was significant (P=0.011).

3.3.3.3 Effect of Capture and Handling on Stress Physiology

Capture caused a measurable stress response in the pūkeko, with significant elevations occurring over the sampling period in the mean concentration of CORT but no difference was observed between control and treatment group (Figure 3.7).

The mean concentrations of plasma CORT were found to change significantly over time (P<0.001) but did not differ significantly (P=0.652) between control and treatment groups. The increase in the mean plasma CORT concentration between 0 minutes and 30 minutes and overall between 0 minutes and 120 minutes was highly significant (both were P<0.001), while the change in plasma CORT concentration between 30 minutes and 120 minutes was not significant (P=0.306).

3.3.3.4 Effect of Capture and Handling on Selected Organ Function

Capture had a measurable physiological impact on selected organ function of pūkeko with significant changes occurring over the sampling period in the mean concentration of GLDH and UA, but no difference was observed between control and treatment group (Figure 3.7).

The mean concentration of plasma GLDH was found to differ significantly over time (P<0.001) but was not significantly different between the control and treatment groups (P=0.172). There was a significant increase (P<0.001) in the mean plasma GLDH concentration between 0 minutes and 30 minutes, and there was also a significant decrease (P=0.001) in mean plasma GLDH between 30 minutes and 120 minutes. Overall the increase in the mean plasma GLDH concentration between 0 minutes and 120 minutes was found to be significant (P=0.016).

The mean concentrations of plasma UA were found to change significantly over time (P<0.001) but not differ between control and treatment groups (P=0.922). The increase in the mean plasma UA concentration between 0 minutes and 30 minutes was highly significant (P=0.002). In addition the decrease in mean plasma UA between 30 minutes and 120 minutes was found to be highly significant (P<0.001). However the overall decrease on plasma UA between 0 minutes and 120 minutes was not significant (P=0.098).

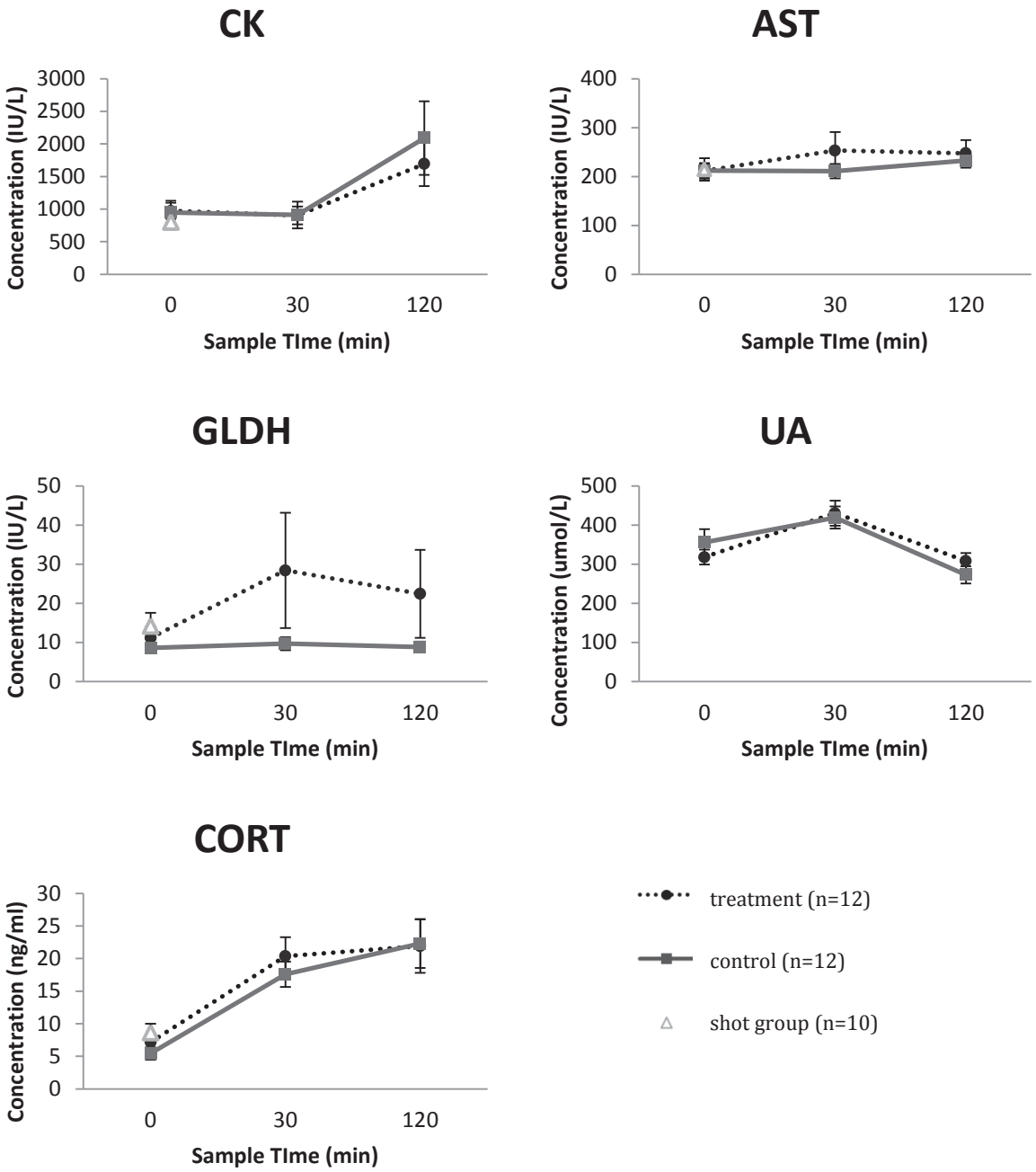


Figure 3.7 The effect of handling on the change in plasma concentrations of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in pūkeko (*Porphyrio porphyrio melanotus*) following capture in New Zealand, July 2011-February 2012. Values shown are means ± S.E.M. Individuals in the control group were blood sampled at capture (0 minutes) and then placed in boxes until 30 minutes when they were removed, sampled again and returned to their box until a final sample was taken at 120 minutes. The treatment group were similarly sampled but they were wrapped in a towel and held between the samples at 0 and 30 minutes before being placed in boxes between 30 and 120 minutes. A further group of pūkeko which were shot dead are also shown at 0 minutes.

3.3.4 Physiological Responses of Pūkeko to Capture in Flight

As no significant differences were found between control and treatment pūkeko, birds from both groups were pooled together *post hoc* and then analysed for differences between birds captured in flight ('flight group') or on the ground ('ground group') using only the data from birds captured at night. Thirteen birds were captured upon the ground and seven birds were captured in flight. Four birds were removed from this analysis as they were captured while sitting in small trees and shrubs and did not fit the classification of either group.

The '0 minutes' samples of both the flight and ground groups could still be considered baseline with no significant differences found in the '0 minutes' concentration of each of the biochemical analytes between the flight, ground and shot groups of pūkeko (CK, P=0.207; AST, P=0.253; GLDH, P=0.107; CORT, P=0.354), or between flight and ground groups for UA (P=0.572).

3.3.4.1 Effect of Capture Type on Muscle Physiology

Capture type (capturing bird in flight compared to on the ground) had a measurable physiological impact on the enzymes indicative of muscle damage in pūkeko (Figure 3.8).

Pūkeko captured in flight had a significantly greater elevation in the mean plasma concentration of CK compared to those captured on the ground (P=0.01). *Post hoc* analysis revealed that while the birds captured in flight started at a similar mean concentration of plasma CK to the ground group (P=0.119) and they were not significantly different at 30 minutes (P=0.055), they had increased concentration of plasma CK at 120 minutes (P=0.002) compared to the birds captured on the ground. The mean concentration of plasma CK of both groups increased significantly over time (P<0.001) with *post hoc* analysis revealing significant changes occurring between 30 and 120 minutes (P<0.001) and between 0 and 120 minutes (P<0.001).

Pūkeko captured in flight also had a significantly greater elevation of mean plasma concentration of AST compared to those captured on the ground (P=0.022). *Post hoc* analysis also revealed that while they started at a similar mean concentration of plasma AST (P=0.066), the birds captured in flight had significantly greater concentrations of plasma AST at both 30 minutes (P=0.033) and 120 minutes (P=0.009) than those captured on the ground. The mean concentrations of plasma AST in both groups increased significantly over time

(P=0.022) with *post hoc* analysis revealing that while no significant changes occurred between 0 and 30 minutes (P=0.429) or 30 and 120 minutes (P=0.487), there was a significant change between 0 and 120 minutes (P=0.012).

3.3.4.2 Effect of Capture Type on Stress Physiology

Capture type had a measurable physiological impact on the stress response of pūkeko with significantly increased elevations of plasma CORT occurring in the birds captured in flight compared to those captured on the ground (Figure 3.8).

Pūkeko captured in flight also responded with significantly greater elevations in the mean concentrations of plasma CORT compared to those birds captured upon the ground (P=0.042). *Post hoc* analysis revealed that while the birds captured in flight started at a similar mean concentration of plasma CORT (P=0.565), and the two groups were not significantly different by 30 minutes (P=0.176), the two groups had diverged significantly by 120 minutes (P=0.024) and the flight group had higher concentrations of plasma CORT. The mean concentration of plasma CORT changed significantly over time (P<0.001) in both groups with *post hoc* analysis revealing significant changes occurring between 0 and 30 minutes (P<0.001) and between 0 and 120 minutes (P<0.001) but not between 30 and 120 minutes (P=0.137).

3.3.4.3 Effect of Capture Type on Selected Organ Function

Capture type did not have a measurable physiological impact on the selected organ function of pūkeko and neither GLDH (P=0.141) nor UA (P=0.570) were significantly different between flight and ground groups (Figure 3.8).

3.3.4.4 Effect of Capture Type on Handling Protocol

As pūkeko captured in flight were found to have significant elevations of plasma analytes, this may have confounded the initial analysis on pūkeko handling where control and treatment control birds were compared. Therefore, the treatment and control groups were compared separately within the flight capture group and within the ground capture group. There were 7 control birds and 6 treatment birds within the ground captured pūkeko, and 3 control birds and 4 treatment birds within the flight captured pūkeko. However, further

analysis based on the flight capture and ground capture distinctions did not alter the findings, suggesting that control birds and treatment birds responded similarly to capture.

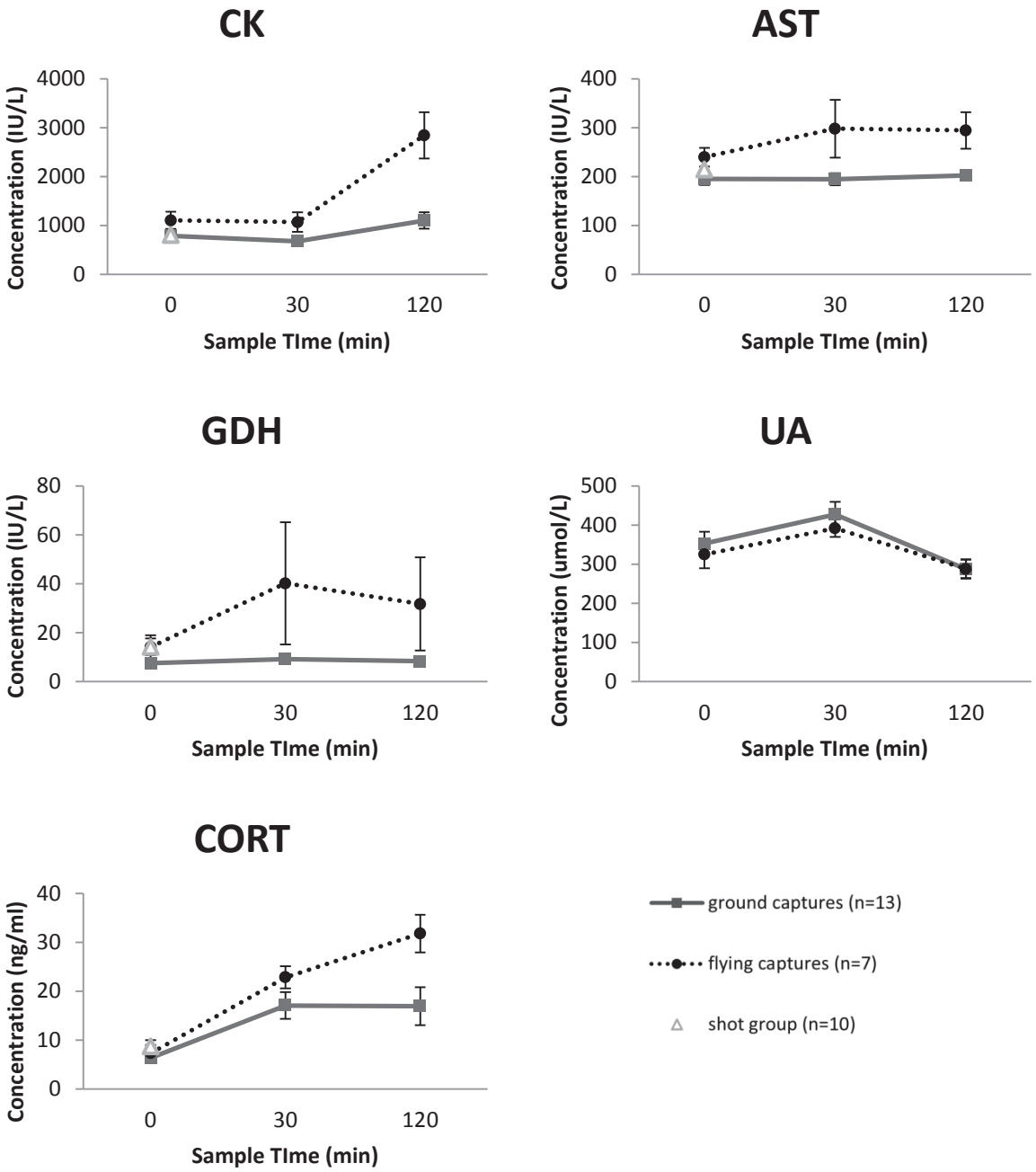


Figure 3.8 The effect of capture in flight compared to ground capture on the changes in plasma concentrations of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT) in pūkeko (*Porphyrio porphyrio melanotus*) following capture by net-gun at night in New Zealand, July 2011-February 2012. Values shown are means ± S.E.M. The control and treatment groups were pooled and the results were distinguished by whether birds were captured in flight or on the ground. Another group of pūkeko which were shot dead and tested to establish a baseline are also shown at 0 minutes.

3.3.5 Effect of Initial Entanglement Time during Capture on the Biochemical Analytes

Pūkeko captured in flight were observed as being more difficult and taking longer to untangle from the net (n=6, x = 502 ± SE 71 seconds), compared to pūkeko captured on the ground (n=13, x = 306 ± SE 100 seconds). However this increased time was not found to be significant. Unfortunately there was no data by which to compare pūkeko that were captured whilst running compared to stationary birds. While the difference between entanglement time in flight vs. ground captures was not significant it was hypothesised that the entanglement time might be a contributing factor to stress and muscle damage and potentially the cause of the biochemical differences found between the pūkeko captured in flight and on the ground. Therefore the length of entanglement of the pūkeko in the net was investigated as a contributing factor to the elevations in the biochemical parameters.

There was no linear relationship evident between the length of time a pūkeko spent entangled in the net and the concentration of any the biochemical parameters at either 30 minutes (CK P=0.736; AST P=0.505; GLDH P=0.224; UA P=0.121; CORT P=0.271) or 120 minutes sampling point (CK P=0.519; AST P=0.779; GLDH P=0.217; UA P=0.234; CORT P=0.598).

3.4 DISCUSSION

3.4.1 Impacts of Capture & Handling on Pūkeko Muscle Physiology

The pūkeko captured in this study responded with significant elevations in plasma CK and AST indicating that capture and sampling resulted in some degree of skeletal or cardiac muscle damage. The handling protocol subsequent to capture was found to be irrelevant regarding the degree of muscle damage the birds experienced. This is a similar finding to a study by Dabbert and Powell (1993) which found that the degree of muscle damage that the wild birds in their study experienced due to capture overrode any additional effects that handling might have. Therefore factors surrounding capture play a primary role in the development of muscle damage in pūkeko rather than handling protocols.

The increase in plasma CK is likely a combination of the physical trauma caused by capture and metabolic and/or localised acidosis in the muscles. The impact of the net, potential fall to the ground, and straining of the pūkeko against the net may have caused physical damage to the muscle tissue. The majority of pūkeko did not fall to the ground on capture; no pūkeko were observed being struck with net projectiles and the impact force of the nylon net itself was probably minor. Therefore the straining and struggling of entangled birds is probably the most frequent mode of physical muscle damage in these captures. The other aspect of muscle damage in these birds is through capture myopathy. For animals that are affected by capture myopathy, the capture results in a biochemical cascade whereby the stress and exertion of the animal through capture and handling caused a build-up of heat and lactic acid in the muscles of the birds. This then causes degeneration and necrosis in the muscle cells and releases muscle enzymes such as CK and AST into the bloodstream (Bartsch *et al.*, 1977; Spraker, 1993; Williams & Thorne, 1996). The difficulty is determining whether plasma elevations in muscle enzymes in the early period following capture are simply due to physical muscle trauma or if they signify a developing capture myopathy.

In this study, the mean plasma concentrations of CK did not significantly raise until between 30 and 120 minutes following capture. Plasma CK responds relatively rapidly to muscle damage and even small amounts of muscle damage may be reflected in the plasma as elevations in CK concentration (Williams & Thorne, 1996). In birds maximum plasma CK concentrations are relatively consistently found to occur around the 24 hr mark following capture or muscle injury (Bailey *et al.*, 1997; Ward *et al.*, 2011). Although the pūkeko were

not released until 120 minutes after capture, this study, as with most sampling of wild animals, is only looking at the initial stages of the CK response curve as the plasma enzyme concentrations are likely to continue to increase to peak concentrations at around 24 hr in the absence of on-going muscle injury.

Similarly, plasma AST concentrations were also found to increase significantly over the sampling period. This change was only significant when looked over a longer time frame (120 minutes) and was not apparent when the change between capture (0 minutes) and 30 minutes or 30 minutes and 120 minutes were looked at in isolation. AST is less specific to muscle than CK (Franson *et al.*, 1985; Cardinet, 1989; Williams & Thorne, 1996). Therefore elevation in plasma AST may also be due to the damage of other tissues such as liver and if the pūkeko captured had liver disease this may have confounded the results. However when looked at in conjunction with CK, it indicates that the most likely source of the elevated plasma AST concentration is damaged muscle tissue.

Pūkeko netted in flight were found to respond to capture with greater elevations of CK and AST than those birds captured upon the ground, suggesting this is a more traumatic method of capture. This finding reinforces my conclusion that the capture event is of central importance in the development of subclinical capture myopathy rather than subsequent handling protocol. Increased muscle damage in birds captured in flight could potentially have been a result of the increased physical trauma a pūkeko netted in flight might experience when falling to the ground. However, plasma CK has been reported to become elevated very rapidly following muscle damage (Williams & Thorne, 1996) and in this study the pūkeko show no significant elevations in plasma CK by the 30 minutes mark. Additionally when looking at each sampling point individually the difference in the plasma CK concentration between ground and flight netted birds only becomes significant at the 120 minutes sampling point. Therefore an alternative hypothesis is that the increased exertion of birds captured in flight promoted a higher degree of acidosis in the muscles and therefore CK and AST leakage. Indeed, in other animal species studied, short, high-intensity chases result in a more severe metabolic acidosis than longer pursuits of a lesser intensity (Harthoorn & Van der Walt, 1974 as cited in Williams & Thorne, 1996). A useful comparison may be between pūkeko that had been caught whilst running compared to birds that were stationary. However, these data were not recorded in this study and all birds captured upon the ground were pooled together.

An alternative explanation for the differences observed is the result of behavioural or personality types within the pūkeko captured. Avian personalities can be defined as a set of related behaviours that a bird expresses across different situations (Cockrem, 2007). That is, the birds that were caught in flight might be individuals that have similar personality types and respond similarly to a perceived threat or stressor. Personalities that consist of the avoidance behaviour such as taking flight rather than freezing may also be linked with fighting behaviours once captured and the greater struggling upon capture may have caused a higher prevalence of muscle injury rather than the flight behaviour.

It was thought that the increase in plasma CK found in pūkeko captured in flight might be partially due to the increased length of time these birds spent entangled in the net. Bollinger *et al.* (1989) found a positive relationship between the length of time wild mallard ducks (*Anas platyrhynchos*) spent struggling in the net and their plasma CK concentration. Ponjoan *et al.* (2008) also found handling and restraint time to be a significant factor in the risk of little bustards (*Tetrax tetrax*), developing clinical signs of capture myopathy. However, no correlation was found between the length of time spent untangling a pūkeko from the net and the plasma CK or AST concentrations, so it is unlikely that the length of time a pūkeko spent tangled in the net was a major factor in the muscle damage. The pūkeko in the current study were caught singly and were reached very quickly (usually within seconds) of being captured and so they were unable to thrash around in the net for long periods of time. The effective entanglement period of the pūkeko in this study may therefore be better considered as the time between capture and the handler reaching the bird. Further research comparing birds held in nets and unrestrained in nets is needed to investigate this as a factor in capture.

The baseline plasma biochemical values measured in pūkeko in the current study (netted and shot) exhibit higher mean values then other biochemical information available on an unknown subspecies of captive swamphens, *Porphyrio porphyrio* (Polo *et al.*, 1994). The mean plasma CK and AST concentrations of the captive birds in Polo *et al.* (1994) was lower than the wild birds sampled by either shooting or net-gun capture in the current study. It is possible that the difference seen could be a result of a peracute rise in plasma enzymes in the pūkeko due to subclinical muscle damage from shooting or capture. However, it could due to naturally occurring differences in baseline CK concentration between the two populations. In humans baseline levels of CK have been shown to be influenced by a number of factors including race, climatic condition and physical activity (Brancaccio *et al.*, 2007). The captive

swamphens and pūkeko that are being compared here may be different subspecies and are likely subjected to different climatic conditions which may have caused the differences. Also human athletes have a higher baseline CK concentration when at rest compared to sedentary individuals at rest (Brancaccio *et al.*, 2007). The wild pūkeko sampled likely have a greater physical activity rate compared to the captive swamphens and this may have resulted in the observed differences in basal CK.

In comparison with normal values reported for waterfowl, the baseline plasma CK levels found in the current study are relatively high (Bollinger *et al.*, 1989). Normal plasma CK concentrations for captive waterfowl were reported as 50-200 IU/L, while waterfowl with capture myopathy are reported having CK concentrations over 1000 IU/L (Bollinger *et al.*, 1989). However while a correlation has been observed between elevated concentrations of plasma CK and AST and clinical signs of capture myopathy (Ward *et al.*, 2011), the values of CK that indicate debilitating muscle damage in birds are not known (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). Generic reference values of CK that may indicate debilitating muscle damage and/or death are likely not able to be determined due to inter-species differences in baseline concentrations of these plasma enzymes. Even birds which have with similar or greater elevations of plasma CK compared to birds of the same species which have suffered from clinical signs of capture myopathy may not exhibit symptoms (Bailey *et al.*, 1997). The one factor which must be taken into account if there is ever to be a reliable reference range of muscle enzymes which reflect debilitating muscle damage is the period of time between capture and the sampling for the given reference range of CK and AST.

It can therefore be concluded that net-gun capture has the potential to cause myopathy in pūkeko, although no birds in this study displayed any overt clinical signs of muscle injury. In addition, capturing pūkeko in flight causes greater muscle damage then capturing them on the ground. In the measured in the current study, pūkeko did not respond differently to the different handling protocols, suggesting that capture is the main event initiating muscle damage in the birds. The routine handling used did not significantly contribute to any muscle damage the birds experienced.

3.4.2 Impacts of Capture & Handling on Pūkeko Stress Physiology

The pūkeko captured in this study responded with significant elevations in plasma CORT indicating that capture elicited a stress response. There were no significant differences found

between the plasma CORT between control and treatment pūkeko, suggesting that handling protocols played little if any role in the CORT response

All pūkeko responded to capture with significant elevations in plasma CORT by the first sampling point, but appeared to have reached a plateau by the second sample at 120 minutes. This is in accordance with the literature where plasma CORT concentrations have been shown to quickly rise in response to capture and handling in a range of wild birds (Dawson & Howe, 1983; Gratto-Trevor *et al.*, 1991; Romero & Romero, 2002; Cockrem *et al.*, 2008). Pūkeko captured in flight showed a greater stress response than those captured on the ground. This emphasises that the capture event had a greater impact on the physiology of the birds than the different handling protocols used following capture.

Capture is likely both an emotional and a physical stressor. While CORT responses have not been assessed in predation events, it is usually assumed the experience of a bird during capture and handling approximates that of predation (Cockrem, 2007). Prior to any physical trauma when a pūkeko becomes aware of human presence, is chased or even is entangled in the net the pūkeko is likely to identify a potential predation event and responds with an increase in plasma CORT. This is the emotional aspect of the stress response (Cockrem, 2007). Following the physical trauma of capture and/or physiological capture myopathy, a second aspect of the stress response likely comes into play where the physical damage acts as a stressor and may cause more CORT to be released (Cockrem, 2007).

The results of the present study support this. Both birds captured on the ground and in flight experienced elevations in CORT between 0 minutes and 30 minutes which is likely the result of the initial emotional stress of capture and also possibly partially due to physical stressors of capture. In birds captured in flight there was a higher degree of muscle damage apparent as discussed in the previous section. This may have acted as more of a physical stressor then in the ground captured birds and therefore prompted the increased CORT elevation in the birds captured in flight due to the muscle damage acting as a stronger physical stressor.

An alternative hypothesis to this is that the greater CORT elevation observed in the birds captured in flight was the cause rather than the result of the increased level of muscle damage in this group. Stress plays a pivotal role in the development of capture myopathy (Basson & Hofmeyr, 1973) and the higher elevations of plasma CORT in the birds captured in flight may have promoted the development of subclinical capture myopathy. If this is the case it is not clear whether capturing a pūkeko in flight caused it to have a higher elevation of plasma

CORT or whether those pūkeko which would respond to capture (regardless of capture type) with higher elevations of plasma CORT are more likely to take flight when they see humans and thus are more likely to be captured in flight.

Characteristic patterns of behaviour which are used to cope with the different challenges or stressors an animal faces have been called personalities (Cockrem, 2007). The pūkeko that responded to capture attempts by taking flight may all be of the same or similar personalities. That is, in the face of an immediate threat such as attempted predation or in this case capture, they have a lower threshold for taking flight. Personalities vary along a continuum but individuals are often divided into either active or passive coping styles based on their behavioural response to a range of situations (Cockrem, 2007). Based solely on the behavioural observation of flight at capture, pūkeko could be classified into those captured on the ground as having passive personalities and those captured in flight as having active personalities. However, passive type personalities have been associated with having higher CORT response compared to active type personalities which is the opposite of what was observed in these pūkeko under these personality types. In order to determine if the responses seen in the current study reflect true personality differences, multiple observations of the individuals involved would be necessary.

If the difference between the pūkeko capture on the ground and in flight was a result of different personality types it may be that the active personality of these birds meant they responded to capture not only with flight but also by struggling more. The increased muscle damage of birds captured in flight might not be the actual result of flying behaviour or directly that of high CORT but simply behaviour associated with these factors that promoted muscle damage. A higher percentage of pūkeko captured in flight (compared to those captured on the ground) were observed to struggle more against the net or during handling. The behavioural response, the CORT response and avian personalities are all interrelated and it may be relationship between these factors which caused the increased degree of muscle damage measured in pūkeko that were captured in flight.

It can therefore be concluded that the capture event was the major factor in provoking a stress response in the pūkeko and this overrode any effect handling protocols might have had.

3.4.3 Impacts of Capture & Handling on Selected Organ Function

While the plasma biochemical enzymes previously discussed are linked to acute effects on muscle and stress responses, the physiology of other body systems is also affected by a capture event. This study also recorded the response of plasma concentrations of UA and GLDH to capture.

UA is the major nitrogenous waste in birds. It is mainly produced in the liver and is eliminated from the body in the urine via the kidneys (Fudge, 1999). Plasma UA concentrations were not significantly different between control and treatment groups of pūkeko, or between pūkeko captured in flight and those captured upon the ground. Pūkeko captured in the daylight/twilight hours exhibited higher levels of plasma UA compared to those pūkeko that were captured at night. Following capture the plasma UA of the pūkeko significantly rose by the 30 minutes sampling point. The concentration returned to approximately the original concentration of plasma UA by the 120 minutes sampling point.

There are several possible mechanisms that may explain the rise of plasma concentrations of UA. Digestion of a high protein meal is a physiologically normal cause of elevated plasma concentrations of UA in birds (Fudge, 1999). Alternatively, elevated plasma concentrations of UA may also indicate failure of the kidneys to clear UA (Fudge, 1999). The initial rise of plasma UA in these pūkeko could potentially be a result of digestion of food in the gizzard, dehydration or from renal failure. However, the secondary decrease in mean plasma UA between the 30 and 120 minutes sampling point largely rules out renal damage or dehydration.

Renal damage can be associated with capture myopathy when myoglobin released from the damaged muscle cells induces acute renal failure (Williams & Thorne, 1996; Herraez *et al.*, 2007). Ward *et al.* (2011) found plasma UA to significantly increase in bar-tailed godwits diagnosed with clinical capture myopathy in the days following capture. Godwits that died also had significantly higher concentration of plasma UA then those that were released. However if the pūkeko in the present study exhibited myoglobinuria, the plasma UA would be expected to become increasingly elevated rather than peaking at 30 minutes and decreasing in concentration by the 120 minutes sample. Therefore renal damage is unlikely to be the cause of the increase in plasma UA between the 30 minutes and 120 minutes sample. The simplest explanation is that the birds initially had post-prandial UA elevations associated

with physiologically normal nitrogen metabolism (Lumeij & Remple, 1991) and the capture event limited digestion or diverted energy to more immediate concerns (flight/fright). This may be due to a sympathetically mediated diversion of blood flow away from the digestive system to the musculoskeletal system (Spraker, 1993; ter Steege & Kolkman, 2012). Digestion of protein following foraging activity would also explain the differences in plasma UA concentrations that were observed between birds that were captured during daylight/twilight and those birds captured at night.

In times of physical exertion in humans, blood flow is directed away from splanchnic organs such as the kidneys and diverted to the working muscles (ter Steege & Kolkman, 2012). The stress associated with the capture of an animal due may also cause this to happen (Spraker, 1993). Avian physiology may follow a similar pathway and the physical exertion and stress a pūkeko experiences during capture may have caused blood to be directed away from the kidneys and towards other areas of the body. With reduced blood flow to the kidneys, the kidneys would not process UA as efficiently and the plasma UA concentration would rise. As the bird recovered from the exertion of capture normal blood flow would resume to the kidneys and they would return to efficiently processing blood UA, decreasing the mean plasma UA concentration back to the normal. However, if reduced blood flow to splanchnic organs affected kidney function then it should also affect the digestion of protein and therefore the blood UA should not rise between 0 minutes and 30 minutes either.

The baseline UA concentrations in our study were much lower than was found for the 6 captive swamphens *Porphyrio porphyrio* from Barcelona zoo (Polo *et al.*, 1994). As plasma UA is affected by ingesting animal protein, the difference could be due to the captive birds receiving a diet with higher animal protein content.

In the present study, plasma GLDH concentrations were not significantly different between control and treatment groups of pūkeko, or between pūkeko captured in flight and those captured upon the ground. There was a large amount of variation in the plasma GLDH concentration between pūkeko captured in the daylight/twilight hours compared to those pūkeko that were captured at night. The variation in baseline GLDH between the day and night in this study support the hypothesis that while marked elevations of plasma GLDH concentration are a sensitive marker of hepatocellular damage, more subtle changes may be indicative of changes in metabolism. Once variation between day and night was taken into account, seasonal variation in plasma GLDH was also apparent, with pūkeko captured in

winter exhibiting higher concentration of plasma GLDH compared to those birds captured in the summer. Plasma GLDH was found to become significantly elevated over the sampling period however the aforementioned factors may have confounded the analysis.

The pattern of change in GLDH concentration seen in the pūkeko was similar to the changes in plasma concentrations of UA, and was different to that seen for plasma concentrations of AST. Taken together, these changes in the plasma enzyme concentrations suggest that the GLDH changes seen were due to nitrogen metabolism, similar to the pattern seen in UA, rather than reflecting liver damage, in which case it would be expected to mirror AST elevations. However, it is still possible that acute hepatic damage was responsible for the transient elevations in GLDH, as the enzyme is eliminated in a comparatively very short time from the blood, with a mean half-life of 0.68 hours and standard deviation of 0.17 hours in racing pigeons (Lumeij *et al.*, 1988).

The process whereby the capture event could have caused the transient elevation of plasma GLDH concentrations is unclear. Potentially the increased exertion of the pūkeko at capture may have caused the pūkeko physiology to redirect blood flow away from the splanchnic organs such as the liver and towards the working muscles (ter Steege & Kolkman, 2012). In mammals GLDH activity is relatively specific to liver tissue. There is some GLDH activity in other tissue such as kidneys and brain but it is unlikely that plasma GLDH would rise transiently due to kidney or nervous damage (Clampitt & Hart, 1978). In mammals the level of GLDH activity in skeletal and cardiac muscle is much smaller again compared to the kidneys therefore damage to these tissue is extremely unlikely to have caused elevations in plasma GLDH (Clampitt & Hart, 1978).

3.4.4 How the Results Compare With Other Studies on Capture Myopathy

Many papers do not differentiate between the capture and handling processes and do not consider the two processes independently (Bailey *et al.*, 1997; Cox & Afton, 1998). This is likely because the impacts of events such as capture and tagging may be hard to separate from each other (Southern & Southern, 1983) unless specifically designed to examine these effects. The present study suggests that in future studies on capture related conditions, each technique within the processes of capture, handling and tagging should be looked at in

isolation as well as the combined effect. Alternatively, it may take longer handling durations to show a significant impact than that used in this study.

Findings appears to conflict with one study on little bustards (*Tetrax tetrax*) where handling time was found to be a significant factor in the development of clinical signs of capture myopathy (Ponjoan *et al.*, 2008). It may be that handling techniques play a more significant role in some circumstances in which a bird experiences more severe muscle damage or alternatively that handling has different effects in different species.

Bollinger *et al.* (1989) also found that the capture event was of central importance in the development of muscle damage in birds and that different capture techniques resulted in more or less muscle damage. The method the birds were restrained by and the duration of the restraint were identified as the key element of capture that affected CK elevation.

While both the present study and that undertaken by Bollinger *et al.* (1989) are in agreement about the importance of capture techniques, the specific factors that Bollinger *et al.* (1989) identified as major contributors to muscle damage were not seen in the current study on pūkeko. Bollinger *et al.* (1989) found the time mallard ducks spent struggling under a rocket net was related to the degree of muscle damage in the bird. No relationship was found in the current study between the elevation in CK and length of time a pūkeko spent entangled in the net. In addition, Bollinger *et al.* (1989) reported that the extra handling time that birds captured using a decoy trap had resulted in higher CK concentrations than birds captured by a bait trap, which required less handling time. In contrast the present study on pūkeko found that handling time did not increase the amount of muscle damage compared to free confinement in a box. The differences may be because the birds spent on average over ten times longer time under the rocket net then they did under the net-gun net. No major factor is apparent as to why handling was not a factor in my study but it may the handling of the birds in the Bollinger *et al.* (1989) study is not directly comparable to that carried out in my study. For example, the birds in Bollinger's study were noted as being carried up to 40 m prior to processing.

Seemingly in contrast to findings in the current study, Nicholson *et al.* (2000) found that the probability of mortality in Eastern wild turkeys following capture went down (insignificantly) with increased concentrations of plasma CORT, while the probability of mortality in Eastern wild turkeys following capture went up with increased concentrations of plasma CK. Nicholson *et al.* (2000) hypothesise that this may be due to birds that experience higher levels

of CORT and therefore stress being more likely to freeze and struggle less, thereby causing less muscle damage. The present study appears to give evidence to the contrary as plasma CK and plasma CORT were both significantly elevated in the group of pūkeko captured in flight. This may reflect a species variation in response to capture. Further investigation into the relationship between muscle damage and stress levels is clearly warranted.

3.4.5 Assessment of the Use of Net-guns to Capture Pūkeko

The capture of pūkeko in this study using a net-gun did not result in significant observable injuries or clinical capture myopathy, although the biochemical analysis indicated that a measurable physiological response occurred which included a stress response, some degree of subclinical muscle damage, and an alteration in to visceral function.

Netting pūkeko in the air was found to significantly increase the chance of subclinical muscle damage and the magnitude of plasma CORT elevations. It is therefore advisable that where possible, pūkeko (and potentially other avian species) should be netted on the ground rather than flight. It is possible that moving birds may be more at risk of subclinical capture myopathy then stationary birds.

The two major advantages of net-guns are that they allow the specific targeting of individuals and prompt sampling of the bird. Furthermore they can be used with caution so that potentially at risk individuals may not be captured or less than ideal scenarios such as birds captured in flight may be avoided.

None of the birds captured in this study were injured or killed during capture. This is in contrast to some early studies using net-guns (Mechlin & Shaiffer, 1980; Huschle *et al.*, 2002; Herring *et al.*, 2008), but in agreement with some more recent studies (Edwards & Gilchrist, 2011). This is likely due to the lesser power of the model used in this study, the smaller size of the net projectiles, and the closer distance that the birds were netted at. Combined, these factors meant that there was a lower chance of injury if a projectile struck a bird and it was easier to be accurate the closer the shooter was to the bird. The reduced power and therefore effective range of this model of net-gun also meant that it was likely harder to capture birds as they had to be approached closer.

3.4.6 Assessment of Using Pūkeko as a Surrogate Species for Takahē

As discussed in the initial sections of this chapter, Pūkeko were used as a surrogate species to model capture and handling impacts on takahē because the sampling protocol required in this study would not be permitted on such rare birds. In order to effectively draw management conclusions from this study that can be applied to takahē, some sort of validation of the suitability of the results must be made.

Pūkeko may not be an ideal substitute for modelling takahē capture and handling impacts with notable differences between the two species being the takahē having a more robust build and lack of flight. However the baseline concentrations of the biochemical analytes CK, AST and UA in pūkeko found in this study are similar to normal values found in takahē (Rose, 2000; Youl, 2008).

It should be noted that levels of CK and AST that were found in the pūkeko captured in this study at the 30 minutes mark are comparable to the concentrations found in takahē captured by hand or by using hand-nets or loose nylon nets (Rose, 2000) and takahē captured by hand or by using hand-nets or capture pens with food lure (Youl, 2008).

In the absence of any further biochemical information on takahē following capture and handling the conclusions below are based on the assumption that pūkeko and takahē would physiologically respond in a similar way.

3.4.7 Management Recommendations Regarding Takahē Capture and Handling Protocols

3.4.7.1 Assessment of Takahē Handling protocol

The takahē handling protocol used in this study was found not to affect the elevations of CK, AST and CORT in pūkeko that resulted from capture. These results indicate that current handling protocol does not cause additional stress or muscle damage to birds. Whether the larger mass of takahē and more robust build of a takahē would affect these results cannot be known without directly sampling takahē. However, the evidence indicates that the handling

protocol of takahē is likely to be appropriate with regard to its potential to cause clinical or subclinical capture myopathy.

3.4.7.2 Recommendations Regarding Capture protocols of Takahē

The capture event of pūkeko was the most important factor affecting the birds' physiological response to handling. This is also likely to be true of takahē. Pūkeko captured in flight were the most at risk of higher elevations of plasma CK, AST and CORT. Takahē are flightless and while this mode of capture is not directly applicable, the exertion of an animal during capture is recognized as key factor in the development of clinical capture myopathy (Williams & Thorne, 1996). The maximum metabolic rate a bird may experience when running is lower than that achieved during flight (Vaillancourt *et al.*, 2005). However it may be that birds captured during or immediately after running activity are more likely to suffer greater elevations of plasma CK, AST, CORT and therefore increased subclinical capture myopathy compared to those birds captured when stationary. Further research should therefore be carried out to investigate this. In addition, future studies would be useful to compare the effects of net-gun capture compared to current wild takahē capture techniques. Net-guns have the potential to reduce the chase times and thus may actually reduce the impact of capture on the animal.

Rose (2000) found two takahē which exhibited extremely high plasma CK and AST that were excluded from the normal values reported in that study. None of the pūkeko captured in this study showed enzyme concentrations which approached the concentrations reported from these two birds. The plasma CK concentration of these two takahē were 73720 IU/L and 118480 IU/L, while their plasma AST concentrations were 1640 and 2600 IU/L within 14-24 minutes of capture (Rose, 2000). The plasma enzymes in this study in comparison reached an uppermost range of 7300 IU/L of plasma CK in one pūkeko at the 120 minutes sample and 608 IU/L of plasma AST in a different pūkeko at 30 minutes.

In the present study, neither plasma CK nor AST became significantly elevated until after the 30 minutes sampling point. The much higher plasma enzyme concentrations seen in the two takahē as reported by Rose (2000) may suggest either some traumatic event prior to capture, or that the muscle damage experienced by these takahē during capture was so significant that it caused a massive and very rapid elevation of CK and AST. The events leading up to

capture of these two birds were not noted, in particular how the birds were captured. Takahē are a critically endangered species and given that in most cases the capture of birds does not appear to cause any negative clinical signs, there may be a reluctance to proceed with further research on sampling wild takahē over an extended period as this may be seen as causing more harm than benefit. Therefore it is suggested that in addition to the visual cues of stress already looked for during takahē captures, a maximum chase time should be established for takahē and that capture scenarios or techniques that require pursuits are largely avoided.

References

Anderson, C. M., & Swanson, R. A. (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia*, *32*(1), 1-14.

Anonymous. Wildlife Act 1953. http://www.legislation.govt.nz/act/public/1953/0031/latest/whole.html#whole (accessed 23 May 2013). Public Act 1953 No 31. New Zealand Government, Wellington, NZ, 1999.

Anonymous (2012a). *IUCN Red List of Threatened Species*. Version 2012.2. www.iucnredlist.org (accessed 19 August 2012).

Anonymous (2012b). *Sun or Moon Rise/Set Table for One Year. from U.S. Naval Observatory*. http://aa.usno.navy.mil/data/docs/RS_OneYear.php (accessed 20 May 2013). The United States Naval Observatory (USNO).

Anonymous (2013). Open season for game in Fish and Game regions. *New Zealand Gazette. Issue No. 30*, pp 893-920. http://www.dia.govt.nz/diawebsite.nsf/wpg_URL/Services-New-Zealand-Gazette-2013-Gazettes?OpenDocument (accessed 23 May 2013). New Zealand Government, Wellington, NZ, 2013.

Bailey, T. A., Wernery, U., Naldo, J., & Samour, J. H. (1997). Plasma concentration of creatine kinase and lactate dehydrogenase in houbara bustards (*Chlamydotis undulata macqueenii*) immediately following capture. *Comparative Haematology International*, *7*(2), 113-116.

Bartsch, R. C., McConnell, E. E., Imes, G. D., & Schmidt, J. M. (1977). Review of exertional rhabdomyolysis in wild and domestic animals and man. *Veterinary Pathology*, *14*(4), 314-324.

Basson, P. A., & Hofmeyr, J. M. (1973). Mortalities associated with wildlife capture operations. In E. Young (Ed.), *The Capture and Care of Wild Animals; The Work of Eighteen Veterinary, Medical, and Wildlife Experts* (pp. 151-160). Cape Town and Pretoria: Human and Rousseau.

Bollinger, T., Wobeser, G., Clark, R. G., Nieman, D. J., & Smith, J. R. (1989). Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by 3 methods for banding. *Journal of Wildlife Diseases*, *25*(2), 225-231.

Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport medicine. *British Medical Bulletin, 81-82*, 209-230.

Bunin, J. S., & Jamieson, I. G. (1996). A cross-fostering experiment between the endangered takahe and its closest relative, the pukeko. . *New Zealand Journal of Ecology, 20*, 209-213.

Cardinet III, G. H. (1989). Skeletal muscle function. In J. J. Kaneko (Ed.), *Clinical Biochemistry of Domestic Animals* (4th ed., pp. 463-495). New York: Academic Press, Inc.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A., & Reynolds-Hogland, M. L. (2008). An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy, 89*(4), 973-990.

Clampitt, R. B., & Hart, R. J. (1978). Tissue activities of some diagnostic enzymes in 10 mammalian species. *Journal of Comparative Pathology, 88*(4), 607-621.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology, 148*, S169-S178.

Cockrem, J. F., Potter, M. A., Barrett, D. P., & Candy, E. J. (2008). Corticosterone responses to capture and restraint in emperor and Adelie penguins in Antarctica. *Zoological Science, 25*(3), 291-298.

Cox, R. R., & Afton, A. D. (1998). Effects of capture and handling on survival of female northern pintails. *Journal of Field Ornithology, 69*(2), 276-287.

Crouchley, D. (1994). Takahe Recovery Plan (*Porphyrio [Notornis] mantelli*). Wellington: Department of Conservation.

Dabbert, C. B., & Powell, K. C. (1993). Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases, 29*(2), 304-309.

Dawson, A., & Howe, P. D. (1983). Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *General and Comparative Endocrinology, 51*(2), 303-308.

Edwards, D. B., & Gilchrist, H. G. (2011). A new means of catching shorebirds: the Super Talon Net Gun. *Wader Study Group Bulletin, 118*(2), 134-136.

Franson, J. C., Murray, H. C., & Bunck, C. (1985). Enzyme activities in plasma, kidney, liver, and muscle of 5 avian species. *Journal of Wildlife Diseases, 21*(1), 33-39.

Fudge, A. M. (1999). *Laboratory Medicine: Avian and Exotic pets*: Saunders.

Gratto-Trevor, C. L., Oring, L. W., & Fivizzani, A. J. (1991). Effects of blood sampling stress on hormone levels in the semipalmated sandpiper. *Journal of Field Ornithology, 62*(1), 19-27.

Herraez, P., Sierra, E., Arbelo, M., Jaber, J. R., de los Monteros, A. E., & Fernandez, A. (2007). Rhabdomyolysis and myoglobinuric nephrosis (capture myopathy) in a striped dolphin. *Journal of Wildlife Diseases, 43*(4), 770-774.

Herring, G., Gawlik, D. E., & Beerens, J. M. (2008). Evaluating two new methods for capturing large wetland birds. *Journal of Field Ornithology, 79*(1), 102-110.

Hudson, R. C., & Daniel, R. M. (1993). L-glutamate dehydrogenases distribution: properties and mechanism. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 106*(4), 767-792.

Huschle, G., Toepfer, J. E., Brininger, W. L., & Azure, D. A. (2002). Capturing adult American bitterns. *Waterbirds, 25*(4), 505-508.

Kilduff, L., Dodson, H., & Marsh, P. (2011). Takahe (*Porphyrio hochstetteri*) Husbandry Manual. Te Anau: Department of Conservation (DOC),.

Kreeger, T. J., White, P. J., Seal, U. S., & Tester, J. R. (1990). Pathological responses of red foxes to foothold traps. *Journal of Wildlife Management, 54*(1), 147-160.

Lee, W. G., & Jamieson, I. G. (2001). *The Takahe: Fifty Years of Conservation Management and Research* Dunedin: University of Otago Press.

Lumeij, J. T., Debruijne, J. J., Slob, A., Wolfswinkel, J., & Rothuizen, J. (1988). Enzyme activities in tissues and elimination half-lives of homologous muscle and liver enzymes in the racing pigeon (*Columba livia domestica*). *Avian Pathology, 17*(4), 851-864.

Lumeij, J. T., & Remple, J. D. (1991). Plasma urea, creatinine and uric-acid concentrations in relation to feeding in peregrine falcons (*Falco peregrinus*). *Avian Pathology, 20*(1), 79-83.

Mechlin, L. M., & Shaiffer, C. W. (1980). Net firing gun for capturing breeding waterfowl. *Journal of Wildlife Management, 44*(4), 895-896.

Miskelly, C. M., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Powlesland, R. G., Robertson, H. A., Sagar, P. M., Scofield, R. P., & Taylor, G. A. (2008). Conservation status of New Zealand birds. *Notornis 55*(3), 117-135.

Nicholson, D. S., Lochmiller, R. L., Stewart, M. D., Masters, R. E., & Leslie, D. M. (2000). Risk factors associated with capture related death in eastern wild turkey hens. *Journal of Wildlife Diseases, 36*(2), 308-315.

Nissim, I. (1999). Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *American Journal of Physiology-Renal Physiology, 277*(4), F493-F497.

Polo, F. J., Celdran, J., Viscor, G., & Palomeque, J. (1994). Blood chemistry of captive herons, egrets, spoonbill, ibis and gallinule. *Comparative Biochemistry and Physiology a-Physiology, 107*(2), 343-347.

Ponjoan, A., Bota, G., De La Morena, E. L. G., Morales, M. B., Wolff, A., Marco, I., & Manosa, S. (2008). Adverse effects of capture and handling little bustard. *Journal of Wildlife Management, 72*(1), 315-319.

Ripley, S. D., Lansdowne, J. F., & Olson, S. L. (1977). *Rails of the World: A Monograph of the Family Rallidae*: M. F. Feheley.

Romero, L. M., & Romero, R. C. (2002). Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor, 104*(1), 129-135.

Rose, K. (2000). Takahe (*Porphyrio hochstetteri*) Health Investigation Project. Te Anau Department of Conservation.

Seddon, P. J., Launay, F., Van Heezik, Y., & Al Bowardi, M. (1999). Methods for live trapping houbara bustards. *Journal of Field Ornithology, 70*(2), 169-181.

Sener, A., Owen, A., Malaisselagae, F., & Malaisse, W. J. (1982). The stimulus secretion coupling of amino acid-induced insulin release .11. kinetics of deamination and transamination reactions. *Hormone and Metabolic Research, 14*(8), 405-409.

Southern, L. K., & Southern, W. E. (1983). Responses of ring-billed gulls to cannon-netting and wing-tagging. *Journal of Wildlife Management, 47*(1), 234-237.

Spraker, T. R. (1993). Stress and capture myopathy in artiodactylids. In M. E. Fowler (Ed.), *Zoo and Wild Animal Medicine: Current Therapy* (Vol. 3, pp. 481-488). Philadelphia: W.B. Saunders Company.

Spraker, T. R., Adrian, W. J., & Lance, W. R. (1987). Capture myopathy in wild turkeys (*meleagris gallopavo*) following trapping, handling and transportation in Colorado. *Journal of Wildlife Diseases, 23*(3), 447-453.

Suttie, J. M., & Fennessy, P. F. (1992). Organ weight and weight relationships in takahe and pukeko. *Notornis, 39*(1), 47-53.

ter Steege, R. W. F., & Kolkman, J. J. (2012). Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Alimentary Pharmacology & Therapeutics, 35*(5), 516-528.

Town, M. H., Gehm, S., Hammer, B., & Ziegenhorn, J. (1985). A sensitive colorimetric method for the enzymatic determination of uric acid. *Journal of Clinical Chemistry and Clinical Biochemistry, 23*(9), 591-591.

Trewick, S. A. (1996). The diet of Kakapo (*Strigops habroptilus*), Takahe (*Porphyrio mantelli*) and Pukeko (*P. porphyrio melanotus*) studied by faecal analysis. *Notornis, 43*(2), 79-84.

Trewick, S. A. (1997). Flightlessness and phylogeny amongst endemic rails (Aves: Rallidae) of the New Zealand region. *Philosophical Transactions of the Royal Society B: Biological Sciences, 352*(1352), 429-446.

Vaillancourt, E., Prud'Homme, S., Haman, F., Guglielmo, C. G., & Weber, J. M. (2005). Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running. *Journal of Experimental Biology, 208*(2), 317-325.

Ward, J. M., Gartrell, B. D., Conklin, J. R., & Battley, P. F. (2011). Midazolam as an adjunctive therapy for capture myopathy in bar-tailed godwits (*Limosa lapponica baueri*) with prognostic indicators. *Journal of Wildlife Diseases, 47*(4), 925–935.

Wickes, C., Crouchley, D., & Maxwell, J. (2009). Takahe (*Porphyrio hochstetteri*) Recovery Plan 2007–2012. Wellington: Department of Conservation.

Williams, E. S., & Thorne, E. T. (1996). Exertional myopathy (capture myopathy). In A. Fairbrother, L. N. Locke & G. L. Hoff (Eds.), *Noninfectious Diseases of Wildlife* (2nd ed., pp. 181-193). London: Manson Publishing Ltd.

Youl, J. M. (2008). *An investigation into lead exposure in free-ranging kea (Nestor notabilis), takahe (Porphyria hochstetteri) and Australasian harriers (Circus approximans) in New Zealand.* Masters of Veterinary Science, Massey University, Palmerston North.

CHAPTER FOUR

A STUDY ON WILD MALLARD DUCKS (*Anas platyrhynchos*) AS A SURROGATE FOR ASSESSING THE PHYSIOLOGICAL IMPACTS OF CAPTURE AND HANDLING ON THREATENED NATIVE WATERFOWL

Abstract

The management of waterfowl may require the capture and handling of certain individuals for monitoring, translocations and research purposes. These procedures likely impact the physiological homeostasis of the bird and therefore this study investigate these potential impacts by using wild mallard ducks (*Anas platyrhynchos*) as a model for threatened species such as pateke/brown teal (*Anas chlorotis*), or whio/blue duck (*Hymenolaimus malachorhynchos*).

Thirty-seven wild mallard ducks were captured using a net-gun at Massey University's Turitea campus over the months of January and February, 2012. Captured mallards were held and blood sampled immediately once captured and twice more 30 and 120 minutes following capture. Birds in the control group were placed in a box between blood samples and birds in the treatment group were held using pateke best practice protocol between the first and 30 minute blood sample. During this process the bird was held upright with the handler's hands on either side around the wings and body. After the 30 minute sample treatment birds were placed in the box also.

To assess the physiological impact of capture, biochemical analytes measured included plasma concentrations of uric acid (UA), creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and the stress hormone corticosterone (CORT).

Capture caused a significant stress response in the mallards as seen by the increase in plasma CORT concentrations but did not have a significant effect on the plasma concentrations of CK, GLDH or UA. Plasma AST concentrations decreased significantly following capture albeit by a very small amount. Handling protocols of treatment birds did not cause any measurable effects on the biochemical analytes. Season and the time of day the sample was taken caused variation in concentration of plasma CK in the ducks.

4.1 INTRODUCTION

Anatidae, commonly called waterfowl, are a family of birds including ducks, geese and swans that are found throughout the world in wetland habitats a number of which are threatened and require intensive conservation management. The number of extant species in the family changes as more genetic and taxonomic information is revealed but there are around 151 species in 45 genera (Johnsgard, 2010) to 173 species in 55 genera (Livezey, 2010 as cited in Johnsgard, 2010). The International Union for Conservation of Nature (IUCN) Red List of Threatened Species lists 162 extant species of Anatidae including 6 critically endangered species, 11 endangered species, 13 vulnerable species and 8 near threatened species (Anonymous, 2012).

Endangered and vulnerable waterfowl species often require intensive conservation management to slow, halt or reverse population declines. These interventions may include translocations, where wild caught or captive bred animals are released at new locations to propagate new populations or sustain existing populations of the threatened species. (IUCN, 1987; Holmes & Caskey, 2001; Gummer & Berry, 2007). Species management often requires detailed information about their biology, health and population dynamics to be able to meet husbandry requirements of captive populations, assess the trends in the populations and how management process and/or threats are affecting the populations (O'Connor *et al.*, 2007; Glaser *et al.*, 2010). While indirect sampling and monitoring of animals is preferred there is some information that can only be obtained by capturing the animal (Powell & Proulx, 2003).

In all management procedures that require the capture and handling of wild or free-living waterfowl there is the potential for injury or altered physiology or behaviour to the birds in question (Mechlin & Shaiffer, 1980; Bollinger *et al.*, 1989; Wobeser, 1997). The process must not only be justified but the techniques used should be continually improved to minimise the impact the methods are having on the animals (Powell & Proulx, 2003). This is important for animal welfare and becomes increasingly more vital for endangered species as the conservation value of each individual to the survival of the species is more significant.

Given the potential for capture techniques to have detrimental effects on the target animal and evidence of capture myopathy in waterfowl following capture I wished to address the question of whether capture and handling protocols currently used for pateke/brown teal (*Anas chlorotis*), whio/blue duck (*Hymenolaimus malachorhynchos*) and other intensively managed waterfowl species has the potential to cause the birds muscle damage and stress. To

do this I investigated biochemical markers of muscle damage and stress in wild mallard ducks (*Anas platyrhynchos*) following capture, as a model system for native waterfowl.

4.1.1 Status, Ecology & Management of Native Waterfowl in New Zealand

Waterfowl of the family Anatidae in New Zealand consist of 10 native extant species, 9 extinct ones (Miskelly *et al.*, 2008) and 5 species that were introduced and naturalised (Anonymous, 2013a). Extant species of the family Anatidae in New Zealand include grey teal (*Anas gracilis*), New Zealand shoveler (*Anas rhynchotis variegata*), New Zealand scaup (*Aythya novaeseelandiae*), black swan (*Cygnus atratus*) and paradise shelduck (*Tadorna variegata*). The family also includes many threatened species listed under the Under the New Zealand Threat Classification System (Miskelly *et al.*, 2008) such as the 'recovering' North Island wide population of pateke/brown teal. The 'nationally vulnerable' Auckland Island teal (*Anas aucklandica*) and whio/blue duck. Those most at risk are the 'nationally critical' South Island wide population of pateke, Campbell Island teal (*Anas nesiotis*) and grey duck (*Anas superciliosa superciliosa*). The IUCN Red List of Threatened Species list similarly lists Auckland Island teal as vulnerable and whio, pateke and Campbell Island teal as endangered (Anonymous, 2012). Grey duck are a subspecies of pacific black duck, which is considered of least concern by IUCN Red List (Anonymous, 2012). Of these native waterfowl species grey duck, New Zealand shoveler, paradise shelduck and black swan are currently hunted recreationally (Anonymous, 1953, 2013c).

A number of historic and on-going factors have contributed to the decline of waterfowl species in New Zealand. One of the major causes of decline, and the biggest contemporary threat to threatened waterfowl species, is from introduced mammalian predators (O'Connor *et al.*, 2007; Glaser *et al.*, 2010; Whitehead *et al.*, 2010). On islands which have had these predators eradicated this is no longer a problem. Native species that prey on threatened waterfowl include weka, pūkeko and eel (on eggs or ducklings) and Australasian harriers (*Circus approximans*) or New Zealand falcon (on adult birds) (Barker & Williams, 2002; O'Connor *et al.*, 2007; Glaser *et al.*, 2010). Predation has resulted in decline in current populations of some species, and has resulted in the loss of some species from certain areas.

Reductions in the number and size of populations threaten the ability of a species to respond adequately to environmental change, disease and further predation. Intensive management procedures may then become necessary and supplementing current populations and propagating new populations has/and continues to play an important role in species recovery.

One such technique to establish new populations are translocations, in which wild birds are captured and relocated to areas with small populations or where the species has become locally extinct. This has been used for Campbell Island teal (Gummer & Berry, 2007) and whio (Holmes & Caskey, 2001). Translocations tend to use captive bred or reared birds rather than wild caught birds, but some wild birds are captured and included as breeding stock to increase the genetic diversity in breeding programmes (O'Connor *et al.*, 2007; Glaser *et al.*, 2010). Captive breeding programmes are used for Campbell Island teal (Gummer & Berry, 2007), pateke (O'Connor *et al.*, 2007) and whio (Glaser *et al.*, 2010).

Good conservation management requires accurate information with which to base management decisions on. Obtaining biological and physiological information about species that can only be discovered through capture often happens in parallel with other interventions such as disease screening or attaching transmitters during translocations (Holmes & Caskey, 2001; Gummer & Berry, 2007). However sometimes wild birds are captured with the sole purpose of expanding knowledge about a species. Wild birds are captured and may be physically sampled, banded or have transmitters attached to aid in the collection of information about a species (Dumbell *et al.*, 1988; Williams, 1995; King & Caskey, 2010).

All of these methods require capture and/or handling of the birds whether they are wild or living in aviaries. Therefore any potential negative effects that these procedures may cause to these individuals, could affect the health of new or current populations.

4.1.2 Status, Ecology & Management of Mallard Ducks (*Anas platyrhynchos*)

Mallard ducks (*Anas platyrhynchos*) were imported in to New Zealand by various Acclimatisation Societies as a suitable sport hunting species. The first birds introduced in 1867 were of Great Britain origin and these were supplemented with further introductions of birds from both Great Britain and the USA. Active breeding and release of the birds by different Acclimatisation Societies and also by private individuals allowed mallards to

become widespread (Dyer & Williams, 2010). By 1980 mallards made up 80% of the mallard/grey duck population (Caithness *et al.*, 1991) and their population in New Zealand is now about 4.5 million (Anonymous, 2013b). Within New Zealand the paradise shelduck is now the only species of waterfowl that is more widely distributed than Mallard ducks (Dyer & Williams, 2010).

Globally mallards are currently listed as of 'Least Concern' on the IUCN Red List (Anonymous, 2012). In New Zealand they are classified as a game bird under the Wildlife Act 1953 (Anonymous, 1953) and hunted recreationally each year over a set hunting season (Anonymous, 2013c). As such their management falls under the jurisdiction of Fish & Game New Zealand. In New Zealand mallards have contributed to the decline of the endemic grey duck populations through extensive hybridisation (Rhymer *et al.*, 1994). There is also evidence of some hybridisation with pateke (O'Connor *et al.*, 2007).

4.1.3 Capture and Handling Protocols Used on New Zealand Waterfowl

4.1.3.1 Capture and Handling Techniques of Waterfowl

4.1.3.1.1 Capture techniques

Waterfowl are captured in New Zealand and around the world using a range of techniques. Captive birds in aviaries may be captured using a soft hand net (Gummer & Evans, 2003) and wild species are also captured using hand nets or even by hand (Weller, 1975; Williams, 1995; Milani *et al.*, 2012). Spotlighting birds at night or the use of indicator dogs may be used to help locate the birds for capture (Gummer & Berry, 2007). Waterfowl are also captured using trapping techniques such as clap traps (Rickett, 2010), decoy traps or bait traps (Bollinger *et al.*, 1989). Rocket nets are another method of capture used, which uses a launcher to fire nets over groups of birds and either envelop or entangle the birds (Bollinger *et al.*, 1989; Dabbert & Powell, 1993; Cox & Afton, 1998). Net-guns have even been used to capture waterfowl in the past with mixed results (Mechlin & Shaiffer, 1980; Quinlan & Hughes, 1992).

4.1.3.1.2 Handling techniques

Pateke handling protocol recommends holding the bird firmly with both hands on either side of the birds around the wings and body, holding the bird the right way up. Alternatively the

bird can be tucked under one arm with a hand around the birds' lower neck (Gummer & Evans, 2003). In the field birds may be weighed by placing them in a cotton bag and suspending the bag from scales (Rickett, 2010).

4.1.3.2 Capture Impacts

The capture and handling of individuals is a key component of many management strategies but it can be an invasive process and have significant behavioural, physical and physiological effects on the animal. Capture myopathy is a recognised potential complication of waterfowl captures (Wobeser, 1997). This is where the stressful nature of capture and the physical exertion of the animal can cause a physiological cascade which results in muscle damage and potentially sickness and death (Williams & Thorne, 1996; Wobeser, 1997).

When muscle tissue is damaged it release the enzymes creatine kinase (CK) and aspartate aminotransferase (AST) into the bloodstream (Williams & Thorne, 1996). The plasma concentrations of CK that are thought to reflect capture myopathy are >1000 IU/L (Bollinger *et al.*, 1989). The exact relationship between the concentration of CK and AST and the degree of muscle damage for waterfowl is not known (Wobeser, 1997), however a correlation between elevated concentrations of plasma CK and AST and clinical signs of capture myopathy is well established (Ward *et al.*, 2011). Capture techniques, methods of restraint and restraint duration have been identified as the major contributing factors in the elevation of plasma CK and AST and development of clinical signs in waterfowl (Bollinger *et al.*, 1989; Dabbert & Powell, 1993).

Clinical signs of capture myopathy such as an impaired ability to walk or take flight have been observed in a several waterfowl species following capture, such as lesser snow (*Anser caerulescens caerulescens*) and Ross' geese (*Anser rossi*) (Wobeser, 1997), and mallard ducks (Bollinger et al., 1989). Many capture operations of wild waterfowl result in small numbers or birds exhibiting clinical signs of capture myopathy such as stiffness or an inability or reluctance to fly away on release (Wobeser, 1997). Some other studies on waterfowl species which have noted no signs of capture myopathy have found high concentrations of plasma CK and/or AST which reflect some degree of muscle injury even in absence of clinical signs (Driver, 1981; Dabbert & Powell, 1993; Milani *et al.*, 2012).

While capture myopathy can cause animals to die suddenly or exhibit signs immediately, clinical signs may take hours, days or even weeks to develop (Basson & Hofmeyr, 1973).

This may mean that the condition is never recognised in a species. Capture myopathy itself may not be fatal for a bird however the injury and behaviour of the bird following capture induced muscle injury may mean the bird is more susceptible to predation. Cox and Afton (1998) found that survival of northern pintails (*Anas acuta*) in the days following release after capture and radio tagging was positively related to the bird's flight quality on release. This is obviously very relevant in New Zealand because of the impact of predators on translocations (Holmes & Caskey, 2001; Gillies *et al.*, 2003). In addition to the clinical form of the disease there is also the potential for a subclinical level of muscle damage to negatively affect the bird and cause changes to the birds' behaviour or reduce the birds' mobility. This has been shown to occur in other animals and may result in a reduction of an animal's chances of survival and reproductive success (Cattet *et al.*, 2008). The potential for current capture and handling protocols to cause capture myopathy should therefore be investigated.

An important aspect of any capture and handling event is the stress caused to the animal during the process. The avian stress response allows birds to respond to stressful events with specific and non-specific regulatory processes which govern behavioural and physiological changes in the bird. These changes allow the bird to respond accordingly to stressful events in the environment (Siegel, 1980). The avian nonspecific stress response is characterised by increases in plasma corticosterone (CORT) secreted from the adrenal glands (Siegel, 1980; Carsia & Harvey, 2000).The changes in the concentration of plasma CORT can therefore be used as an indication of the degree of stress the bird is experiencing (Cockrem *et al.*, 2004) and therefore are used in this study to assess the impact of capture and handling protocols on the stress response of mallards.

4.1.4 Suitability of Mallard Ducks (*Anas platyrhynchos*) as Surrogates for New Zealand waterfowl.

Due to the logistical and ethical difficulties of working directly on endangered wildlife, mallards were thought to be an appropriate surrogate species for evaluating the effects of capture and handling on native New Zealand waterfowl. They are closely related species and have been observed to hybridise with some species (eg. pateke and grey ducks) (Rhymer *et al.*, 1994; O'Connor *et al.*, 2007). Like all experimental work that uses surrogate species and cross species extrapolations there is a fundamental assumption that there are minimal differences in the way these species will physiologically respond to capture and handling techniques. They share morphological and behavioural similarities to some species of threatened New Zealand waterfowl.

4.1.5 Specific Aims of the Study

As stated, the main aim of the study is to assess current capture and handling practices used for threatened waterfowl and if possible suggest refinements to the process based on the results in mallards. To this end, this study investigated the physiological changes in wild mallards in response to capture and handling.

The specific aims of the study were to:

1) capture wild mallard ducks and obtain serial blood samples following capture, handling each bird according to either the control or treatment methodology;
2) determine plasma biochemical changes indicative of muscle damage, selected organ functions and the stress response to capture and handling;
3) analyse whether the treatment methodology had a significant effect on a bird's potential for muscle damage, selected organ functions and stress response by comparing the biochemical changes shown in the results of the testing;
4) from the analyses, assess the field practicality and potential health impacts of using a net-gun to capture wild mallards; and
5) extrapolate from the mallard study conclusions on the current capture and handling process for threatened waterfowl and, if relevant, suggest refinements or further research.

4.1.6 Approval for Study

All procedures were carried out with permission from the Massey University Animal Ethics Committee (MUAEC 11/33). An *Authority to Disturb and/or Kill Game Birds* was granted by Fish & Game New Zealand (File No: 1181).

4.2 MATERIALS AND METHODS

To assess physiological effects of capture in mallard ducks (*Anas platyrhynchos*) following capture and handling I sampled 39 mallard ducks using a net-gun (Super Talon Net-Gun, Advanced Weapons Technology, La Quinta, California, USA), between August 2011 and February 2012.

4.2.1 Study Site

The study took place on two large ponds within Massey University's Turitea Campus, Palmerston North, New Zealand 40°38'748''S, 175°61'90''E where there is a small stable population and a larger transient population of habituated mallard ducks. Most of the ducks have become accustomed to close human proximity and occasionally are fed. Sampling took place during the hours of daylight between 0800 and 2100 hours.

Figure 4.1 A map of showing sampling site within New Zealand (left) and a close up showing the sampling site at Massey University (right).

4.2.2 Field Methodology

I captured mallards using a net-gun with five cm mesh nets (Super Talon Net-Gun, Advanced Weapons Technology, La Quinta, California, USA), with another one or two people acting as spotters and handlers. I captured mallards during daylight hours while they were feeding on

or near the pond. Poultry pellets were used to attract birds on to dry land or to within firing range.

Once the net-gun had been fired, the shooter and handler went immediately to the bird and restrained it from further movement and entanglement. If the bird was relatively untangled then it was removed immediately from the net and wrapped in a towel. In cases where it was more entangled the bird was wrapped in a towel while still in the net and untangled after the first sample was taken. In this study the first sample is referred to as "0 minutes."

I allocated birds alternately into either a control group or a treatment group. All the mallards sampled in either the control group or and treatment group were handled by necessity for removal from the net and again at each further blood sample. I used heparinised 25 gauge needles and 1 ml syringes. All blood samples were taken in less than 5 minutes after capture. Birds which were sampled from the brachial veins on either wing were held on their backs with their bodies supported and one wing extended. Birds which were blood sampled from the medial metatarsal veins on either leg were held on their sides with their bodies supported and one leg outstretched. Following the blood sample, the birds were held for a short period to ensure haemostasis of the sampling site was achieved using digital pressure and a swab

Following the first sample birds in the control group were placed unrestrained in ventilated cardboard boxes after the first sample, and removed for further blood samples after 30 minutes and 120 minutes. Birds allocated to the treatment group were placed into a clean pillowcase after the first sample and elevated to simulate a weighing event (Rickett, 2010). The treatment birds then underwent additional handling compared to the control group, during which they were held upright by the handler who used a hand on either side of the bird encircling the wings and the body in a manner prescribed by pateke handling protocol (Gummer & Evans, 2003). After 30 minutes I took the second blood sample. The bird was then placed into a ventilated box unrestrained and removed for a further sample at 120 minutes. Once the final blood sample was taken at 120 minutes each bird in either treatment group had a short physical examination for injuries and was temporarily marked with white paint on its bill. I released the bird in the same location as capture. I observed the bird and recorded its behaviour on release. Throughout the sampling and at release the behaviour of the bird was recorded for both treatment groups, in particular struggling, vocalisations and respiration. Other notes taken included the length of time between capture (or removal of the

bird from the box) and a blood sample was recorded for each bird as was the age group of the bird (based on feather growth and colouration).

4.2.3 Laboratory Methodology

The heparinised blood samples were kept on ice and within 12 hours of being taken the samples were centrifuged at 5 000 rpm for five minutes. The plasma was then pipetted off into a separate microcentrifuge tube before being frozen and stored at -20°C until biochemical analysis could be performed.

A sub-sample of plasma was submitted to a commercial diagnostic laboratory (New Zealand Veterinary Pathology, Palmerston North, New Zealand) and batch-analysed for plasma concentrations of CK, AST, glutamate dehydrogenase (GLDH) and uric acid (UA).

The methods used to analyse CK, AST and GLDH has been previously described in Chapter Two and the method used to analyse UA has been previously described in Chapter Three.

Plasma CORT concentrations were measured at the Institute of Veterinary, Animal & Biomedical Sciences, Massey University, Palmerston North, New Zealand. The concentration of CORT was measured by radioimmunoassay as described in Chapter Two.

4.2.4 Statistical methodology

I used PASW Statistics 18 for Windows 7.0 (SPSS Inc., Chicago, Illinois, USA) to analyse the data. All results are presented as means (±S.E.M) unless otherwise stated.

I used a repeated-measures analysis of variance (ANOVA) to assess the samples for significant differences over time (0 minutes, 30 minutes, 120 minutes) and between the treatment and control groups. To exclude other confounding factors I assessed differences due to the season samples were taken and the time of day and age of bird (adult and juvenile). *Post hoc* analysis was performed if there was a significant difference in the parameter over time. Where appropriate a Bonferroni correction was applied.

This method of analysis does not allow for missing values, so only birds that had all three samples taken were included in the repeated-measures analysis. This resulted in an effective sample size of 32 mallard ducks.

When analysing the effects of season on the physiological response of mallards the GLDH and AST data were not homogenous between winter and summer and homogeneity could not

be achieved by transforming the data. A Mann-Whitney test was therefore used to compare the concentrations of GLDH between seasons at each sampling point.

4.3 RESULTS

During the study 37 mallard ducks were successfully captured using a net-gun and blood sampled. There were 16 birds in the control group and 21 birds in the treatment group. The mallards were captured while on the ground, on the water or while flying.

4.3.1 Field Observations

During the sampling process, from capture through to release, three of the 37 birds (8%) captured and sampled were heard to vocalise, five of the birds (14%) were noted to have struggled during handling, 4 of the birds were observed panting (11%). None of the birds suffered any significant injury or exhibited any clinical signs of muscle injury during sampling or upon release. There were some minor feather abrasions on the flight feathers of one bird and two birds lost a couple of feathers as a result of entanglement in the net. The extent of feather damage and loss in the birds was, however, extremely minor.

Mallards were usually captured at a range of three to seven metres, but the effective range of the net-gun was three to ten metres depending on the wind direction and strength and also on whether the bird was stationary or in flight. Mallards were usually captured individually, however when there was more than one handler present I often targeted pairs of birds. It was necessary to separate the target bird(s) from the main group so that the projectiles surrounding the net would not contact the surrounding birds and would contact the target bird(s) with the centre portion of the net.

Of the 37 birds captured, three birds were not able to be successfully blood sampled within 5 minutes of capture and were excluded from analysis. A further two birds were excluded from analysis due to a sample labelling error. This left 32 birds that were suitable for analysis of blood samples, with 15 birds in the control group and 17 birds in the treatment group.

Within these 32 birds it took an average of 2 minutes 43 seconds ± 9 seconds (mean ± SE) from capture to obtain the first blood sample. The average time it took before a bird was

untangled from the net was 6 minutes 43 seconds ± 27 seconds (mean ± SE). To ensure that a fast blood sample was obtained it was desirable to blood sample a bird prior to untangling, but occasionally birds that were barely tangled in the net could be removed quickly before sampling.

4.3.2 Potential Confounding Factors in the Analysis

In order to ensure that the analysis between control and treatment groups was as robust as possible the data set was analysed *a priori* to establish if there were any potentially confounding factors. The potentially confounding factors that were identified and tested to determine the effects on the data included the age class of the bird, time of sampling and any seasonal variation in the data.

4.3.2.1 Effects of Age on the Physiological Response of Mallard Ducks to Capture

Adult (n=11) and juvenile (n=21) mallard ducks were captured during this study. There were no significant differences found in the plasma concentrations of any of the biochemical analytes when adult ducks were compared with juveniles. Age was therefore deemed not to be a confounding factor in the comparison between control and treatment groups.

4.3.2.2 Effect of Season and Time of Day on the Physiological Response of Mallard Ducks to Capture and Handling

4.3.2.2.1 Effect of Season

The sampling period spanned several seasons therefore the birds were divided into two groups referred to as the 'winter group' (August to October) and 'summer group' (January to February) for further analysis to determine whether there was a seasonal difference in the physiological response of mallard ducks to capture. The winter group consisted of 5 birds (A+C as shown in table 1) and the summer group of 27 birds (B+D as shown in table 1).

Table 4.1 The number of birds sampled in each season and at what time of day

Sampled During	Winter	Summer
Morning	5 birds (A)	13 birds (B)
Afternoon	0 birds (C)	14 birds (D)

The physiological response of mallard ducks to capture was found to be affected by the season the bird was sampled in (Figure 4.2). Mallards captured in summer (B+D) had significantly higher plasma CK concentrations then compared to those captured in winter (A+C), (P=0.002). *Post hoc* analysis showed mallards sampled in summer had consistently higher CK concentrations at all sampling times (0 minutes (P=0.031), 30 minutes (P=0.020) and at 120 minutes (P=0.028)). No seasonal variation was found for any of the other biochemical analytes. The plasma concentrations of UA (P=0.699) and CORT (P=0.648) were not significantly different between mallards captured in winter compared to those birds captured in summer. There was no significant difference in the concentration of plasma GLDH at 0 P=0.388), 30 (P=0.280), or 120 (P=0.189). There was no significant difference in the concentration of plasma AST at 0 P=0.152), 30 (P=0.252), or 120 (P=0.184). Whether a bird is captured in the winter or summer may therefore be a confounding factor in the analysis of handling techniques in mallards.

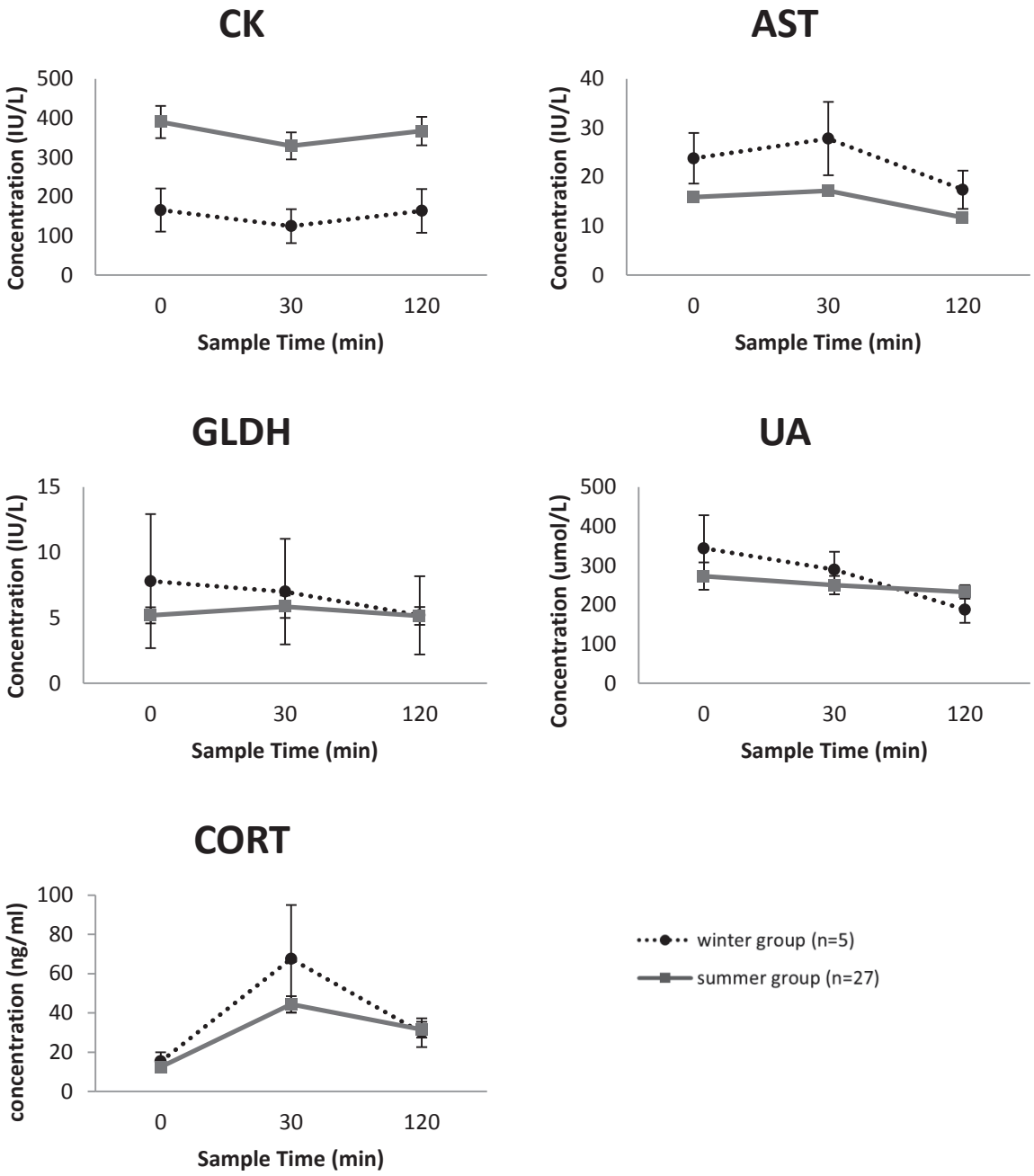


Figure 4.2 Seasonal differences in the plasma levels of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in mallard ducks (*Anas platyrhynchos*) following capture and handling in New Zealand, August 2011-February 2012. Values shown are means ± S.E.M. The birds were separated into a summer group sampled in Jan-Feb 2012, and winter group sampled in Aug-Oct 2011.

4.3.2.2.2 Effect of Time of Day

Sampling was carried out throughout the day therefore the birds were grouped into those captured in the morning (0800-1159 hours) and those captured in the afternoon (1200-2000 hours) for further analysis to determine whether the time of day the samples were taken had affected the biochemical response of mallard ducks to capture. There were 18 ducks sampled in the morning (A+B as shown in table 1) and 14 ducks sampled in the afternoon (D as shown in table 1).

The physiological response of mallard ducks to capture was found to be affected by the time of day the bird was sampled (Figure 4.3). Mallards captured in afternoon (D) had significantly higher plasma CK concentrations then compared to those captured in the morning (A+B), (P=0.005). *Post hoc* analysis showed mallards sampled in the afternoon had consistently higher CK at 0 minutes (P=0.032) and 30 minutes (P=0.040) but not at 120 minutes (P=0.068). The concentrations of the other biochemical analytes showed no difference according to the time of day the bird was captured (AST, P=0.076; GLDH, P=0.979; UA, P=0.327; and CORT, P=0.809). Whether a sample is taken in the morning or afternoon may therefore be a confounding factor in the analysis of handling techniques in mallards.

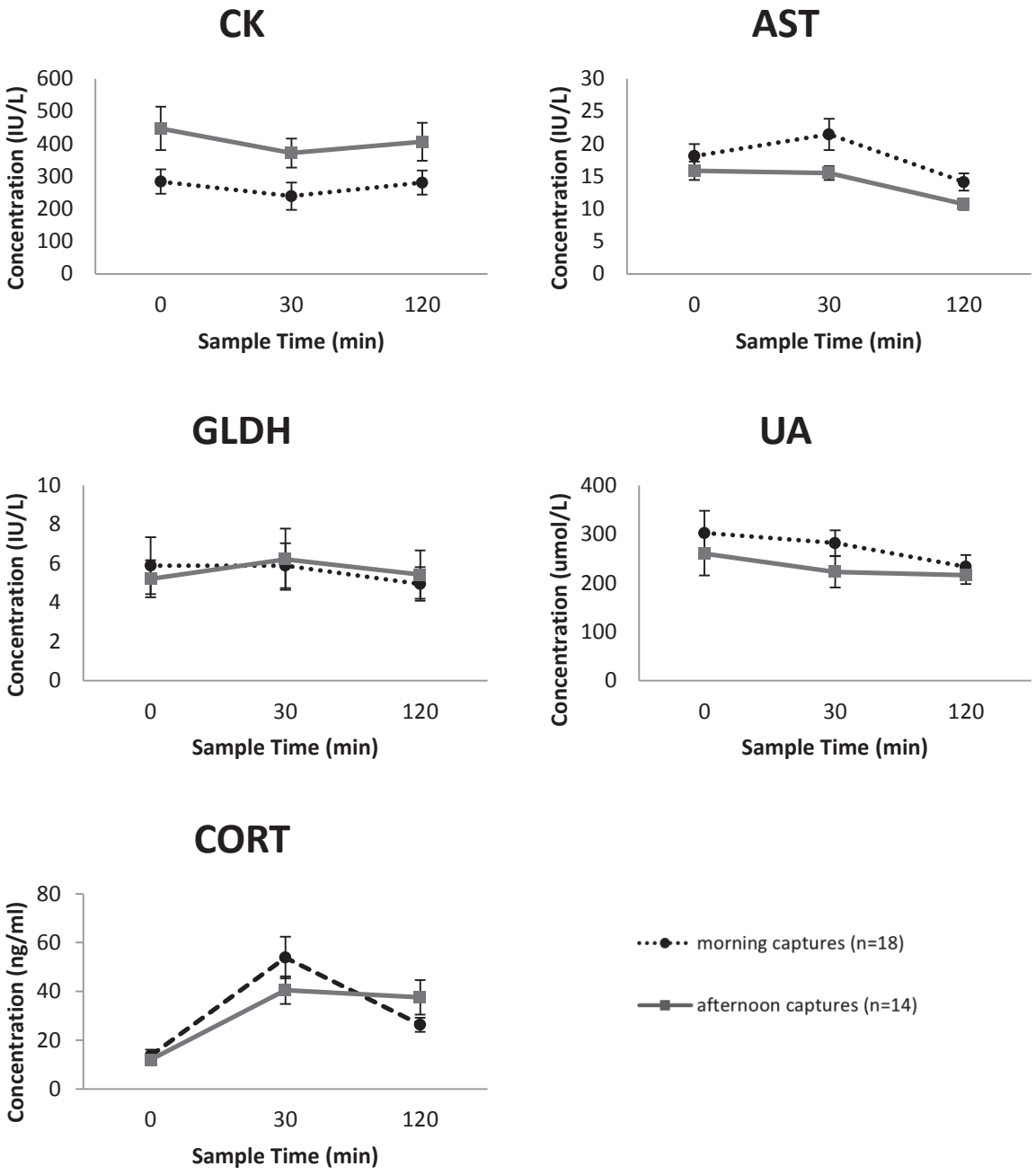


Figure 4.3 Time of day variation in the plasma levels of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in mallard ducks (*Anas platyrhynchos)* following capture and handling in New Zealand, August 2011-February 2012. Values shown are means ± S.E.M. The birds were separated into morning captures sampled between 0800-1159 hours; and afternoon capture sampled between 1200-2000 hours.

4.3.2.2.3 Effects of Both the Season and Time of Day on the Physiological Response of Mallard Ducks to Capture

I concluded that both season and time of day could be potentially confounding factors in the analysis. To eliminate the effect of seasonal differences on the data the five birds captured in winter were excluded from the control vs. treatment analysis, resulting in 27 birds for analysis. In order to minimise the effect of time of day on the data the analysis comparing control and treatment groups was carried out not only on the data from the 27 birds but also separately within birds captured in the morning and afternoon. No significant differences were observed between control and handed groups when analysis was performed within morning or afternoon subsets of the data.

4.3.3 Physiological Response of Mallard Ducks to Capture and Handling

In order to determine the impact of handling on mallard ducks, birds in the summer group were examined in relation to the control (11 birds) and treatment (16 birds) groups.

4.3.3.1 Establishing a Robust Baseline

The delay between capture and blood sampling of both the control and treatment birds had an effect on the mean plasma concentration of CORT (Figure 4.4). There was a highly significant linear relationship evident between the length of time it took to take the first blood sample and the concentration of plasma CORT (P=2.63x10^{-5}). There was also a significant relationship between the length of time it took to take the first sample and the concentration of plasma GLDH (P=0.0421). No significant relationship were observed for the other biochemical analytes tested (CK P=0.684, AST P=0.137 and UA P=0.175). It can be concluded that the longer it took to take the first blood sample the greater the elevation of plasma CORT and plasma GLDH from normal. Therefore some of the mallard ducks captured in the study already had partially elevated plasma CORT and GLDH concentrations by the time the first blood sample (0 minutes) was taken. There was no significant difference, however, between the starting plasma concentrations of the control and treatment groups at 0 minutes (P=0.441). It can therefore be concluded that the time between capture and the 0 minutes blood sample affected both control and treatment groups equally and the effect of handling can still be examined by comparing the control and treatment groups of mallard ducks.

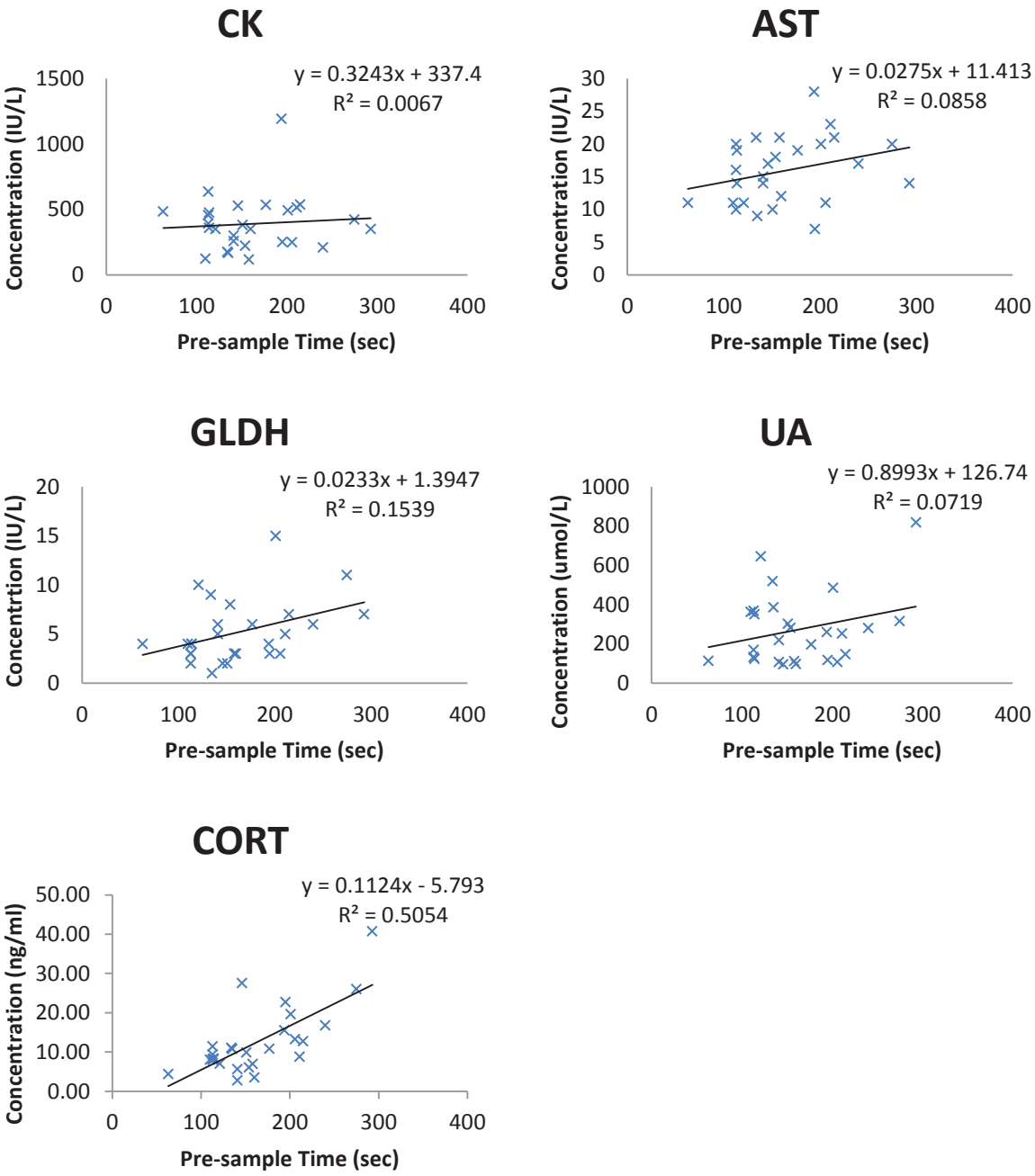


Figure 4.4 Pre-sampling time between capture and 0 minutes samples and the plasma concentration of creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in mallard ducks (*Anas platyrhynchos*) following capture in New Zealand, January-February 2012.

4.3.3.2 Effect of Capture and Handling on Muscle Physiology

Mallard ducks demonstrated a measurable physiological response to capture with significant changes occurring over the sampling period in the mean concentrations of plasma AST. (Figure 4.5). However mallards did not exhibit any differences in their response to capture between the control and treatment groups.

The mean concentration of plasma AST decreased significantly over time ($P < 0.001$) but was not different between control and treatment groups ($P = 0.806$). There was no change in the plasma AST concentration between 0 minutes and the 30 minutes sample (P=0.394), however the decrease in plasma AST from the 30 minutes sample to the 120 minutes sample was highly significant (P<0.001), for both control and treatment groups. There was also a significant overall decrease in plasma AST from the 0 minutes sample to the 120 minutes sample (P=0.003), for both control and treatment groups. The concentrations of plasma CK did not change significantly following capture (P=0.406), nor was it significantly different between control and treatment groups (P=0.937).

4.3.3.3 Effect of Capture and Handling on Stress Physiology

Significant elevations in the mean concentration of plasma CORT occurred over the sampling period showing capture had a measurable physiological impact on the stress response of mallards. However no difference was observed between the control and treatment group (Figure 4.5).

The mean concentration of plasma CORT was found to differ significantly over time ($P < 0.001$) suggesting a strong stress response to capture but this was not significantly different between control and treatment groups ($P = 0.448$). Plasma CORT became significantly elevated between 0 minutes and the 30 minutes sample ($P < 0.001$), however the decrease in plasma CORT from the 30 minutes sample to the 120 minutes sample was not significant (P=0.074). The net result was an overall increase in plasma CORT from the 0 minutes sample to the 120 minutes sample (P=0.001).

4.3.3.4 Effect of Capture and Handling on Selected Organ Function

Capture did not have a measurable physiological impact on selected organ function of mallard duck and no significant changes occurred over the sampling period in the mean concentration of GLDH and UA, and no difference was observed between control and treatment group (Figure 4.5). The concentrations of plasma GLDH and UA did not change significantly following capture (P=0.126 and P=0.290 respectively) nor did they significantly different between control and treatment groups (P=0.546 and P=0.778 respectively).

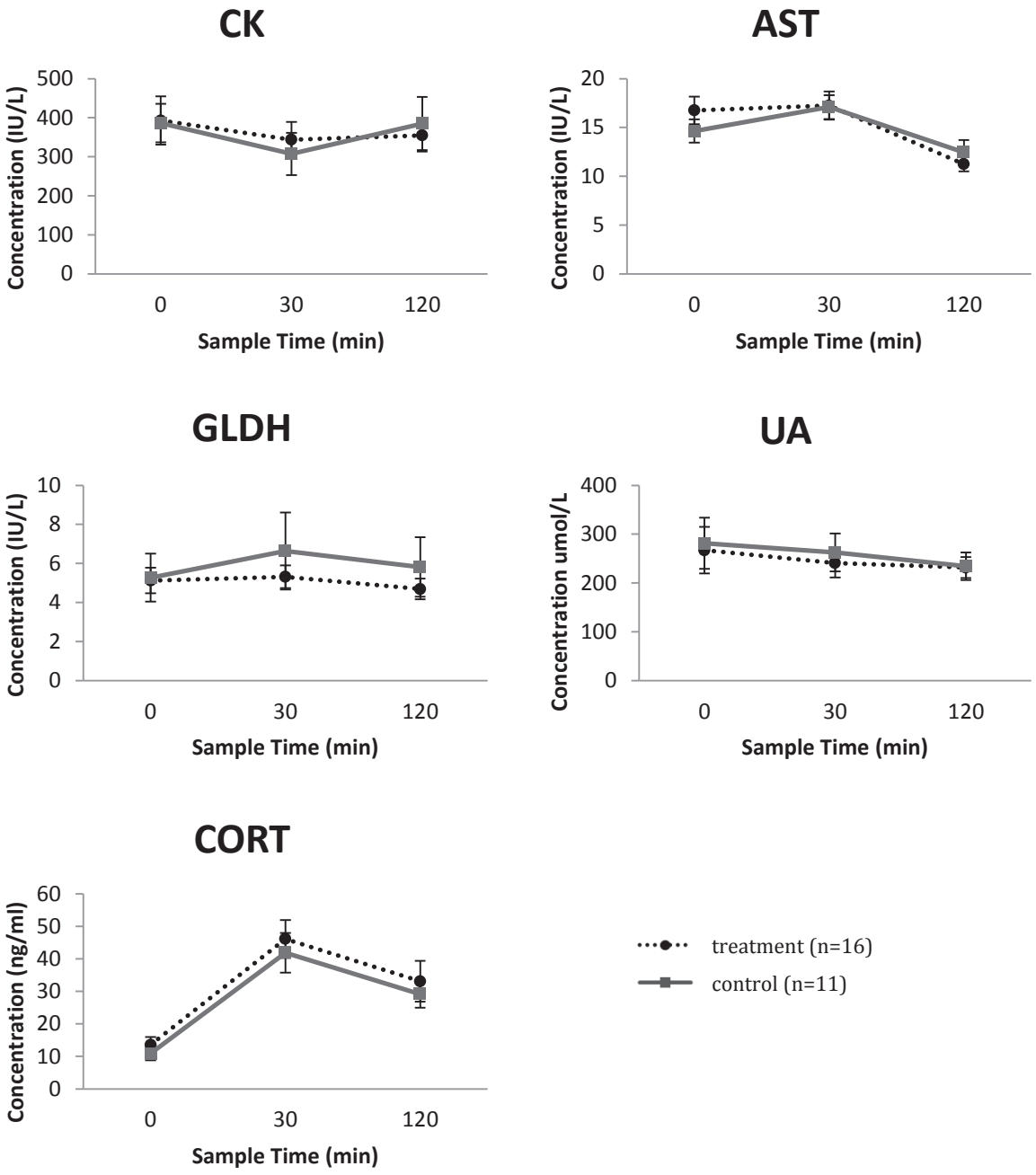


Figure 4.5 Control vs. treatment comparison of the plasma levels of the biochemical parameters: creatine kinase (CK), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), uric acid (UA) and corticosterone (CORT), in mallard ducks (*Anas platyrhynchos*) following capture in New Zealand, January-February 2012. Values shown are means ± S.E.M. The control group were blood sampled at capture (0 minutes) and then placed in boxes until 30 minutes when they were removed, sampled again and returned to their box until a final sample was taken at 120 minutes. The treatment group were similarly sampled however they were wrapped in a towel and held between the samples at 0 and 30 minutes before being placed in boxes between 30 and 120 minutes.

4.4 DISCUSSION

Mallard ducks in this study responded to capture with significant stress response as demonstrated by the elevations in plasma CORT, but showed little other physiological response to capture as demonstrated by plasma biochemical parameters. There was a decrease in plasma AST following capture, but in the absence of any changes in plasma CK the physiological significance of this change with regards to capture is unclear. The treatment group of mallard ducks did not respond differently to capture suggesting that capture is the main event initiating a stress response and handling has a negligible effect on the stress levels a mallard experiences following capture. There was also significant seasonal and time of day variation in the biochemical response of mallard ducks to capture. In particular plasma CK was largely affected by the season or time of day a duck was sampled. It was found that some mallard ducks sampled may have already begun to respond to capture with elevations in plasma CORT by the time the first blood sample was taken.

The findings regarding muscle physiology are in direct contrast to a similar study on pūkeko (Chapter 3) where capture was found to cause increases in the enzymes CK and AST indicating muscle damage had occurred. This difference demonstrates strong differences in the responses of different species to similar capture and handling techniques that may reflect physiological or behavioural differences between the birds studied.

4.4.1 Impacts of Capture & Handling on Mallard Muscle Physiology

The mallards captured in this study did not show any elevations in plasma CK or AST that gave evidence of disturbance or injury to the musculature system following capture and handling. This is in contrast to some studies that observed clinical signs of capture myopathy and/or increases in plasma CK and AST following a range of capture techniques and which listed increased handling time as a significant factor in the development of clinical signs or chemical markers (Bollinger 1989, Ponjoan *et al.*, 2008).

4.4.1.1 Baseline Concentrations of CK and AST

Baseline concentrations of plasma CK and AST for a species often differ because the method by which the bird is captured and sampled may cause muscle injury and therefore alter the so called baseline value. Normal concentrations of plasma CK in captive waterfowl have been reported at between 50-200 IU/L compared to waterfowl suffering from capture myopathy which have CK concentrations over 1000 IU/L (Bollinger et al, 1989). Baseline concentrations of CK and AST specific to mallards are listed as 225±52 and 19±7 respectively (mean ± SD), from aviary housed captive birds with minimal handling (Dabbert & Powell, 1993). Another study gave normal values for mallards much higher than this, with average plasma CK concentrations ranging from 372 ± 56 IU/L to 2212 ± 316 IU/L (mean ± S.E.M) in different study areas (Driver, 1981). However some of the birds in this study were noted by the author to have CK levels suggesting capture myopathy, and it is possible other birds in their study were experiencing elevated CK due to capture. This would therefore likely artificially increase the CK values given and therefore they should not be considered 'normal'.

The baseline AST concentration observed in the mallards in my study matches the baseline concentrations of Dabbert and Powell (1993), while the CK concentration in my study is similar to that found in one sampling group with the lowest CK concentrations by (Driver, 1981). In humans baseline levels of CK have been shown to be influenced by a number of factors including race, climatic condition and physical activity (Brancaccio *et al.*, 2007). The difference between the CK concentration in my study and that found by Dabbert and Powell (1993) could therefore be due to a number of factors such as climatic conditions during sampling, differences in genetics through hybridisation with grey ducks or the difference in physical activity rate of wild mallards compared to captive mallards. The 0 minutes concentrations of plasma CK found in our study can therefore be considered to be within the normal range for mallards in this area.

4.4.1.2 AST Response of Mallards to Capture

Captured mallards responded with a significant decrease in plasma AST, suggesting that AST was elevated at capture. There are two main reasons why concentrations of plasma AST may change while CK remain unaltered. Firstly CK has a shorter half-life than AST and is

eliminated faster and the initial higher 0 minutes concentration of AST could be the result of an event in the previous few days that had caused muscle damage. If so plasma CK could have become elevated then returned to normal whereas AST was still partially elevated and decreasing. However this pattern occurred in more than one bird and it is extremely unlikely all the birds sampled had historic muscle damage making this theory is unlikely. Secondly the initial elevation of AST could be due to damaged liver tissue and not muscle tissue and therefore no CK was released. While damaged liver tissue may release GLDH, it only does so in certain types of severe liver damage, so it is possible to get AST elevations without GLDH elevations. However if liver damage had occurred as part of capture then an increase, not decrease, in AST would be expected. Some other factor is therefore likely the cause.

The actual magnitude AST decreased by over the sampling period was very low, albeit significant. It is probable then that the concentration of AST at capture was not elevated and was physiologically normal for mallards with normal functioning muscles and liver. This is supported by the similar baseline concentrations found in captive mallards (Dabbert & Powell, 1993). When the birds were captured in my study they were usually active and this higher level of muscle activity could have resulted in small but relatively higher concentrations of AST at capture compared to the 120min sample after 2 hours of relative immobility. If this were the case, then CK would also be expected to change also as it is the more sensitive and specific indicator of muscle activity/damage (Williams & Thorne, 1996).

Alternatively the stress of the capture event as seen by the elevation of plasma CORT may have caused blood flow to be directed away from the liver (ter Steege & Kolkman, 2012). Hepatic activity may therefore have been lower and less AST may have been released during normal liver physiology. While this occurred the circulating AST in the plasma would still be metabolised and since less AST was being released by the liver, this could cause the overall plasma AST concentration to drop.

I also found that the concentration of plasma CK in our ducks was affected by the time of the year when sampling took place and also may have been affected by the time of day sampling took place. In contrast Driver (1981) found no significant seasonal variation in plasma CK concentrations when comparing pre-moult, remige moult and post moult stages in mallard drakes. Some of the mallards in Driver's (1981) study were noted to have high CK values thought to be a result of capture and handling. If some birds in this study had been

experiencing elevations in plasma CK due to capture then this may have confounded any possible seasonal variation in CK in that study.

4.4.1.3 Does Muscle Damage Occur in Mallards as a Response to Capture and Handling?

The lack of elevated CK and AST in the mallard ducks captured in this study suggest no muscle damage occurred. This is in contrast to other studies that show wild mallards responding to capture using a range of techniques mallards (decoy traps, bait traps and rocket nets) with increases in CK and AST (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). Even captive aviary house mallards have even been shown to respond to handling with increases CK and AST (Dabbert & Powell, 1993).

The mallards in this study were observed as struggling less during the procedures compared to the birds in the pūkeko study (14% of mallards vs. 50% of pūkeko). It may be that the observed habituation of these mallards to human presence meant they responded to capture with different behaviour than non-habituated birds. Alternatively it could be a species difference in the behavioural response and mallards may be more likely to freeze rather struggle when captured.

The type of method used to capture mallards is recognised as a significant factor in the development of any subsequent muscle damage (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). The use of the net-gun and minimal retrieval time between capture and the handler restraining the bird may cause less muscle damage than other capture methods. Mallards were often left entangled in the nets for relatively long periods of time, 6 minutes 43 seconds ± 27 seconds, (mean ± SE). This was because prompt samples were needed for baseline analyte concentrations and sometimes two birds were captured which meant that untangling times were reduced. The type and length of restrainment of captured birds has been noted as a potential factor in causing muscle damage (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). However the length of time mallards spent in the net (entanglement time) was not found to have an impact on CK or AST. While the mallards spent an average of just 6 minutes 43 seconds entangled in the net they were reached within seconds of the net being fired. At this point the birds were restrained by a handler and was unable to thrash around and in many cases stopped struggling. I hypothesise that rather than the total length of time a bird is

entangled for increasing the chance of muscle damage, it may be the period of time a bird is left entangled in a net and still able to struggle and thrash about. This is likely the time when the bird might cause injury or muscle damage to itself, rather than when a bird is held securely by a handler and happens to still be entangled in the net. An increase in plasma CK and AST following capture cannot be fully ruled out as plasma CK peaks around 24 hours in birds (Bailey *et al.*, 1997; Ward *et al.*, 2011) and AST probably peaks even later (Williams & Thorne, 1996). However you would expect to see some evidence of this trend in these enzymes elevations prior to release. Other studies which have seen elevations in plasma CK or AST have done so within the 120 minutes that these mallards were held for (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). Furthermore plasma AST was actually observed to decrease in concentration in my study.

4.4.2 Impacts of Capture & Handling on Mallard Stress Physiology

The mallard ducks in this study responded to capture with significant elevations of plasma CORT similarly to many other species. This stress response was shown to occur independently of any muscle damage. Stress is considered a component of capture myopathy (Williams & Thorne, 1996) however as my study shows the stress response can occur independently of muscle damage. The capture of these mallard ducks appear to act as an emotional rather than physical stressor. The birds likely identify capture as a potential predation event and respond with increases in plasma CORT (Cockrem, 2007). As the ducks experience no physical trauma (as shown by CK and AST responses) the physical stress of capture was much less and therefore probably had a much lesser impact on the stress response then the perceived threat of capture.

4.4.2.1 Elevated Baseline CORT Values at 0 Minutes Samples

The baseline CORT concentrations of the mallards appear to have a linear relationship with the time between capture and blood sampling. Poisbleau *et al.* (2005) found baseline concentrations of CORT around 9 ± 1 ng/ml in aviary housed mallard's ducks at capture.

The mallards in my study had slightly higher CORT concentrations at capture; 11 ± 2 ng/ml and 14 ± 2 ng/ml (mean ± SE) for the control and treatment groups respectively. The most likely reason for this is that the five minutes cut off period between capture and sampling in my study was too long and that some birds already had elevated plasma CORT due to capture at the first blood sample.

Some studies suggest blood samples should be taken in under three minutes from capture (Romero & Romero, 2002) while others studies suggest sampling within one minute to get true unaltered baseline CORT concentrations (Dawson & Howe, 1983). This would have resulted in extremely small sample sizes in this study and a larger sample size was not within the resources of this project. There were no significant differences between treatment groups at 0 minutes so the elevation at 0 minutes was considered inconsequential when comparing control and treatment groups.

4.4.2.2 CORT Response of Mallards Following Capture

Plasma CORT concentrations increased significantly over the sampling period which indicates that capture and handling is a stressor for mallard ducks. This is as hypothesised because CORT concentrations have been observed becomingly rapidly elevated in response to capture and handling in a range of wild avian species (Dawson & Howe, 1983; Gratto-Trevor *et al.*, 1991; Romero & Romero, 2002; Cockrem *et al.*, 2008; Adams *et al.*, 2010), including waterfowl (Perfito *et al.*, 2002).

A similar stress response has been observed in captive waterfowl. Aviary housed mallards and northern pintails have been shown to experience CORT elevations following capture (Poisbleau et al., 2005). In contrast to my study the maximum CORT elevation in this study was much lower, around 16 ± 2 ng/ml for mallards and 23 ± 2 ng/ml for pintails in Poisbleau *et al.* (2005) study, compared to 42 ± 6 ng/ml and 46 ± 6 ng/ml (mean ± SE) in my study for the control and treatment groups respectively. There are a number of possible reasons for the differences seen in the maximum CORT elevation following capture between my study and that of Poisbleau *et al.* (2005). Firstly the aviary housed mallards in Poisbleau *et al.* (2005) study may have been more habituated to humans which resulted in a lesser stress response upon capture and lower maximum CORT concentrations.

Secondly the technique used to capture the mallards in Poisbleau *et al.* (2005) study was a net (probably a hand net), which may have had a lesser impact on the stress response and resulted in lower maximum CORT concentration compared to capture by net-gun. Romero and Romero (2002) found that if birds were not removed from the net/trap promptly there could be differences in the maximum CORT elevation between capture types. The mallards in my study were not removed from the net until after an average of 6 minutes 43 seconds so this may been a possible cause for the greater maximum CORT concentrations found in my study. Perfito *et al.* (2002) also found the capture technique to affect the CORT response in wild harlequin ducks (*Histrionicus histrionicus*). Box traps proved a more stressful method of capture compared to mist nets provoking a much higher CORT response. Thirdly it could be due to seasonal differences as the CORT response may rise to different maximum concentrations following capture in different life history stages (Perfito *et al.*, 2002).

Differences in maximum plasma CORT of a similar magnitude have been seen between wild male and female wild harlequin ducks, with male ducks exhibiting almost twice the maximum CORT concentration following capture compared to females (Perfito *et al.*, 2002). This is unlikely to be the cause of the difference between studies as both sexes were sampled in both my study and Poisbleau *et al.* (2005).

Without directly comparing the above factors in the same experiment the actual reason for the difference in maximum CORT is not clear. However it clear looking at the literature that many factors can influence CORT response following capture and handling and drawing conclusions when comparing between studies should be done with caution and only if the studies are very similar. Further research in this area could look into these factors. In particular comparing the effect of different capture types on the magnitude and duration of CORT elevation would provide valuable information for management practices. These factors also have implications when using surrogate species to assess the stress impacts of certain techniques on threatened species.

4.4.2.3 The Effect of Habituation of Mallards to Human Presence on the CORT Response

Habituation to a repeated stressor such as handling may lead to a less robust stress response to that stressor (Pitman *et al.*, 1988). However I am interested in whether the habituation of

birds to human presence in general has an effects on their CORT (and other biochemical analytes) response to capture. The mallards captured in my study were in areas regularly frequented by people (almost constantly during the working week) therefore have a certain degree of habituation to people compared to wild birds in more isolated areas. Magellanic penguins (*Spheniscus magellanicus*) living in locations that were visited by tourists had significantly reduced plasma CORT responses following capture and handling compared to penguins in undisturbed areas (Walker *et al.*, 2006). The mallards in this study did show a robust stress response to capture; however there were no wild mallards from isolated areas to compare them too. Therefore it is possible that the mallards in my study had a reduced CORT response to capture compared to wild birds in isolated areas. The mallards in Poisbleau *et al.* (2005) study were aviary housed captive birds and responded as mentioned with much lower maximum CORT elevations then the mallards in this study. Therefore it is possible that an even greater degree of habituation was present in these birds and resulted in reduced CORT response following capture and handling.

The avian stress response provokes behavioural and/or physiological changes which may influence the occurrence of capture myopathy. Therefore if birds habituated to human presence, like the mallards in my study, respond to capture with different behavioural or physiological changes compared to wild birds, than habituation may affect whether capture myopathy occurs. For example since habituated birds may have a less robust stress response this may cause them to struggle less (or more) during capture and handling and therefore decrease (or increase) the chances of muscle damage occurring. This is important as it result in different protocols being required for captive and wild birds.

Alternatively the location and type of capture method we use may have attracted mallards of a specific avian personality. Avian personalities can be defined as sets of related behaviours that a bird expresses across different situations (Cockrem, 2007). The capture method and location used in this study may have artificially selected mallards of a specific personality that were less inclined to fly away during attempted capture and more likely to approach during baiting. If the capture method and sampling location were biased to a particular type of mallard personality then this may have affected not only the CORT response but the response of the other biochemical analytes also.

4.4.3 Impacts of Capture & Handling on Mallard Gastro-Intestinal/Renal Physiology

Neither plasma GLDH nor plasma UA changed significantly as a result of capture or between treatment and control birds. Therefore there is no evidence that capture and handling affected any other physiological system other than the stress response. This was in contrast to the result found for pūkeko and suggests that the same capture technique may cause different species to respond in different ways. This means that some methods may be considered appropriate for one species may not be appropriate for another. It also has implications when using surrogate species as the species may respond differently and the surrogate may not be an adequate model.

4.4.4 Assessment of Using Net-guns to Capture Mallard Ducks

While only one method of capture was used in this study, none of the injuries or fatalities that have been attributed with using net-guns on birds occurred (Mechlin & Shaiffer, 1980; Huschle *et al.*, 2002; Herring *et al.*, 2008). This capture method also didn't result in any evidence of muscle damage that has been seen in mallards using other methods of capture (Bollinger *et al.*, 1989; Dabbert & Powell, 1993). This is not conclusive as only one method of capture was used in this study, but it does suggest that this style of net-gun capture is a safe method of capture with no health impacts on waterfowl muscles. More research is needed in different waterfowl species and in comparing different capture techniques to see if this technique causes a greater, lesser or similar stress response compared to other methods.

4.4.5 Assessment of Using Mallard Ducks as a Surrogate Species for other Waterfowl

There are a number of assumptions that are made when using a surrogate to model another species. In this study the assumptions were that mallards had similar enough morphology, physiology and behaviour to New Zealand waterfowl species that the physiological response of mallards to capture and handling would provide insight as to how New Zealand waterfowl might be responding to these events.

Auckland and Campbell Island teal are flightless and this difference in morphology may cause them to physiologically respond differently to capture and handling. The condition of the mallards used in the study and of the waterfowl I am trying to model is also a potential complication. Both wild and aviary housed birds are 'captured' and handled and these conditions or captive rearing and artificial diet may affect the behaviour, stress response, weight and fitness of the captive birds. For example captive pateke tend to be heavier and fatter then healthy wild birds and have reduced caeca and small intestines (Moore & Battley, 2006). These factors in turn may increase or decrease the development of muscle injury in these birds.

The mallards used were used to human presence and this may have caused a different stress and behavioural response compared to fully wild birds or to captive raised birds, although Campbell Island teal have had no behavioural differences observed between captive raised and wild birds during translocations (Gummer & Berry, 2007). Hand raising grey-faced petrels (*Pterodroma macroptera gouldi*) chicks resulted in fledglings that had a reduced CORT response compared to parent raised chicks (Adams *et al.*, 2005). Further research should be done on fully wild mallards unused to human presence and to captive raised mallards. Captive raised are perhaps more important given the extent of the captive breeding programme, but capture may have greater impacts in fully wild birds.

The validation of mallard as a surrogate is important in order to apply these findings to other waterfowl species. Validation of using surrogates as a method to assess capture and handling impacts in general could be done carrying out the same methodology on similar species under the same conditions. The species physiological response should then be compared to see if the patterns of physiological response were the same/similar for each parameter or whether

there were interspecies differences. Variables such as time of year, time of day and environmental conditions need to be in the same range across all species. This should be done on more common species, for example in New Zealand paradise shelduck, New Zealand shoveler and possibly New Zealand scaup could all be sampled using the same methodology to determine if they respond the same under similar environmental/seasonal conditions. If there are only minimal differences in the patterns of response between these species then it is likely that the threatened species will also respond the same way.

The other type of validation that needs to be performed relates to the living condition of the bird. That is whether the bird is a naïve wild bird, a habituated free living bird, an aviary raised bird or even a hand raised bird. These conditions may affect the physical, physiological or behavioural state of the bird and therefore potentially how it is impacted by capture and handling. In order to validate this, the same capture and handling methodology should be applied to several groups of the mallards (wild, habituated aviary raised etc.) under the same seasonal and environmental conditions.

Sampling threatened species would result in smaller sample sizes, less serial blood sampling and a lesser degree of manipulation. Therefore further studies on other waterfowl would provide validation without resorting to sampling the target threatened species.

4.4.6 Management Recommendation Regarding New Zealand Waterfowl Capture and Handling

4.4.6.1 Assessment of the Waterfowl Handling Protocol

The results of this study do not indicate that the handling protocols of waterfowl contribute to either the magnitude of the stress response or the duration of the elevation over the sampling period. My study therefore finds the current handling protocols to be acceptable although further research is needed including validation of these findings for them to be applicable to other species in different situations.

4.4.6.2 Recommendations Regarding Capture Protocols for Waterfowl

In my study capture did not cause muscle damage in mallards however it has been shown to cause muscle damage in other studies. Therefore I advise caution when capturing waterfowl but that it is possible to capture waterfowl without detectable muscle damage. Capture does provoke a stress response in mallards and different capture techniques may cause a difference in the magnitude and duration of the stress response. Therefore further research is needed to determine what capture techniques have a minimal impact on the magnitude and duration of the CORT response.

I suggest that the key features of the capture method I used that resulted in minimal muscle damage were the use of a human habituated population, a very short interval from envelopment in the net to manual physical restraint and the gentle handling protocol followed. It is recommended that these factors be considered when extrapolating the results to a wild species capture.

References

Adams, N. J., Cockrem, J. F., Taylor, G. A., Candy, E. J., & Bridges, J. (2005). Corticosterone responses of hand-reared and parent-reared grey-faced petrel chicks (*Pterodroma macroptera gouldi*). *Zoo Biology, 24*(3), 283-290.

Adams, N. J., Parker, K. A., Cockrem, J. F., Brunton, D. H., & Candy, E. J. (2010). Corticosterone responses and post-release survival in translocated North Island Saddlebacks (*Philesturnus rufusater*) in New Zealand. *Emu, 110*(4), 296-301.

Anonymous. Wildlife Act 1953. http://www.legislation.govt.nz/act/public/1953/0031/latest/whole.html#whole (accessed 23 May 2013). Public Act 1953 No 31. New Zealand Government, Wellington, NZ, 1999.

Anonymous (1987). *IUCN position statement on the translocation of living organisms: introductions, re-introductions, and restocking.* http://intranet.iucn.org/webfiles/doc/SSC/SSCwebsite/Policy_statements/IUCN_Position_Statement_on_Translocation_of_Living_Organisms.pdf (accessed 20 May 2013). International Union for Conservation of Nature (IUCN), Gland, Switzerland.

Anonymous (2012). *IUCN Red List of Threatened Species*. Version 2012.2. www.iucnredlist.org (accessed 19th August 2012). International Union for Conservation of Nature (IUCN).

Anonymous (2013a). *New Zealand Birds Online. The digital encyclopaedia of New Zealand birds.* http://nzbirdsonline.org.nz/group-search?field_search_genus_name_value=Waterfowl&field_search_family_name_value=Waterfowl&field_search_other_groups_value=Waterfowl&search_sort=field_species_weighting_value&search_filter=introduced (accessed 24th December 2013). Te Papa & the Ornithological Society of New Zealand & the Department of Conservation.

Anonymous (2013b). *Ducks*. http://hunting.fishandgame.org.nz/content/ducks (accessed 2nd April 2013). Fish and Game New Zealand, Wellington, New Zealand.

Anonymous (2013c). Open season for game in Fish and Game regions. *New Zealand Gazette. Issue No. 30*, pp 893-920. http://www.dia.govt.nz/diawebsite.nsf/ wpg_URL/Services-New-Zealand-Gazette-2013-Gazettes?OpenDocument (accessed 23rd May 2013). New Zealand Government, Wellington, NZ, 2013.

Bailey, T. A., Wernery, U., Naldo, J., & Samour, J. H. (1997). Plasma concentration of creatine kinase and lactate dehydrogenase in houbara bustards (*Chlamydotis undulata macqueenii*) immediately following capture. *Comparative Haematology International, 7*(2), 113-116.

Barker, D., & Williams, M. (2002). Breeding of brown teal (*Anas chlorotis*) at Okiwi, Great Barrier Island. *Notornis, 49*(4), 199-208.

Basson, P. A., & Hofmeyr, J. M. (1973). Mortalities associated with wildlife capture operations. In E. Young (Ed.), *The Capture and Care of Wild Animals; The Work of Eighteen Veterinary, Medical, and Wildlife Experts* (pp. 151-160). Cape Town and Pretoria: Human and Rousseau.

Bollinger, T., Wobeser, G., Clark, R. G., Nieman, D. J., & Smith, J. R. (1989). Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by 3 methods for banding. *Journal of Wildlife Diseases, 25*(2), 225-231.

Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport medicine. *British Medical Bulletin, 81-82*, 209-230.

Caithness, T., Williams, M., & Nichols, J. D. (1991). Survival and band recovery rates of sympatric gray ducks and mallards in New Zealand. *Journal of Wildlife Management, 55*(1), 111-118.

Carsia, R. V., & Harvey, S. (2000). Adrenals. In G. C. Whittow (Ed.), *Sturkie's Avian Physiology* (pp. 489-537). San Diego: Academic Press.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A., & Reynolds-Hogland, M. L. (2008). An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy, 89*(4), 973-990.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology, 148*, S169-S178.

Cockrem, J. F., Adams, D. C., Bennett, E. J., Candy, E. J., Hawke, E. J., Henare, S. J., & Potter, M. A. (2004). Endocrinology and the Conservation of New Zealand Birds. In M. S. Gordon & S. M. Bartol (Eds.), *Experiemental approaches to conservation biology* (pp. 101-121). Berkeley and Los Angeles: University of California Press.

Cockrem, J. F., Potter, M. A., Barrett, D. P., & Candy, E. J. (2008). Corticosterone responses to capture and restraint in emperor and Adelie penguins in Antarctica. *Zoological Science, 25*(3), 291-298.

Cox, R. R., & Afton, A. D. (1998). Effects of capture and handling on survival of female Northern Pintails. *Journal of Field Ornithology, 69*(2), 276-287.

Dabbert, C. B., & Powell, K. C. (1993). Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases, 29*(2), 304-309.

Dawson, A., & Howe, P. D. (1983). Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *General and Comparative Endocrinology, 51*(2), 303-308.

Driver, E. A. (1981). Hematological and blood chemical values of mallard, *Anas p. platyrhynchos*, drakes before, during and after remige molt. *Journal of Wildlife Diseases, 17*(3), 413-421.

Dumbell, G. S., McArdle, B. H., & Craig, J. L. (1988). Discriminating the sex of birds: a study of brown teal (*Anas aucklandica chlorotis*). *New Zealand Journal of Zoology, 15*(1), 47-53.

Dyer, J., & Williams, M. (2010). An introduction most determined: Mallard (Anas platyrhynchos) to New Zealand. *Notornis, 57*(Part 4), 178-195.

Gillies, C. A., Leach, M. R., Coad, N. B., Theobald, S. W., Campbell, J., Herbert, T., Graham, P. J., & Pierce, R. J. (2003). Six years of intensive pest mammal control at Trounson Kauri Park, a Department of Conservation "mainland island," June 1996-July 2002. *New Zealand Journal of Zoology, 30*(4), 399-420.

Glaser, A., van Klink, P., Elliott, G., & Edge, K. (2010). Whio/blue duck (*Hymenolaimus malacorhynchos*) Recovery Plan 2009–2019. Wellington: Department of Conservation.

Gratto-Trevor, C. L., Oring, L. W., & Fivizzani, A. J. (1991). Effects of blood sampling stress on hormone levels in the semipalmated sandpiper. *Journal of Field Ornithology, 62*(1), 19-27.

Gummer, H., & Berry, R. (2007). First transfer of Campbell Island teal (Anas nesiotis) to Campbell Island/Motu Ihupuku. Wellington: Department of Conservation.

Gummer, H., & Evans, K. (2003). Brown teal/pateke (*Anas chlorotis*) Husbandry Manual. Ruawai: Brown Teal Recovery Group.

Herring, G., Gawlik, D. E., & Beerens, J. M. (2008). Evaluating two new methods for capturing large wetland birds. *Journal of Field Ornithology, 79*(1), 102-110.

Holmes, T., & Caskey, D. (2001). Translocation of wild-caught and captive-reared blue duck *Hymenolaimus malacorhynchos*. Wellington: Department of Conservation.

Huschle, G., Toepfer, J. E., Brininger, W. L., & Azure, D. A. (2002). Capturing adult American Bitterns. *Waterbirds, 25*(4), 505-508.

Johnsgard, P.A. (2010). *Ducks, Geese, and Swans of the World, Revised Edition*. pp. xxvi. http://digitalcommons.unl.edu/biosciducksgeeseswans/1/ (accessed 20 May 2013). University of Nebraska–Lincoln Libraries.

King, E. J., & Caskey, D. A. (2010). Egmont National Park Blue Duck Programme Progress Report 2008-2010. Whanganui: Department of Conservation.

Mechlin, L. M., & Shaiffer, C. W. (1980). Net firing gun for capturing breeding waterfowl. *Journal of Wildlife Management, 44*(4), 895-896.

Milani, J. F., Wilson, H., Ziccardi, M., LeFebvre, R., & Scott, C. (2012). Hematology, plasma chemistry, and bacteriology of wild tundra swans (*Cygnus columbianus*) in Alaska. *Journal of Wildlife Diseases, 48*(1), 212-215.

Miskelly, C. M., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Powlesland, R. G., Robertson, H. A., Sagar, P. M., Scofield, R. P., & Taylor, G. A. (2008). Conservation status of New Zealand birds. *Notornis 55*(3), 117-135.

Moore, S. J., & Battley, P. F. (2006). Differences in the digestive organ morphology of captive and wild brown teal *Anas chlorotis* and implications for releases. *Bird Conservation International, 16*(3), 253-264.

O'Connor, S. M., Maloney, R. F., & Pierce, R. J. (2007). Pateke (*Anas chlorotis*) Recovery Plan, 2005–10. Wellington: Department of Conservation.

Perfito, N., Schirato, G., Brown, M., & Wingfield, J. C. (2002). Response to acute stress in the Harlequin Duck (*Histrionicus histrionicus*) during the breeding season and moult: relationships to gender, condition, and life-history stage. *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 80*(8), 1334-1343.

Pitman, D. L., Ottenweller, J. E., & Natelson, B. H. (1988). Plasma corticosterone levels during repeated presentation of 2 intensities of restraint stress: chronic stress and habituation. *Physiology & Behavior, 43*(1), 47-55.

Poisbleau, M., Fritz, H., Guillon, N., & Chastel, O. (2005). Linear social dominance hierarchy and corticosterone responses in male mallards and pintails. *Hormones and Behavior, 47*(4), 485-492.

Powell, R. A., & Proulx, G. (2003). Trapping and marking terrestrial mammals for research: Integrating ethics, performance criteria, techniques, and common sense. *Ilar Journal, 44*(4), 259-276.

Quinlan, S. E., & Hughes, J. H. (1992). Techniques for capture and radio tagging of marbled murrelets. *Proceedings of the Western Foundation of Vertebrate Zoology, 5*(1), 117-121.

Rhymer, J. M., Williams, M. J., & Braun, M. J. (1994). Mitochondrial Analysis of Gene Flow Between New Zealand Mallards (*Anas platyrhynchos*) and Grey Ducks (*Anas superciliosa*). *Auk, 111*(4), 970-978.

Rickett, J. (2010). *The dispersal and survivorship of pateke (Anas chlorotis) in relation to experimental release techniques; supplementary feeding and wing-clipping.* Masters of Science, Massey University, Auckland.

Romero, L. M., & Romero, R. C. (2002). Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor, 104*(1), 129-135.

Siegel, H. S. (1980). Physiological stress in birds. *Bioscience, 30*(8), 529-534.

ter Steege, R. W. F., & Kolkman, J. J. (2012). Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Alimentary Pharmacology & Therapeutics, 35*(5), 516-528.

Walker, B. G., Boersma, P. D., & Wingfield, J. C. (2006). Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology, 20*(1), 146-154.

Ward, J. M., Gartrell, B. D., Conklin, J. R., & Battley, P. F. (2011). Midazolam as an adjunctive therapy for capture myopathy in bar-tailed godwits (*Limosa lapponica baueri*) with prognostic indicators. *Journal of Wildlife Diseases, 47*(4), 925–935.

Weller, M. W. (1975). Ecological studies of Auckland islands flightless teal. *Auk, 92*(2), 280-297.

Whitehead, A. L., Elliott, G. P., & McIntosh, A. R. (2010). Large-scale predator control increases population viability of a rare New Zealand riverine duck. *Austral Ecology, 35*(7), 722-730.

Williams, E. S., & Thorne, E. T. (1996). exertional myopathy (capture myopathy). In A. Fairbrother, L. N. Locke & G. L. Hoff (Eds.), *Noninfectious Diseases of Wildlife* (2nd ed., pp. 181-193). London: Manson Publishing Ltd.

Williams, M. (1995). Social structure, dispersion and breeding of the Auckland Island teal. *Notornis, 42*(4), 219-262.

Wobeser, G. A. (1997). *Diseases of Wild Waterfowl* (Second Edition ed.). New York: Plenum Press.

CHAPTER FIVE

GENERAL DISCUSSION

5.1 THREATENED SPECIES' HANDLING PROTOCOLS

The capture of all three species used in this investigation elicited a stress response and in pūkeko also had an effect on the muscle physiology, and other, less clearly defined effects on gastrointestinal and renal physiology. However the handling protocols used for kiwi, takahē and pateke and investigated in this study in surrogate species showed minimal additional physiological impacts subsequent to the birds' capture. This is in contrast to some of the literature which has found handling and restraint time to be a factor in the development of muscle damage, clinical signs of capture myopathy or on post release survival in wild animals (Spraker, 1993; Williams & Thorne, 1996; Nicholson *et al.*, 2000; Ponjoan *et al.*, 2008). It is also in contrast to other studies which have found different handling protocols have different effects on the stress response (Cockrem *et al.*, 2008). However it is in partial agreement with a study by Dabbert and Powell (1993) which found that the degree of muscle damage that the wild birds in their study experienced due to capture overrode any additional effects that handling might have. It is very hard to fully separate the effects of capture, handling and sampling on wild animals as one of these procedures cannot occur without the other. Damage which may be associated with increased handling/restraint may simply be a result of a particular capture type requiring more handling and/or restraint (Bollinger *et al.*, 1989) and as the pūkeko study showed factors surrounding capture type can influence the degree of muscle damage. Alternatively the handling protocol used in my research may not have fully reflected how rough handling in the field may be. Instead of being held predominantly in one position as were the control birds in my research, birds are usually moved about and have their limbs or wings stretched out for samples, measurements or attachments. Handling protocols that more accurately reflect field practices should therefore be tested in future research. Another possibility is that handling protocols may have a larger effect following a greater degree of muscle damage. In cases where capture results in a greater degree of muscle then handling protocol may play a much more significant role in the further development of muscle damage/capture myopathy.

5.2 THE IMPORTANCE OF CAPTURE TECHNIQUES

Factors surrounding capture had a significant effect on the development of the stress response and degree of muscle damage birds experienced following capture. Namely pūkeko captured in flight experienced a greater stress response and increased degree of muscle damage compared to pūkeko captured on the ground. This is finding is in agreement with other studies which have found different capture methods to have different effects on the development of the stress response (Romero & Romero, 2002) and the degree of muscle damage (Bollinger *et al.*, 1989).

This research did not set out to test the impacts of different capture techniques on wild birds. Some of the techniques used to routinely capture takahē such as capture by hand, hand-net, loose nylon nets or by using capture pens (Rose, 2000; Youl, 2008; Kilduff *et al.*, 2011) were not used in this study because the flighted nature of pūkeko made most of the methods unsuitable. The techniques used to routine capture waterfowl in New Zealand were also not used because I wanted to use a similar protocol to the pūkeko study to see if there were inter-species differences in the response to the same technique. An assumption was therefore made that the physiological responses of the wild birds would be similar when using a net-gun to the routine capture techniques normally used. However the differences found between pūkeko captured in flight compared to those captured on the ground suggests that factors associated with capture may have significant effects on physiological responses.

The stressful nature of capturing wild birds is thought to approximate that of predation (Cockrem, 2007). However some techniques may be perceived as more or less stressful than others (Romero & Romero, 2002). In addition the nature of certain capture methods mean they are more likely to capture birds that are exhibiting a particular behaviour. For example, birds which are chased may try to escape and birds which are captured using a fired net may be more likely to be running or flying away. Birds attempting to evade capture will show greater exertion and the restraint of struggling animals may be more stressful compared to capturing a bird that is unaware of the capture attempt or has responded by becoming still. Further research could compare the physiological impacts of different capture techniques currently used on threatened animals. These techniques could be compared by sampling the threatened species or by using a surrogate, but a different choice of surrogate species may be

necessary in some cases because some capture techniques or flightless birds are not suitable for flighted ones (eg. the protocol for capturing takahē by hand to capture pūkeko).

5.3 ASSESSING THE POTENTIAL FOR SUBCLINICAL CAPTURE MYOPATHY IN THE FIELD

The clinical signs of capture myopathy may be delayed in onset and muscle damage may be asymptomatic. Therefore, plasma levels of CK and AST are thought to be suitable measurements to assess muscle damage in birds (Franson *et al.*, 1985; Lumeij *et al.*, 1988). However in the pūkeko the rise in plasma CK and AST was only apparent between 30 to 120 minutes. Threatened species are generally handled for as short a time as possible and are often let go before this time. This means that capture may cause muscle damage in threatened species but go unnoticed because the plasma enzymes were not significantly elevated by the time of the birds' release. This finding complicates assessing the physiological impacts of capture on threatened species in the field as the absence of change in the muscle enzymes over the period the birds are held for does not rule out the occurrence of muscle damage.

The only way to remedy this would be to use a new diagnostic tool that could identify the occurrence of muscle damage earlier or to sample birds over a longer time frame. Sampling threatened species over a longer time frame than they are normally held for is possibly detrimental and so this would have to occur as either part of a translocation where birds are held for a longer duration as part of management or through further use of a surrogate species. Since metabolic acidosis is central to the development of muscle damage caused by capture myopathy a method that may be useful would be to measure blood pH and/or blood lactate concentrations. This can be performed in the field using portable hand-held clinical blood gas analysers and has been used to assess the metabolic and respiratory impacts of capture and handling on mourning doves (*Zenaida macroura*), boat-tailed grackles (*Quiscalus major*) and house sparrows (*Passer domesticus*) (Harms & Harms, 2012). This would have the added benefit of providing instant information to the field team and may identify individuals that are most at risk during events like translocations. This method would allow for the comparison between capture techniques etc. but similarly to measuring

concentrations of plasma CK and AST it does not in itself indicate whether a bird will suffer debilitating or fatal capture myopathy.

5.4 THE ROLE OF STRESS IN THE DEVELOPMENT OF MUSCLE DAMAGE

The stress experienced by a bird during capture is pivotal in the development of capture myopathy but a stress response does occur even in the absence of muscle damage as seen in the mallard study. Pūkeko captured in flight suffered more muscle damage and had greater stress response compared to those captured on the ground. But does a high stress response directly cause a higher amount of muscle damage or does a high amount of muscle damage cause a bird more stress? Further, are individuals that are prone to a higher stress response as a result of their personality more likely to initiate the capture myopathy cascade? Capture of wild birds is thought to be similar to a predation event and causes an emotional stress response in the birds. In addition if muscle damage does occur then it is likely a painful condition (Williams & Thorne, 1996) and therefore probably acts as a physical stressor, further increasing the level of stress experienced by the bird. Therefore it is likely muscle damage is both a cause and effect of the stress a bird experiences during capture. However due to the variation in the stress response between individuals, avian personalities may be a significant factor in the development of muscle damage and further research should investigate this.

In a similar pattern to other species studied, plasma CORT becomes elevated rapidly following capture (Romero & Romero, 2002). The pūkeko study showed that similar CORT elevations occurred in birds captured in flight and on the ground at 30 minutes but the CORT response had diverged much more by the 120 minute sample. This suggests that the variability of the physiological response to capture includes not only the magnitude, but also the duration of the stress response. The relationship between the stress response and the development of capture myopathy is not fully understood and contrary to what this research observed in pūkeko, one study suggested that birds which experience high levels of stress (as reflected by high plasma CORT) may ‘freeze’ and therefore suffer less muscle damage

(Nicholson *et al.*, 2000). The effect of different response strategies such as freeze versus flight on the development of capture myopathy may warrant further investigation.

Further research which compares different capture or handling techniques could look at the different plasma CORT peaks, the time it takes to reach peak plasma CORT and the duration that plasma CORT remains elevated above baseline levels. While this has been done in threatened birds to some extent already (Adams, 2000), the use of surrogate species may allow a greater number of blood samples and a greater sampling period to more fully investigate this. This is provided that the stress response of the surrogate species is validated and found to be similar to the species being modelled.

5.5 ASSESSING DELAYED IMPACTS OF CAPTURE, STRESS AND SUBCLINICAL CAPTURE MYOPATHY

Measuring several biochemical analytes in this study allowed the acute physiological impacts of capture and routine handling protocol on birds to be assessed. This research did not address what wider effects these changes may have on the birds once they were released. The physiological impacts of capture observed in this research, (increased stress, muscle damage and altered digestive physiology in some species) may prove to be negligible to the survival and reproductive efforts of the birds or they may have significant but subtle effects reducing body condition and making birds more susceptible to predation (Cox & Afton, 1998; Mueller, 1999; Nicholson *et al.*, 2000).

One of the main components of the "emergency life history stage" is the flight or fight response, which involves a very rapid response to events such as a sudden attack by a predator (Wingfield, 2003). The capture of wildlife for management or research is in its essence very similar to predation. Using Wingfield's (2003) definition, capture can be considered an indirect labile perturbation factor, because it is a rapid event that should only affect the animal in the short term. Therefore it would only trigger a flight or fight response in the bird. In the event that capture resulted in the bird being injured or if it suffered from clinical capture myopathy then sickness behaviour may follow (Wingfield, 2003). If the sampling pressure on the population was high and consistent over a sustained period of time

then this process may be considered a direct labile perturbation factor, similar to an influx of predators and it may therefore trigger other emergency life history responses also.

The emergency life history stage has evolved to be beneficial to wild animals as behaviours or physiological functions that are unnecessary of even deleterious in the current situation are supressed and other alternative behaviours and physiological functions which enhance survival are promoted (Wingfield, 2003). However the capture of wildlife is not a natural event and the survival of the animal is not intentionally at risk, therefore any intervention which causes an emergency life history stage needs to not only be justified but changed or refined so that it may have a minimal effect on the animal. While the immediate effects of a stressor may be very short, the recovery from them is not well studied (Wingfield, 2003).

Therefore further research should investigate what impacts capture, stress and subclinical muscle damage may be having on threatened species over a longer time frame then the current research. Additional physiological or behavioural measurements may be used to determine what the effects are. Further research could look into the movement and survival rates of the birds in the days and weeks following release to compare the effects of different capture techniques. If common game bird species are used as surrogates again then this allows certain sampling techniques that cannot be done with threatened species, such as post mortem examination. Further research could look at the relationship between the physiological changes in the birds at capture and the presence, absence and/or severity of muscle lesions in the birds one (or more) week following capture. This type of information would be very useful from a management perspective as the impact of different methods of capture could be compared and the relationship between measurable physiological change and the impacts on the bird better understood.

One other finding from the pūkeko study is the possibility that capture may cause blood flow to be re-directed to the musculoskeletal system and away from the kidneys and digestive system due to stress and exertion. The potential impacts this may have on these organs may warrant further investigation.

5.6 USING SURROGATES SPECIES

Surrogate species were used in this research to model the physiological impact that routine capture and handling may be having on threatened avian species in New Zealand. The characteristics that make a suitable surrogate species for endangered species research is that they should respond similarly to the threatened species in question, and the surrogate should be more available for research purposes, and as such are usually a common or pest species. Before inter-species extrapolations can be made, validation of the response is needed. However during these studies, no validation of the selected surrogates was performed due to the difficulty in sampling threatened species ie. the very reason surrogates were required in the first place. In an ideal situation, I would have repeated my experiments on a limited sample of the endangered species in question to determine if they were in fact showing similar physiological responses to capture and handling.

A comparison between the pūkeko and mallard studies reveal major inter-species differences in the physiological response of the two species to very similar capture protocols (handling was found to not influence the response). It is therefore apparent that one surrogate avian species cannot be used to extrapolate across all birds. In two of the three studies, the surrogates in this research were chosen to be closely related taxonomically and have similar morphology to the threatened species they were chosen to model. However, it became apparent during this research that other factors such as season and time of day also affected the physiological response of birds to capture. This has the potential to limit the use of surrogates as there may be too many external confounding factors that may influence the physiological response of birds to capture for it to be accurately modelled between species.

The choice of a particular species or population of birds likely has bearing on its suitability for being a surrogate. While layer hens were used in this research, I determined that they are unsuitable surrogates for wild birds due to the prior husbandry conditions and the extreme metabolic demands of high egg production. In addition to these husbandry related issues, captive birds may also not be suitable to model physiological impacts on wild birds due to potential differences in stress that captive and wild birds may experience (Bollinger *et al.*, 1989). Even though ‘capture’ and handling may still cause a stress response in captive birds, as seen in the layer hens in this study and birds in other studies (Cabezas *et al.*, 2013) the habituation of captive or wild birds to human presence may cause a reduction in the acute

stress response of a bird (Walker *et al.*, 2006). Since stress plays a pivotal role in the development of capture myopathy (Basson & Hofmeyr, 1973) this may also effect the development of muscle damage potentially reducing the risk of it occurring in captive or habituated birds too. It is therefore advisable that the birds selected to be surrogate species have experienced similar living conditions to the species it is trying to model. For instance, captive aviary housed mallards may be a suitable surrogate for captive aviary housed pateke (brown teal) but may not be suitable for free living wild whio (blue duck). These factors and the results of this research again indicate that some validation should be performed to confirm or refute the suitability of each surrogate for the species it is attempting to model.

This research was not considered vital by conservation management authorities because there is no evidence of the negative impacts of subclinical capture myopathy on the threatened species studied in this research. The perceived lack of importance that the subclinical impacts of capture and handling may be having and the difficulty of removing the effects of blood sampling were considered grounds to reject the sampling of wild kiwi. Based on the literature results, I contend this research is important as the impacts of capture may not be readily observable whilst still having considerable impacts on the birds due to late onset of symptoms (Basson & Hofmeyr, 1973; Spraker, 1993), reduced survival probability due to increased risk of predation (Cox & Afton, 1998; Mueller, 1999) and decreased movement rate and body condition (Cattet *et al.*, 2008). As predation (McLennan *et al.*, 1996; O'Connor *et al.*, 2007; Wickes *et al.*, 2009; Glaser *et al.*, 2010) is such major factor in the decline of New Zealand birds any factors which may increase the chances of predation should be investigated further. My results show some support that subclinical muscle damage may occur in the absence of symptoms in pūkeko but showed no evidence of this in mallards. Whether the damage that occurred in pūkeko was enough to significantly alter behaviour or survival is unknown. My thesis topic is especially relevant as one of the ways to combat a declining population is management practices which involve capture techniques. However it requires further testing to be of more practical use in management.

One of the concerns that threatened species recovery groups may have is the potential risk that blood sampling poses to the animal through risk of injury or infection or because of an increased sampling time. However my research did not find increased handling time to be an issue in either the development of either muscle damage or the stress response and previous studies have found limited volume blood sampling has no major negative effects on wild birds (Sheldon *et al.*, 2008). Therefore, my results suggest that blood sampling on wild

threatened species can be safely used as part of the assessment of routine management actions that require capture and handling.

5.7 USING NET-GUNS TO CAPTURE THREATENED SPECIES IN NZ

The net-gun was found to be a safe method of capturing birds in this study, which is a similar result to other studies using this model of net-gun (Edwards & Gilchrist, 2011; Johnson *et al.*, 2011). No injuries were encountered through its use, which is in contrast to some previous studies using different models of net-gun which have caused injuries and fatalities (Mechlin & Shaiffer, 1980; Huschle *et al.*, 2002; Herring *et al.*, 2008). The lack of trauma (as measured by plasma enzymes) associated with the net-gun capture of mallards in this research is different from what has been reported in other studies (Bollinger *et al.*, 1989). The plasma concentrations of plasma CK and AST observed in pūkeko was similar to that seen in takahē over a comparative time frame within 30 minutes of capture (Rose, 2000; Youl, 2008). This is not conclusive as no other method of capture was compared to it in this study, but it does suggest that the net-gun has a similar or possibly lesser impact on birds' muscles then some other capture techniques used on takahē such as capture by hand, hand-net, loose nets or corral pens.

Net-guns can be a useful method of capture for a number of reasons: they allow individuals of a specific species, sex, age etc. to be targeted; they allow prompt sampling of the bird; they may reduce pursuit times by increasing the strike distance compared to capturing animals by hand or hand-net; and they may reduce the time birds are able to struggle freely against the net compared to techniques such as cannon netting. They do require a higher level of operator skill compared to some other methods and the more experience the operator has with the technique the better they will be at successfully capturing and quickly untangling birds and the less risk posed to the bird due to judgement of which shots to take and avoiding projectile strike. Further research could compare the physiological impacts of net-guns directly with other methods of capture currently used.

5.8 MANAGEMENT RECOMMENDATIONS

The results of this study suggest that the capture of a bird may not always result in muscle damage but will usually provoke a stress response. Factors surrounding the capture event are of great importance in the development of the stress response and in the event of muscle damage occurring, the extent of that damage. In particular the presence of a chase during capture, increased exertion of the target bird and possibly escape behaviour of the bird have all been highlighted as risk factors. I therefore suggest that where possible the use of capture methods that require chases are minimised and where these techniques are necessary a pre-planned cut off point is established in best practice guidelines based on the time of the pursuit and state of exhaustion of the bird. Further research should address what these cut off points should be. While not used for the species in this study, research or management elsewhere has used capture techniques that use chase tactics designed to exhaust the bird and therefore aid in its capture (Ellis, 1975; O'Gara & Getz, 1986; Ellis *et al.*, 1998). The intentional exhaustion of the bird is not advisable and should only be used as a last resort if at all. As the handling protocol was not found to be a factor in the development of stress or muscle damage in this study I suggest no changes to the current methods of handling or restraint.

5.9 FUTURE RESEARCH

While this research has found the handling protocol of several threatened species has only a minimal impact following capture it did raise several questions about the use of surrogate species, the assessment of muscle damage at capture, the role of capture type and the effects of capture following release. Further study in this area is needed to address the research questions:

1) are surrogate species an appropriate method for evaluating the physiological impacts of different techniques in threatened species?
2) What physiological effects are different routine capture protocols having on threatened avian species?

3) What field techniques can be used to reliably indicate that a bird is susceptible to developing muscle damage following release? And
4) what is the relationship between the changes in certain biochemical parameters caused by capture and the survival probability, body condition and movement rate of a bird?

References

Adams, D. C. (2000). *Corticosterone responses of captive and wild Northern brown kiwi (Apteryx mantelli)* Unpublished masters thesis, Massey University, Palmerston North.

Basson, P. A., & Hofmeyr, J. M. (1973). Mortalities associated with wildlife capture operations. In E. Young (Ed.), *The Capture and Care of Wild Animals; The Work of Eighteen Veterinary, Medical, and Wildlife Experts* (pp. 151-160). Cape Town and Pretoria: Human and Rousseau.

Bollinger, T., Wobeser, G., Clark, R. G., Nieman, D. J., & Smith, J. R. (1989). Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by 3 methods for banding. *Journal of Wildlife Diseases, 25*(2), 225-231.

Cabezas, S., Carrete, M., Tella, J. L., Marchant, T. A., & Bortolotti, G. R. (2013). Differences in acute stress responses between wild-caught and captive-bred birds: a physiological mechanism contributing to current avian invasions? *Biological Invasions, 15*(3), 521-527.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A., & Reynolds-Hogland, M. L. (2008). An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy, 89*(4), 973-990.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology, 148*, S169-S178.

Cockrem, J. F., Potter, M. A., Barrett, D. P., & Candy, E. J. (2008). Corticosterone responses to capture and restraint in emperor and Adelie penguins in Antarctica. *Zoological Science, 25*(3), 291-298.

Cox, R. R., & Afton, A. D. (1998). Effects of capture and handling on survival of female northern pintails. *Journal of Field Ornithology, 69*(2), 276-287.

Dabbert, C. B., & Powell, K. C. (1993). Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases, 29*(2), 304-309.

Edwards, D. B., & Gilchrist, H. G. (2011). A new means of catching shorebirds: the Super Talon Net Gun. *Wader Study Group Bulletin, 118*(2), 134-136.

Ellis, D. H. (1975). 1st experiments with capturing golden eagles by helicopter. *Bird Banding, 46*(3), 217-219.

Ellis, D. H., Hjertaas, D., Johns, B. W., & Urbanek, R. P. (1998). Use of a helicopter to capture flighted cranes. *Wildlife Society Bulletin, 26*(1), 103-107.

Franson, J. C., Murray, H. C., & Bunck, C. (1985). Enzyme activities in plasma, kidney, liver, and muscle of 5 avian species. *Journal of Wildlife Diseases, 21*(1), 33-39.

Glaser, A., van Klink, P., Elliott, G., & Edge, K. (2010). Whio/blue duck (*Hymenolaimus malacorhynchos*) Recovery Plan 2009–2019. Wellington: Department of Conservation.

Harms, C. A., & Harms, R. V. (2012). Venous blood gas and lactate values of mourning doves (*Zenaida macroura*), boat-tailed grackles (*Quiscalus major*), and house sparrows (*Passer domesticus*) after capture by mist net, banding, and venipuncture. *Journal of Zoo and Wildlife Medicine, 43*(1), 77-84.

Herring, G., Gawlik, D. E., & Beerens, J. M. (2008). Evaluating two new methods for capturing large wetland birds. *Journal of Field Ornithology, 79*(1), 102-110.

Huschle, G., Toepfer, J. E., Brininger, W. L., & Azure, D. A. (2002). Capturing adult American bitterns. *Waterbirds, 25*(4), 505-508.

Johnson, O. W., Fielding, L., Fox, J. W., Gold, R. S., Goodwill, R. H., & Johnson, P. M. (2011). Tracking the migrations of Pacific golden-plovers (*Pluvialis fulva*) between Hawaii and Alaska: New insight on flight performance, breeding ground destinations, and nesting from birds carrying light level geolocators. *Wader Study Group Bulletin, 118*(1), 26-31.

Kilduff, L., Dodson, H., & Marsh, P. (2011). Takahe (*Porphyrio hochstetteri*) Husbandry Manual. Te Anau: Department of Conservation (DOC),.

Lumeij, J. T., Debruijne, J. J., Slob, A., Wolfswinkel, J., & Rothuizen, J. (1988). Enzyme activities in tissues and elimination half-lives of homologous muscle and liver enzymes in the racing pigeon (*Columba livia domestica*). *Avian Pathology, 17*(4), 851-864.

McLennan, J. A., Potter, M. A., Robertson, H. A., Wake, G. C., Colbourne, R., Dew, L., Joyce, L., McCann, A. J., Miles, J., Miller, P. J., & Reid, J. (1996). Role of predation in the decline of kiwi, Apteryx spp, in New Zealand. *New Zealand Journal of Ecology, 20*(1), 27-35.

Mechlin, L. M., & Shaiffer, C. W. (1980). Net firing gun for capturing breeding waterfowl. *Journal of Wildlife Management, 44*(4), 895-896.

Mueller, J. M. (1999). *Effect of red imported fire ants on reproduction, health, and survival of northern bobwhites.* Texas Tech University, Lubbock.

Nicholson, D. S., Lochmiller, R. L., Stewart, M. D., Masters, R. E., & Leslie, D. M. (2000). Risk factors associated with capture related death in eastern wild turkey hens. *Journal of Wildlife Diseases, 36*(2), 308-315.

O'Gara, B. W., & Getz, D. C. (1986). Capturing golden eagles using a helicopter and net gun. *Wildlife Society Bulletin, 14*(4), 400-402.

O'Connor, S. M., Maloney, R. F., & Pierce, R. J. (2007). Pateke (*Anas chlorotis*) Recovery Plan, 2005–10. Wellington: Department of Conservation.

Ponjoan, A., Bota, G., De La Morena, E. L. G., Morales, M. B., Wolff, A., Marco, I., & Manosa, S. (2008). Adverse effects of capture and handling little bustard. *Journal of Wildlife Management, 72*(1), 315-319.

Romero, L. M., & Romero, R. C. (2002). Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor, 104*(1), 129-135.

Rose, K. (2000). Takahe (*Porphyrio hochstetteri*) Health Investigation Project. Te Anau Department of Conservation.

Sheldon, L. D., Chin, E. H., Gill, S. A., Schmaltz, G., Newman, A. E. M., & Soma, K. K. (2008). Effects of blood collection on wild birds: an update. *Journal of Avian Biology, 39*(4), 369-378.

Spraker, T. R. (1993). Stress and capture myopathy in artiodactylids. In M. E. Fowler (Ed.), *Zoo and Wild Animal Medicine: Current Therapy* (Vol. 3, pp. 481-488). Philadelphia: W.B. Saunders Company.

Walker, B. G., Boersma, P. D., & Wingfield, J. C. (2006). Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. *Conservation Biology, 20*(1), 146-154.

Wickes, C., Crouchley, D., & Maxwell, J. (2009). Takahe (*Porphyrio hochstetteri*) Recovery Plan 2007–2012. Wellington: Department of Conservation.

Williams, E. S., & Thorne, E. T. (1996). Exertional myopathy (capture myopathy). In A. Fairbrother, L. N. Locke & G. L. Hoff (Eds.), *Noninfectious Diseases of Wildlife* (2nd ed., pp. 181-193). London: Manson Publishing Ltd.

Wingfield, J. C. (2003). Control of behavioural strategies for capricious environments. *Animal Behaviour, 66*, 807-815.

Youl, J. M. (2008). *An investigation into lead exposure in free-ranging kea (Nestor notabilis), takahe (Porphyria hochstetteri) and Australasian harriers (Circus approximans) in New Zealand.* Masters of Veterinary Science, Massey University, Palmerston North.