Capture myopathy in migratory shorebirds: An investigation of risk factors and treatment methods

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ABSTRACT

Capture myopathy is a syndrome seen as a complication of capture and handling in many species of birds and mammals. Muscular trauma and necrosis leads to ataxia, paralysis and pain, while metabolic disturbances can result in death of the animal. We investigated risk factors and a new ancillary treatment for three species of shorebirds that are reportedly susceptible to capture myopathy: bar-tailed godwits (*Limosa lapponica*), red knots (*Calidris canutus*) and great knots (*C. tenuirostris*). Serial blood samples were examined for changes in the plasma concentrations of creatine kinase (CK) and aspartate aminotransferase (AST), uric acid (UA) and potassium (K+). Comparisons were made for two capture methods: mist-netting and cannon-netting. Environmental factors were investigated by comparing cannon-net captures in 3 locations with differing ambient temperatures. Sex and body mass were also investigated as potential risk factors in godwits. We found marked increases in plasma concentrations of CK in godwits and great knots following capture and banding. While some muscle damage was evident by both methods of capture, cannon-net captures showed greater evidence of muscle damage in godwits and a greater occurrence of capture myopathy in godwits and red knots. Entanglement nets were especially risky, associated with the most problematic capture and a greater number of CM cases. Sex or body mass differences did not appear to influence godwit susceptibility to muscle damage. Surprisingly, hot environmental temperatures in Australia did not exacerbate muscle damage when compared with cooler New Zealand locations, but elevated plasma concentrations of AST suggested greater generalized tissue or organ damage. Plasma concentrations of uric acid showed species variability, but all species showed a significant decline in the post-capture period that may relate to interruption of digestion due to acute stress. Sixteen godwits that developed capture myopathy after a cannon-net capture in New Zealand were hospitalised and split into two groups of eight birds. Midazolam, a benzodiazepine with the effects of anxiolysis, muscle relaxation and sedation, was used as an ancillary treatment for one of the groups. Both groups were treated with subcutaneous fluid therapy, non-steroidal anti-inflammatories (meloxicam), gavage feeding, and sling therapy twice daily. Six of the 8 birds in the treatment group survived to the point of release compared to 3/8 of the control group. Birds treated with midazolam showed subjective benefits including improved tolerance of handling and sling therapy, but did not show any significant differences in any of the clinical parameters we measured. However, we found the birds' body mass, packed cell volume (PCV), plasma UA, and peak plasma CK showed potential as prognostic indicators for survival. Inability to counteract weight loss in captivity was the most significant problem encountered in the treatment of both groups of birds. Lack of

waterproofing and predation were contributing causes to death of at least two godwits subsequent to release.

Our results imply that common capture techniques have significant effects on the muscular, digestive and homeostatic physiology of shorebirds. Based on this study, we recommend the use of mist-nets or light, fine mesh nets for cannon-net capture of shorebird species known to be susceptible to capture myopathy. Entanglement (large mesh) cannon-nets should be avoided for any susceptible species. Treatment of capture myopathy remains challenging, yet midazolam shows potential as an ancillary treatment for capture myopathy in birds and is worthy of continued study and use.

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I loved this project. I fell for the godwits, which seem like plain brownish birds in the distance, but are elegant, courageous and beautiful once you get to know them. The knots are not so bad either (excuse the pun). I sincerely hope that any small morsels of new information I gleaned from this research can be utilised to help minimise the occurrence of capture myopathy in birds: it is something that no animal should have to go through.

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Chapter One

INTRODUCTION, LITERATURE REVIEW and OBJECTIVES

Chapter One: *Introduction, Literature Review and Research Objectives*

1.1 INTRODUCTION

The capture of wild animals has been integral to the success of humankind. The use of traps to capture live animals actually pre-dates hunting and farming, yet modern trap types are not far removed from those utilised thousands of years ago (Fitzwater, 1970). Historically, wild animals were trapped for human consumption, trade, trophies, medicines and their pelts (Corlett, 2007). While wild animal exploitation still continues in the often illegal wildlife trade (Corlett, 2007), modern motivations for wildlife capture are often science based. Examples include wildlife research; screening for infectious exotic diseases, management of wild populations, or to aid in conservation work. The translocation of wildlife, which involves both capture and transportation of an animal prior to release, is a common conservation practice (Seddon, 2010). Wildlife translocation aims to introduce or reinstate endangered species to suitable habitats, or to supplement declining populations of animals (Dickens, et al., 2010).

Capturing a wild animal is a potentially traumatic event that can cause physical exhaustion, acute or chronic stress (Dickens, et al., 2010), injuries or death (Jacques, et al., 2009). Of all of the possible concerns with wild animal capture, capture myopathy (CM) is one of the most significant (Williams & Thorne, 1996). Capture myopathy results when stress, trauma and overexertion due to pursuit, trapping, handling and/or transportation leads to muscular damage and physiological imbalances resulting in muscular stiffness, disability and potentially death (Williams & Thorne, 1996).

Shorebirds, including bar-tailed godwits (*Limosa lapponica*), red and great knots (*Calidris spp.)* (Rogers, et al., 2004); curlews and whimbrels (*Numenius spp.*) (Green, 1978), have developed clinical signs consistent with capture myopathy after capture and banding, however there have been relatively few peer-reviewed studies to assess the extent of the problem in these birds.

This research aims to further the knowledge of the pathophysiology of capture myopathy in selected shorebird species and determine if there are significant relationships between capture myopathy occurrence and environmental conditions, methods of capture, signalment and stage of migration cycle. A new treatment option will also be explored for birds that develop clinical signs of capture myopathy during the course of this study.

Section 1 presents a review of background information on capture and capture complications in birds. Current knowledge of capture myopathy in birds is discussed, including the definition; clinical signs and syndromes; pathogenesis of the disease; known aetiological factors and current knowledge of the prevention, management and treatment of affected individuals.

Sections 2 and 3 will present publications of the results from this study.

Section 4 will discuss the results and offer interpretations that could be utilised in creating standards or improvements in wild bird capture to prevent capture myopathy, and to improve treatment outcomes.

1.2 DEFINITION OF CAPTURE MYOPATHY

For the purpose of this thesis, capture myopathy is defined as morbidity and/ or mortality due to direct and secondary effects of muscular damage, resulting from the capture (also handling and transportation) of wild birds.

1.3 SCOPE OF THESIS

This thesis investigates capture myopathy as a result of capture in wild birds. Therefore, chemical immobilisation and capture techniques used in mammals are beyond the scope of this dissertation and the following will discuss only wild bird capture techniques. Likewise, the review of current knowledge on the pathophysiology, clinical syndromes, risk factors and treatments will focus on birds, with reference to human and mammalian texts where avian knowledge is lacking, or for comparative purposes.

1.4 CAPTURE OF WILDLIFE

1.4.1 Purpose of Capture

Wild animal capture has been practiced for as long as humanity has existed (Day, et al., 1980). Traditionally, mammals and birds were hunted for food and by-products such as medicines, fur and bone (Corlett, 2007). In more recent history, wild animals were caught alive to introduce into captive populations and zoological parks (Hofmeyr, 1973). Today, hunting and wildlife capture for the pet trade, "bush meat" and traditional medicines, remains prominent in some countries such as China and South East Asia (Corlett, 2007). However, a large proportion of present-day wildlife capture is due to biological research and wildlife management activities (Day, et al., 1980). These motives are driven by dwindling habitats, declining species (Myers, et al., 2000), increasing human-wildlife interactions (White & Ward, 2010) and emerging infectious diseases (Rhyan & Spraker, 2010). Wildlife management activities may include: translocation of rare or endangered species (Kock, et al., 2010); marking (/banding) (Day, et al., 1980) or telemetry studies (Cochrane, 1980); disease investigation or mitigation (Rhyan & Spraker, 2010); and capture of oiled (Goldsworthy, et al., 2000), injured or ill individuals (Van der Welt, 1973) for treatment by veterinarians or humane euthanasia.

Careful planning is required for any capture operation and the aim is to ensure safe, reliable, cost-effective and successful captures (Day, et al., 1980). In the planning stages, wildlife capture teams must take into account factors such as: the species and numbers of animals to be caught, environmental factors such as terrain and weather conditions, numbers of available and trained operators, funding and time limitations (Wilbur, 1967); as well as holding facilities and transportation; release sites and release factors such as the presence of predators or scavengers (Cox Jr. & Afton, 1998). Additionally, scientific studies must gain the approval of University or governmental animal ethics committees and may require permits issued by local and national government agencies, such as the Department of Conservation (Department of Conservation, 2012). Animal ethics committees are a more formal process that govern the use of animals in research, ensuring that welfare standards and legislation is upheld (Animal Research Review Panel, 2011).

Banding and morphometric data collection are common reasons for capture by ornithologists. Birds are captured in small or large numbers, usually via nets. Each bird may have several measurements taken and leg bands fitted. These may be coloured, alphanumeric, or metal imprinted leg bands and records are made when these birds are sighted or recaptured by other 'birders' nationally or globally (Department of

Conservation, 2012). By knowing details of when and where a bird was caught and banded, and subsequent re-sightings, knowledge can be gained about a bird's age and movement patterns (Woodley, 2009). For instance ornithologists proposed that godwits could be flying direct from Alaska to New Zealand using the information gained from resighting banded birds (Gill Jr., et al., 2005). This lead to further studies using satellite transmitters, with the historical event of godwit 'E7' tracked flying non-stop over 8 days from Alaska to northern New Zealand (Battley, et al., 2012).

However, Woodley (2009) mentions that recovery of band information may only occur sporadically, often with long time periods between banding and recovery, and few recoveries when compared to numbers banded. This begs the question: Is the possible harm done to birds, during capture for research or banding purposes, worth it for the actual information gained? And how do we judge this when we cannot predict the future gains, nor the likelihood that the birds will be harmed? Research that gives insight into the risk factors or susceptibility of species to CM will allow wildlife managers, permit operators and ethics committees to make informed decisions about capture of wild birds.

1.4.2 Capture methods for wild birds

The type of trap used to capture birds varies according to the species and its habits. Some ground nesting or terrestrial birds can be tracked and caught by hand e.g. kakapo (*Strigops habroptilus*) (Lloyd & Powlesland, 1994). Other species may be caught in their burrows e.g. kiwi, (*Apteryx spp.*) (Robertson, et al., 2011), and petrels (family Procellariidae) (Miskelly, et al., 2009). For most species, more complex methods of capture are required and some of the more common techniques and known complications are discussed below.

Mist-nets

Mist-netting is "perhaps the most versatile and widely used method" (pg 44.) for capture of passerines, shorebirds and many other species (FAO, 2007). Fine mesh nets are camouflaged within normal vegetation, or by putting them up in the dark if it is an open site, such that birds do not see them (Melville, 2011). Shelf strings (trammels) are of stronger thread and have loops on either end to attach the nets to lightweight poles such as bamboo, aluminium or steel (Melville, 2011). The nets form hammock-like pouches that the birds fall into and become entangled in the folds (Bub, 1991) **(Figure 1)**. The mesh comes in various sizes, to target the species caught (Day, et al., 1980). Large birds may bounce off if the mesh is too fine, while small birds may slip through larger holes and escape (Keyes & Grue, 1982). Modifications of mist-nets allow their use high up in the canopy (Meyers & Pardieck, 1993), in or over water (Heath & Frederick, 2003).

Studies have shown the most common causes of mortality or injury during mist-net capture included handling trauma, damage from the net, and predation (Spotswood, et al., 2012). These authors reviewed data of over 600,000 mist-net captures by 22 organisations and calculated average injury rates of 0.59% and mortality of 0.23%. A lack of experienced handler's, prolonged holding times in bags and decreased monitoring of nets lead to increased mortality rates from 0.5% to 2.8% in another study by Recher et al. (1985). Predominant causes of mortality were leaving the birds in the net too long (26.4%) unknown causes (17%), died in holding bags (15%), struggling in the net (11.3%) and predation (9.4%), with the other causes including various handling injuries and shock.

Figure 1. A mist net has been strung between two bamboo poles via shelf-strings (trammels). Pockets are formed by the net folds, which the bird falls into after encountering the net. *Photo Janelle Ward*

Cannon Nets

Cannon-nets (also termed rocket nets) use gunpowder to project a large, light, net over a flock of birds on the ground (Day, et al., 1980) via projectiles attached to the leading net edges (Gratto-Trevor, 2004) **(Figure 2)**. Phutt nets are similar but use compressed air for a firing mechanism and have a lesser range than cannon-nets (Fair, et al., 2010).The net is camouflaged and birds may need to be encouraged to roost in the catching area or baits and decoys may also be used to attract birds to the capture site (Heath & Frederick, 2003; FAO, 2007). Once the net has been fired, hidden personnel rush out to cover the birds, before beginning rapid extraction from the net and placing birds into keeping cages prior to processing. Modifications have included rockets nets set up on floating apparatus to catch in water (Cox Jr & Afton, 1994) and even a mobile cannon-net attached to the front of a jeep (Lacher & Lacher, 1964). Several hundred birds can be caught at once with cannon-nets. This capture method is frequently used for shorebirds, waterbirds and waterfowl (Fair, et al., 2010), as well as some game birds (FAO, 2007). 'Enveloping' type nets refer to those nets that have smaller sized mesh such that the net effectively drapes over the bird. 'Entanglement' nets have larger mesh holes, in which birds can become entangled with their wings, legs or heads (Dabbert & Powell, 1993).

Cannon-netting is a more expensive method of trapping (Heath & Frederick, 2003), including the need for large amounts of equipment and trained personnel. Operators need to ensure there are enough personnel and keeping cages for extracting and processing the birds promptly (Melville, 2011). The use of explosives and projectiles risks injury or death to the birds and human operators should something go wrong (Bub, 1991; FAO, 2007; Melville, 2011). Only very experienced personnel should attempt this method and permits are usually required (Fair, et al., 2010), as well as Animal Ethics Committee approval in New Zealand (Melville, 2011).

Records found in the literature describe traumatic injuries, capture myopathy and deaths directly or indirectly caused by cannon-netting. King et al. (1998) reported one death due to direct hit by a projectile and a broken wing (requiring euthanasia of the bird) in 5 cannon-net capture events of 142 American White Pelicans (*Pelecanus erythrorhynchos*). Mortalities of 0-2.1% and one event of 10.7% mortality was reported for shorebird captures over several years (Jurek, 1974 as cited in FAO, 2007). Cox and Afton (1994) reported a 1% mortality rate for over a thousand waterfowl captured by cannon-net over water, however 11/12 died indirectly from drowning. In a later study by the same authors, 25/347 female northern pintail ducks (*Anas acuta*) died after capture by cannon-net, radio-tagging and release.

Figure 2. A cannot-net is prepared for firing. This involves camouflaging a mesh net behind where the birds are expected to roost. Cannons, like the one shown here, are partially buried in the sand. Projectiles are tied to the leading edge of the net. A

gunpowder firing mechanism fires the leading edge of the net over the group of birds, trapping them beneath. *Photo Janelle Ward.*

In cannon-net captures of ring-billed gulls at nesting, 14.5% of the total captured birds were not seen again in the colony (Southern & Southern, 1983). Although no direct mortalities were observed and the authors concluded the birds had "deserted' the colony, I would be suspicious that post-capture complications or mortality were possible contributors. However, confirmation would only be possible if natural mortality rates were known for the colony. Ponjoan, et al. (2008) concluded that the use of cannon nets increased the chance of mobility problems (thought to be due to capture myopathy) by a factor of 10 times in little bustards (*Tetrax tetrax*). Ten of 23 (43%) affected birds (including birds caught by leg nooses) died over the next 11 days, suspected to be due to the effects of CM and predation. Unfortunately in this study, they did not reveal mortality rates by capture method alone. In another study 20/130 (15.4%) of eastern wild turkey hens (*Meleagris gallopovo silvestris*) were found dead within 14 days of capture with cannon-nets. Birds that died had significantly higher CK levels than those that survived, suggesting muscle damage was a factor in their deaths (Nicholson, et al., 2000). Hayes et al. (2003) summarised reports for captures of sandhill cranes (*Grus canadensis*) by several authors over the period 1972-1991 (Table 4, Page 864). Rocket net captures resulted in crane mortalities of 43/618 (6.9%), 4/41 (10%), 3/34 (9%), 84/828 (10.1%), 216/1456 (14.8%) and 1/186 (0.5%), compared with zero deaths (0/19 and 0/11) by trough blind and walk in traps (respectively). It is surprising that people continued to use cannon-nets in this species with such high death rates reported.

These studies suggest that mortalities due to cannon-netting can be dramatic in some species, whereas in other species mortality is relatively low. It appears that many deaths occur at a later stage following capture and often follow observed mobility problems, which suggests capture myopathy or traumatic injuries are occurring. In most cases of cannon-net captures, released birds are only detected if they are re-caught, have radio-transmitters, or are flag or colour-banded and identified in the field. True mortality figures can only be reached by individually tracking each released bird. Deaths following capture with cannon nets are therefore likely under-reported.

Nooses

Numerous small slip nooses can be made from nylon or monofilament and attached to a mesh matting to form a carpet (Gratto-Trevor, 2004), or to form lines or rows of nooses at perching sites, or used with food bait or decoy live prey (FAO, 2007). While noose mats can be very effective in some situations, they can be very time consuming to construct

(Plumpton & Lutz, 1992; Gratto-Trevor, 2004). When birds walk over the mats, or land on or attack decoys, their legs get entangled in the nooses. This technique can be used for parrots, raptors, wading birds and shorebirds (FAO, 2007). Numerous modifications have been used successfully by researchers for many species, such as houbara bustards (*Chlamydotis undulata macqueeni*) (Seddon, et al., 1999), passerines, raptors and others (Schemnitz, 1996). For instance, New Zealand falcon (*Falco novaeseelandiae*) have been caught by attaching nooses to a hat that the bird attacked when a person entered their territory (Fox, 1977a as cited in Melville, 2011). Bal-chatri traps have a live bird or rodent in a small cage with nooses on the top of the cage to catch attacking raptors and were used to successfully capture burrowing owls (*Athene cunicularia*) with no reported injuries (Plumpton & Lutz, 1992). Use of live decoys requires animal ethics approval (Melville, 2011). Larger nooses can also be used at the end of poles to catch the likes of kea (*Nestor notabilis*) and gannets (*Morus serrator*) (Melville, 2011). Fish- eating raptors can be caught on the water using snare 'vests' attached to fish bait (Hollamby, et al., 2004). Constant supervision of noose traps is recommended (Gratto-Trevor, 2004) as birds may get potentially be caught by their heads and strangled (Melville, 2011) or otherwise injured.

Gratto-Trevor (2004) found no reports of injuries or mortalities due to noose mats and they are considered a safe method for reducing wild bird capture injuries (McGowan & Simons, 2005). Indeed, noose snares were the method of choice for houbara bustards when compared with 7 other methods. No injuries occurred and the authors found the snares to be cost effective with acceptable levels of capture (Seddon, et al., 1999). In contrast, 14% of little bustards (*Tetrax tetrax*) caught by leg nooses developed clinical signs of myopathy. The authors concluded the increased handling time was the more significant factor rather than the leg noose method per se (Ponjoan, et al., 2008). Yet a further study by Marco et al. (2006) in the same species revealed CM and death in 4 of 37 birds caught by leg noose, then handled and fitted with backpack transmitters. This either suggests little bustard are extremely susceptible to complications regardless of capture method, or that leg nooses may not be as innocuous as first suggested.

Walk-in traps

There are many types of walk-in trap, such as funnel traps, cage traps, and corral traps (Bub, 1991). Mostly these traps consist of lead-in fences, narrowing passageways and "one-way" entrances into wire or mesh cages that birds cannot easily escape from (Gratto-Trevor, 2004). The traps can be baited with food to attract birds, and can also be set in water or floating (Melville, 2011). Walk-in traps are useful for waterfowl (FAO, 2007) and for waders at staging sites (Serventy et al., 1962 as cited in Gratto-Trevor, 2004). Swim-in

variations can be used to catch some species of waterfowl (Evrard & Bacon, 1998). Funnel traps are useful for most species of waterfowl (Bub, 1991). Birds may be lured with bait (Fair, et al., 2010) and the narrow funnel entrance protruding into the midst of the cage is usually missed as an escape route by the birds as they pace the perimeter (Day, et al., 1980). Funnel traps can also be used in water (FAO, 2007). Corral, drive and drift traps are used for flocks of birds such as grouse or temporarily flightless (moulting) waterfowl, which can be herded by several personnel into a gradually narrowing entrance to the trap or corral (Day, et al., 1980).

Walk-in traps can generally be operated by less experienced personnel and can be used in more weather conditions than can other methods (Gratto-Trevor, 2004). While less monitoring is required for some walk-in trap variations (Buck & Craft, 1995), Gratto-Trevor (2004) recommend regular monitoring to prevent predator attacks or drownings due to high tides. These traps require a significant amount of materials for construction (Day, et al., 1980).

Walk in traps are considered to have a lower risk of causing injury and have the benefit of catching many birds simultaneously. Buck and Craft (1995) reported no injuries or mortalities in 56 captures of great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) caught by walk-in traps. Fuertes et al (2002) reported one mortality (2.2%) - due to predation - and 3 birds with minor skin abrasions of 45 marsh birds captured in a modified funnel trap system. In a study by Evrard and Bacon (1998), mortality of ducks caught by 4 methods of swim-in traps averaged 4.5% and was not significantly different between 3 different duck species. Of the known causes of death 16/21 (76.2%) were due to predation and the rest due to trap injuries. Kearns et al (1998) reported 1.6% (48/2639 – I calculate it to be 1.8% from the data given) capture mortalities in soras (*Porzana carolina*) and Virginia rails (*Rallus limicola*) using modified funnel traps from 1993-1997, with nearly half due to predation, a third from drownings and the rest to trauma, heat stress and unknown causes. The authors made modifications over the years to predator proof the cages and also suggested greater monitoring and providing shade to reduce losses. As close to 80% of these mortalities were essentially preventable, this proves the point made by Gratto-Trevor (2004) that traps need to be closely monitored. These findings suggest that indirect losses can be as dramatic as net-related capture trauma.

Nest traps

Nest-traps can be set-up around the nests of brooding birds that are off their nests and sprung upon their return, or by trapping them whilst on the nest. Mist-nets can be used to cover over the bird on the nest, or set upright near to the nest prior to flushing the bird towards it (Gratto-Trevor, 2004). Other methods include hand-nets (Gratto-Trevor, 2004), throw-nets, leg nooses and spot-light assisted hand-netting (at night) (Day, et al., 1980). Specialised nest-traps have been designed to sit over or next to a nest and can be passive (no moving parts) or active with some kind of pulley or spring system to close the trap (Gratto-Trevor, 2004).

Huschle (2002) reported 70% success when capturing incubating American bittern (*Botaurus lentiginosus*) females on nests. No injuries were reported. Similarly Blums et al., (2000) reported nil injuries or mortalities for 165 captures of 33 ducks using a multi-capture nest-box trap with a swinging false floor. Loos and Rohwer (2002) reported injuries to 26% of blue-winged teal (*Anas discors*) captured on nests with traps, compared to only 2% ($P<0.001$) when using hand nets (on nests). Two of the nest-trap injuries caused mortality and other injuries consisted of bill and carpal lacerations and feather loss. Disturbance at nesting sites may cause parents to abandon nests for short periods or permanently, depending on the method of capture and incubation stage (Colwell, et al., 1988; Loos & Rohwer, 2002). The other major concern is egg or chick mortality. Egg damage was reported by Loos and Rohwer (2002) and significantly more damage was found with nest-traps compared with hand nets. Southern & Southern (1983) concluded that nest damage and egg breakage was “virtually unavoidable” when capturing nesting gulls by rocket net.

ClapTraps (Bow net trap) & Drop-nets

Clap traps and many variations have been utilised for some shorebird species (Gratto-Trevor, 2004), small passerines, up to large birds such as eagles (Melville, 2011) and bustards (Seddon, et al., 1999). The traps utilise tension strings, pivot hinges and pull ropes or trigger systems to release a set net over birds that come within range of the net. Decoys, bait or live lures (Melville, 2011) can be used to bring birds within range of the net. A modified version called a Q-net, using a live adult decoy, has been used to capture adult Audubon's crested caracara (*Caracara plancus audubonii*) (Morrison & McGehee, 1996); no injuries were reported. One houbara bustard was caught out of 3 attempts and no injuries were sustained, however the authors found them to be labour intensive (Seddon, et al., 1999).

Drop traps are either suspended nets or walk-in traps with hinges or doors that swing shut when the trap is activated by a bird walking in or landing in the trap (Day, et al., 1980) or by a person who closes the trap via a release cord (Melville, 2011). Although there are few reports of injuries due to these traps, there is risk of injury when the nets close suddenly (Seddon, et al., 1999).

Hand nets

Hand or pole nets are useful for individual birds that are injured, ground nesting birds such as pheasant and grouse (Day, et al., 1980), to catch waterbirds from a boat at night using spot-lights (FAO, 2007) and for some flightless birds such as the takahe (*Porphyrio hochstetteri*)(pers. obs.). The frame and net size may vary according to the target species, however the nets should be lightweight with a long handle (Gratto-Trevor, 2004). Lures or baits may be needed, or spotlights can also be used to dazzle birds at night (Day, et al., 1980; Huschle, et al., 2002).While a cheap, simple and portable method that is useful for many bird species, only individual birds can be caught at a time and sufficient operator skill is required (Gratto-Trevor, 2004). Birds may also quickly learn to avoid this form of capture (Huschle, et al., 2002).

Hofle et al (2004) reported deaths due to self-injury and CM following capture and tagging of red-legged partridge (*Alectoris rufa*) following hand-net capture. As all of the birds were radio-tagged, differentiation between the capture event itself and radio-tagging could not be determined. The birds were spotlighted in the dark, and capture events lasted an average of 4+/-2.0 minutes, compared with handling times of 20+/- 7 mins. Additionally, all of the deceased birds had self-inflicted wounds due to struggling with the transmitter. While the authors concluded that other trap methods such as bait-traps would be less harmful (Höfle, et al., 2004), I believe the capture method per se would have been less harmful than the handling and radio-necklace placement.

Other methods

There are many variations of nets and trapping methods that have been devised to target specific species over the years and can be found in grey and peer-reviewed literature (e.g. Day, et al., 1980). Some more extreme methods of capture have been attempted e.g. Seddon et al. (1999) found an unacceptable level of mortality of 38-86% for capture of Houbara bustards using falcons. Even using falcons with talons and beaks disarmed had mortality rates of 13%. Huschle et al. (2002), found one third of captured bitterns had to be euthanized due to wing fractures following net gun capture. Helicopters are increasingly being used to capture difficult species such as cranes (Ellis, et al., 1998).

These authors found 1/6 cranes developed signs of overheating and was thought to have possibly died post-capture though neither it's body nor transmitter were found. These authors reported the bird ran post-capture but would not fly, so capture myopathy was a possibility in my opinion. Net guns have been used to capture golden eagles (*Aquila chrysaetos*) chased by helicopters with 9/19 successful captures and no injuries reported (O'Gara & Getz, 1986).

1.4.3 Capture complications

The capture of wild animals is an inherently risky and often complex practice; the continual refinement of capture methods is important to minimise harm to the animals. Complications can be as simple as a failure to catch the target animals, to more serious consequences such as multiple mortalities (Basson & Hofmeyr, 1973). As described in previous sections of this discourse, injuries and mortalities due to capture are not uncommon and secondary complications such as predation and capture myopathy may result in mortality for some birds. The following is a breakdown and summary of some of the more common capture complications reported in the literature.

Mortalities

Common causes of death include severe trauma, shock, hyperthermia or hypothermia, respiratory failure or cardiac arrest. However, in many cases the cause of death may be undetermined and often the cause of death can be multi-factorial (Basson & Hofmeyr, 1973). Additionally, post-mortem examination may not reveal the underlying causes of death if the carcase is predated or scavenged (Cox Jr. & Afton, 1998). Secondary effects of capture, such as capture myopathy and injuries subsequent to radio transmitter placement, have also resulted in mortalities of wild caught birds (Höfle, et al., 2004; Marco, et al., 2006). Predation of birds whilst confined in unmonitored traps can be a concern, especially with certain methods such as cage traps (Evrard & Bacon, 1998). Predation of birds following release, secondary to observed mobility problems (likely capture myopathy) has also been reported (Cox Jr. & Afton, 1998; Ponjoan, et al., 2008).

Mortality data in birds –reviewed in the preceding section "Capture methods"- ranged from 0% right up to 86% for various capture methods. Direct harm from the nets during capture, and trauma suffered by birds trying to escape the net/trap appeared to be the most common causes of mortality. Drownings (e.g. Kearns, et al., 1998) were a more common cause of death in water-based capture operations. In the study by Spotswood et al. (2012), mortalities of mist-net caught birds were predominantly due to stress, predation or unknown causes, with internal trauma and strangulation the next most common.

In some cases, mortality could be prevented with closer monitoring and predator proofing, and several studies have showed reduced rates of mortality once the capture method was refined. For example Evrard and Bacon (1998) had a reduction in mortality from 14.3% to 4.7% for duck captures once they started trapping raccoons at the site. Spraker (1993) suggested that a 2% mortality rate (or greater) due to capture of wildlife was

unacceptable, calling for refinement of the capture techniques involved. However, this guideline will be dependent on species e.g. a 2% loss would be unacceptably high in endangered species programmes. The natural mortality rate of the population should be taken into account when considering the mortality rates due to capture. Unfortunately this is difficult to calculate, as natural mortality rates cannot usually be ascertained in unmarked populations (FAO, 2007).

Non-fatal injuries

"…captured waders occasionally suffer from wing strain, or wing droop and cannot fly when they are released. Provided they have no obvious injuries such birds should be left in peace on undisturbed coast where they can feed. They will probably recover…" (Green, 1978: p.26)

Birds that cannot fly could have any number of problems relating to the capture and handling event that may not be "obvious" externally. Pectoral muscle trauma or capture myopathy, nerve damage, undiagnosed broken bones or coracoid fractures are a few possibilities. No injured bird should be taken lightly and expected to recover on its own and we have little data to know whether released birds with any physical signs are capable of recovering. For example, an anecdotal report by Purchase and Minton (1982) described 25 bar-tailed godwits that developed signs of paresis after cannon-net capture and banding but were released. Six newly banded birds were subsequently found in various states of incapacitation (including one dead) over the next 25 days around the bay and although it was unknown if they were the same birds, it seemed probable. It is likely that others were also dead or incapacitated but not found.

Reported injuries from net or trap-based captures range from mild skin abrasions (Fuertes, et al., 2002), feather loss, bill injuries, carpal injuries (Loos & Rohwer, 2002) to more significant injuries such as wing fractures, which may require euthanasia of the bird (e.g. King, et al., 1998). A review of over 600,000 mist-net captures, by 22 organisations, revealed average injury rates of 0.59%. When the data was categorised, the most common injuries were wing trauma, stress (how they classified the 'injury' as stress was not elucidated), cuts and broken bones (in decreasing order) (Spotswood, et al., 2012). Interestingly, the authors found a significant correlation between size of the bird and types of injuries – heavier birds were more prone to predation, leg trauma, internal bleeding and lacerations, while smaller birds were more prone to stress, tangling injuries and wing strain. It is of interest in this report that only 22 out of 70 organisations that were contacted were able to provide tallies of captures, injuries and mortalities. Of those 22 organisations, only 5 (7.1%) were able to provide individual incident reports for

injuries and mortalities, which is where the breakdown data originated. An additional 11/70 organisations could not provide data as they did not record incidents, and 10 groups did record incidents but did not wish to share the data or couldn't due to difficulty in accessing the data. It is presumed that the other 27 organisations did not reply to the author. Similarly to the US situation, no formal reporting is required in New Zealand for injuries or mortalities sustained from capture operations (G. Taylor, pers. comm.). It is therefore likely that injuries and deaths are significantly under-reported.

Capture Myopathy

Capture myopathy (CM) is known to be one of the greatest threats to a wild animal following capture, handling and/or transportation (Basson & Hofmeyr, 1973; Williams & Thorne, 1996). While some authors postulate that any species could develop myopathy (Williams & Thorne, 1996), certain species and groups of birds appear more commonly in the literature. As more researchers look for the disease, it is likely that CM will be found in more species and families of birds. For instance at the time of writing this discourse, the first report of CM in woodpeckers (family *Picidae*) was recorded (Ruder, et al., 2012). The following briefly reviews the known literature on the main species affected at the time of writing. Details of the known pathophysiology, clinical syndromes, risk factors, prevention and treatment of capture myopathy will be discussed in later sections of this review.

Cranes & Flamingos

One of the earliest reports of myopathy was in flamingos, with leg paralysis after capture and transportation (Young, 1967). One of ten greater sandhill cranes (*Grus canadensis tabida*) captured by rocket net and radio-tagged died 5 days after release, with a diagnosis of CM on necropsy (Windingstad, et al., 1983). Four of 12 (33%) African crowned cranes (*Balearica regulorum*) died within 10 days following prolonged restraint of up to 6 hours (Brannian et al., 1981, as cited in Bollinger, et al., 1989). Three of 56 (5.4%) greater sandhill cranes developed paresis and severely elevated muscle emzymes after α-chloralose baiting and physical capture. All three were treated and survived to release (Businga, et al., 2007). Three whooping cranes (*Grus americana)* were diagnosed with CM on necropsy – two had signs of paresis following hand-capture, while the other had sustained injuries following collision with a light aircraft. All three birds died despite rehabilitation attempts (Hanley, et al., 2005). Finally, 7/166 sandhill cranes captured with alpha-chloralose baiting developed clinical signs of CM – 3 died in the field while 4 were successfully rehabilitated and released (Hayes, et al., 2003).

Quail & Partridge

Ninety four northern bobwhite quail (*Colinus virginianus*) that were trapped and handled showed muscle enzyme elevations. The survival rate of released birds was as low as 15% which was thought to be due to CM, although no post-mortems were conducted (Abbott, et al., 2005). Fourteen of 46 (30.4%) juvenile red-legged partridge (*Alectoris rufa*) captured with hand nets and radio-tagged were found dead within a few hours of capture; all of the deaths were diagnosed as CM, self-injury or both (Höfle, et al., 2004).

Waterfowl

In a study of muscle enzyme elevations in mallards (*Anas platyrhynchos*), 13/77 (17%) of the birds were found to have weak flight following release, thought likely related to muscle injury. However, a clear correlation to muscle enzyme elevations was not found (Bollinger, et al., 1989). Twenty five of 347 (7.2%) female pintail ducks died within 10 days of cannon-net capture. The mortalities were significantly linked to mobility problems observed at release, but there was no proof of CM - largely due to scavenging of the carcases (Cox Jr. & Afton, 1998).

Bustards

Ponjoan et al. (2008) found 23/151 (15.2%) little bustards showed impaired mobility following capture by 3 methods, with cannon-nets causing greater problems than leg nooses or funnel traps. Following release, nearly half (10/23) of the affected birds died within 11 days, while the others appeared to recover. CM was diagnosed in 4 of the carcases (Marco, et al., 2006; Ponjoan, et al., 2008). A retrospective study of post-mortem databases for the period 1979-1994 revealed capture myopathy as the cause of death (or reason for euthanasia) in 29.1% (according to the numbers it should be 14/51 = 27.4%) of adult captive houbara bustards (*Chlamydotis undulate macqueenii*), 5/8 (62.5%) of adult captive kori bustards (*Ardeotis kori*) and 1/10 (10%) of juvenile kori bustards (Bailey, et al., 1996).

Ratites

While not directly capture related, a captive emu (*Dromaius novaehollandiae*) developed CM subsequent to being entangled in a fence, diagnosed on necropsy following unsuccessful rehabilitation (Tully, et al., 1996). A captive rhea (*Rhea americana*) developed severe progressive limb paresis after being chased by the male in its enclosure. This bird was successfully rehabilitated (Smith, et al., 2005).

Turkeys

Elevated muscle enzymes were a significant risk factor for death of 20 wild turkey hens following cannon-net captures (Nicholson, et al., 2000), though post-mortem examinations were not conducted. This is corroborated by an earlier study where post-mortem analyses of wild turkeys euthanized following capture, handling and transportation revealed gross lesions of myopathy in 13/27 (48%) birds and microscopic muscle damage in the majority (89%) of the birds, even though clinical signs were not observed (Spraker, et al., 1987). The findings of this study suggest that subclinical CM is likely to be occurring in more cases than previously recognised.

Shorebirds

Cannon-net captures of 4 shorebird species (red knots *Calidris canutus*, great knots *C. tenuirostris*, bar-tailed godwit *Limosa lapponica* and red-necked stint *C.ruficollis*) resulted in paresis of 27 birds, of which 21 were successfully rehabilitated and released (Rogers, et al., 2004). One of 343 (0.3%) semipalmated sandpiper (*Calidris pusilla*) and 2/131 (1.5%) red-necked phalarope (*Phalaropus lobatus*) were 'flightless' up to 30 days after capture (Colwell, et al., 1988). While likely to be CM but this was not investigated further and the authors did not state which capture method (of four used) resulted in the handicapped birds. Two bar-tailed godwits were treated for suspected CM in Australia and New Zealand, but died despite treatment. The Australian bird had been captured by cannon-net (Mulcahy, et al., 2011) and the New Zealand bird by mist-net (B. Gartrell, pers. Comm.). A cannon-net capture operation of curlews (*Numenius sp.*) observed approximately 20% to have paralysis and difficulty flying at release (Stanyard, 1979); Other species reportedly affected by myopathy include bar-tailed godwits (Purchase & Minton, 1982), whimbrels (*Numenius phaeopus*) (Green, 1978), redshanks (*Tringa tetanus*), red-necked Avocet (*Recurvirostra novaehollandiae*) (Minton, 1993) and oystercatchers (*Hamatopus ostralegus*) (Piersma, et al., 1991).

Capture stress

Dickens et al. (2010) suggest that a capture event may be the most distressing event in a wild animal's life, presuming that the animal associates capture with predation. Stress can be a term that is difficult to define, due to individual variation in response to stressors, the many physiological responses that may be involved, as well as relating these responses to the welfare of the animal (Moberg, 1987 as cited in Spraker, 1993). Captured wildlife may be fearful, excited, overheated, and/ or exhausted from the chase or capture. Awareness of danger, unfamiliar environments (Spraker, 1993), captivity, transport and handling are all further stressors to a wild animal (Dickens, et al., 2010). Stress responses can be

divided into acute or chronic stress (Dickens, et al., 2010). Some authors divide stress into categories that are beneficial (eustress); neutral; or detrimental (distress) for the animal (Breazile, 1987).

Regardless of category, all stress responses involve the initial "fight-or-flight" response, where the sympathetic nervous system releases adrenaline and nor-adrenaline, followed by the slower, hormonal (glucocorticoid) responses, mediated via the HPA (hypothalamic – pituitary – adrenal) pathway (Sapolsky, et al., 2000). Chronic stress can develop with persistent elevation of glucocorticoids, leading to debilitating consequences such as protein loss, disease susceptibility due to immunosuppression, neuronal cell death and reproductive failure (Wingfield, 1994 as cited in Wingfield, et al., 1998). Many studies have shown that birds (e.g. Lynn & Porter, 2008) and mammals (e.g. Romero, et al., 2008) mount a significant glucocorticoid response following capture and handling. Wingfield et al. (1998) call this response the "emergency life history response" and suggest that it varies between individuals and species. Species can use this response to decide on whether a stressor is a short-term event that once over can be quickly ignored, or of major consequence that requires a diversion of the animal's annual life history cycle. For example, some species are sufficiently responsive to a stress event such as capture that they will delay reproduction or abandon nests (Wingfield, 1988 as cited in Wingfield, et al., 1998). This emergency life history response (ELHR) allows an animal to divert energy from less necessary life stages, such as territorial aggression and mating, to life-saving measures such as seeking shelter and altering energy production. These temporary measures resolve once the perturbation passes, but allow the animal to avoid the longer term effects of chronic stress (Wingfield, et al., 1998).

Therefore, in wild animal capture we can expect all levels of stress, which will vary in severity between individuals and species. For those that are captured and quickly released, the immediate adrenaline release of the flight-or-fright response is induced, as well as the ELHR for the duration of the capture event and associated environmental disturbance. If the capture also involves transportation and release to a new site, or prolonged captivity then we would expect the ELHR to now start diverting the animal's resources and behaviours for days to weeks (Wingfield, et al., 1998). The effects of chronic stress may be seen in a debilitated animal – such as one suffering capture myopathy, a permanent injury, or prolonged captivity. At this time more debilitating effects of stress may be seen, such as muscle wasting and immunosuppression, which are often incompatible with life for a wild animal (Wingfield, et al., 1998).

Weight loss

Weight loss has been reported in several studies of wild caught mammals (e.g. Cattet, Boulanger, et al., 2008) and birds. Purchase and Minton (1982) found that nine bar-tailed godwits that were recaptured a day apart had all lost between 3.8% to 16.9% bodyweight, a loss that could only be attributed to the previous capture event. Similarly, Barter and Minton (1998) reported an average weight loss of 5% of capture weight in 66% (102/154) of several shorebird species (including red and great knots and bar-tailed godwits) recaptured within 10 days of the first capture event. Weight loss was still evident in 48% (44/92) of those re-caught 11-20 days after the initial event. Cattet et al. (2008) conducted an interesting study and found that both grizzly bears (*Ursus arctos*) and American black bears (*U. americanus)* lost significantly more body condition with repeated captures as opposed to those bears caught only once. A direct cause for the weight loss may be difficult to establish, but loss of body weight has been attributed to reduced food intake in stress responses subsequent to singular or repeated stressors (in rats) (Harris, et al., 1998) and increased metabolic requirements of the body subsequent to trauma (Newsome, et al., 1973), contributing to the assumption that stress, reduced food intake or increased metabolic requirements due to injury are related to the observed loss of condition (Cattet, Boulanger, et al., 2008).

Battley et al. (2004) discuss how body condition is essential to the health of migratory birds. Poor body condition can cause knock-on effects, such as delayed reproduction (Descamps, et al., 2011), altered migration patterns (Schmaljohann & Naef-Daenzer, 2011) and reduced survival (Harding, et al., 2011). Poor body condition has also been shown to reduce reproductive success– for instance Wilson's storm petrels (*Oceanites oceanicus*) in poor body condition had reduced egg numbers and later brooding than birds in good body condition (Quillfeldt, et al., 2006). Schmaljohann (2011) found that songbirds in poorer body condition departed significantly later and in differing directions to those birds in good body condition. On the other hand, shorebirds are able to gain body mass at fast rates (Lindstrom, et al., 2000), meaning that depletion of body mass due to capture should be surmounted relatively quickly in an otherwise healthy shorebird.

These studies suggest that loss of body condition due to capture could have ongoing deleterious effects on migratory birds. The severity of these effects would likely depend on the species, life history stage and proximity of capture date to migration departure.

Breeding success

In a study of nesting ring-billed gulls (*Larus delawarensis*) captured by cannon-net, the majority (92.7%) of re-sighted birds continued to breed. However, a significantly lower brood size was found when compared to uncaptured birds in the same colony (Southern & Southern, 1983). Clutch desertions were significantly greater in male Wilson's Phaloropes (*Phalarobus tricolor*) which were captured during the laying period over the incubation period, suggesting that the timing of capture in nesting birds could be important in some species (Colwell, et al., 1988). For birds that annually migrate, sub-lethal injuries, myopathy or weight loss may preclude their migration for that season, leading to failure to breed, or delay the migration (Moller, 1994), causing later arrival in the breeding grounds which may lead to poorer reproductive success (Moller, 1994; Drent, et al., 2003). Legagneux et al. (2012) conducted an interesting study in greater snow geese (*Anser caerulescens atlanticus*), capturing them by cannon net and then either releasing immediately after banding or 2, 3 or 4 days later. They found the bird's reproductive success to be negatively affected by 45-71% up to 2 years later and this was significantly correlated to length of time in captivity. This study implies that capture stress is of less concern than the duration of the stressful event. More studies of this nature could reveal the true relevance of capture stress on the health of birds.

Confounding science results

If complications of capture cause injuries or mortality, then follow up data will have been influenced by the capture event itself. Many authors agree that capture myopathy has the potential to bias data, especially post-release data regarding mortality, movement or long-term survival (Bollinger, et al., 1989; Williams & Thorne, 1996; Abbott, et al., 2005). Many studies try to counter for these effects by having a 'censor' period where deaths, altered behaviour or movements, are not included in the dataset for up to two weeks (White and Garrott, 1990, as cited in Abbott, et al., 2005; Quinn, et al., 2012). Yet this may not account for more insidious effects that cause mortality at a later stage and current censoring periods may not be adequate (Abbott, et al., 2005). For example, Mueller et al. (1998) found that survival of bobwhite quail 16 weeks post-release was negatively correlated with muscle enzyme levels measured at capture. In addition, Cattet et al. (2008) found that the greater number of times a bear was captured, the poorer it's body condition was, suggesting that capture events were additive in terms of detrimental effects. So what does this mean for migratory birds? We know that migratory birds can potentially be captured at any stopover point en-route. Capture events occur regularly in New Zealand, Australia, Korea, China and Alaska and recaptures appear common. For instance, recapture rates of

5/24 (20.8%), 33/151 (21.9%) and 23/61 (37.7%) of bar-tailed godwits were recorded from 3 days of cannon-net captures in Australia (Hassell, 2009). It is unknown whether any researcher has evaluated the number of re-captures of a single shorebird, nor the effect of re-captures on physiological states of birds. Potentially, captures and re-captures could have detrimental long-term effects on shorebirds that could be influencing or confounding the results of survival or reproductive research, but further studies are needed in this area.

1.5 CAPTURE MYOPATHY

Capture myopathy has been known by a wide variety of names historically, including "overstraining disease", "exertional rhabdomyolysis" "polymyopathy", "leg paralysis", "spastic paresis", "transport myopathy", "muscle necrosis", "muscular dystrophy", "white muscle disease" (Williams & Thorne, 1996); "capture disease", "cramp", "incipient myopathy" and "degenerative polymyopathy" (Paterson, 2007). Rhabdomyolysis is the term utilised in human medicine for a wide range of acquired or inherited diseases that can induce skeletal muscle breakdown (Warren, et al., 2002; Elsayad & Reilly, 2010). The most commonly used terms in wildlife medicine are "capture myopathy" or "exertional myopathy" (Williams & Thorne, 1996; Paterson, 2007).

1.5.1 Overview

Capture myopathy is a non-infectious, metabolic syndrome seen as a complication of capture and handling in many species of mammals and birds, which can lead to significant morbidity and mortality. Capture myopathy was first observed and the pathology described by Jarrett, et al. (1964, as cited in Paterson, 2007) in Hunter's hartebeest (*Damaliscus hunter)*. Since then, there have been many reports of the condition affecting a wide range of species and most vertebrate groups, other than reptiles (Paterson, 2007). The stress, struggling and extreme exertion due to pursuit and capture is thought to create a physiological cascade with cardiac and skeletal muscle necrosis, hyperthermia, metabolic acidosis, hyperkalemia and other systemic effects. The disease commonly causes myoglobinuria and acute renal failure in humans (Warren, et al., 2002) and mammals (Spraker, 1993). The occurrence of acute renal failure in birds has not been elucidated in the literature and invites further investigation. Muscular stiffness and necrosis leads to ataxia, weakness, and partial or complete paralysis. Renal failure, circulatory collapse and death may occur in severe cases (Williams & Thorne, 1996). Some animals appear to recover, only to die suddenly days or weeks later following a further stressful or exertive event (Spraker, 1993). Sub-clinical cases of capture myopathy (CM) may go completely undetected (Spraker, et al., 1987) and the short- and long-term survival of these animals is unknown. Leakage of cellular enzymes from damaged muscle tissue into the blood stream causes serum analytes to become elevated, particularly creatine kinase (CK) and aspartate aminotransferase (AST), which are thus used as indicators of muscle damage related to capture (Bollinger, et al., 1989; Dabbert & Powell, 1993). Thus, CM covers a spectrum of muscle damage from mild sub-clinical myodegeneration to severe myonecrosis and catastrophic systemic disorders.

1.5.2 Species affected

Mammals

Initial reports of deaths or morbidity due to capture myopathy commonly cited African game species such as cape buffalo *Synercus caffer*, tsessebe *Damaliscus lunatus,* eland *Tarotragus oryx*, giraffe *Giraffa camelopardalis,* impala *Aepyceros melampus,* zebra e.g. *Equus burchelli* and elephant *Loxodonta africana* (Basson & Hofmeyr, 1973). CM has been reported in cervids such as white-tailed deer *Odocoileus virginianus* (Beringer, et al., 1996) and roe deer *Capreolus capreolus* (Montane, et al., 2002), wild sheep (Kock, Clark, et al., 1987); primates (McConnell et al., 1974 as cited in Chalmers & Barrett, 1982), macropods (Shepherd, et al., 1988), ursids such as grizzly bear *Ursus arctos* (Cattet, Stenhouse, et al., 2008) and large ungulates such as moose (Haigh, et al., 1977). CM has also been found in dolphins (Herraez, et al., 2007), otters (Hartup, et al., 1999), bats (Jung, et al., 2002) and has even been suspected in poikilotherms such as farmed atlantic salmon (Rodger, et al., 1991) and toads (Williams & Thorne, 1996). It should be considered that any vertebrate animal could potentially be affected by CM under certain conditions of stress and exertion (Williams & Thorne, 1996).

Birds

The first description of capture myopathy in birds was described by Young (1967) in greater flamingoes (*Phoenicopterus ruberroseus*) and lesser flamingoes (*Phoeniconaias minor).* While initially thought to be uncommon in birds (Spraker, et al., 1987), increasingly more wild and captive bird species have been reported to be susceptible to CM. Long-legged species appear to be over-represented in the literature. As described in a previous section of this discourse, species affected include ratites (Tully, et al., 1996); turkeys (Spraker, et al., 1987); bustards (Bailey, et al., 1996), waterfowl (Bollinger, et al., 1989) and cranes (Businga, et al., 2007). Shorebirds reported to have been affected with CM include: godwits (*Limosa spp.)* and knots (*Calidris spp.)* (Rogers, et al., 2004); curlews (*Numenius spp.*) and redshanks (*Tringa spp.*)(Green, 1978).

1.5.3 Aetiology

The aetiologial factors driving the onset of muscle breakdown are incompletely understood, perhaps reflecting a complex interplay of external and internal factors. It is not clear why some birds succumb to CM while others are completely fine, under the same conditions, under the same net, at the same time. Spraker (1993) theorised that CM may be an inherent mechanism in the predator-prey relationship, the premise being that prey have a relatively quick death following a chase, while the predator conserves energy.

Nevertheless, several recent studies have shown that predators can also develop CM (e.g. Cattet, Stenhouse, et al., 2008), somewhat refuting the theory that evolution favoured the physiology of a carnivore over a herbivore. Many authors believe that CM is an iatrogenic disease that is not normally seen under normal circumstances in the wild, and that it is related to an unnatural degree of stress and exertion (Williams & Thorne, 1996). This statement is backed up by the lack of literature on 'naturally' occurring myopathy in the wild, with non-capture related reports mainly referring to birds or mammals that become entangled in some other way (Tully, et al., 1996). Perhaps it is simply because this is not looked for in most wild animal studies. Chalmers and Barrett (1982) also suggested that fear and anxiety are triggering mechanisms for CM on top of genetic or acquired predispositions to the disease, further exacerbated by factors such as overexertion, transportation, handling and shock.

In human medicine, many acquired and inherited aetiologies of rhabdomyolysis have been defined. These include crush (re-perfusion) injuries, trauma, hyperthermic syndromes (including exertional heat stroke and malignant hyperthermia), metabolic disturbances, ischaemia, drugs and toxins, infectious diseases (e.g. Influenza A and B; *Streptococci* infections), autoimmune diseases and a range of inherited protein and enzyme deficiencies (Warren, et al., 2002). Interestingly, exertion-related myopathies are the most common cause of rhabdomyolysis in humans and are often triggered by excessive exercise in hot or humid conditions, particularly in untrained persons (Zager, 1996). A study by Newham et al. (1986) showed that eccentric muscle contractions cause a greater degree of muscle injury than concentric contractions in people. Further studies are needed of this type in birds and mammals, but presumably this would be the case in any vertebrate.

It is possible that some of the above aetiological factors could be creating or exacerbating myopathies in birds, such as traumatic injuries, re-perfusion injuries and hyperthermia. Tully et al. (1996) discussed the possibility of trauma related muscle lesions in birds caused by an initiating event (e.g. capture), rather than CM per se. Ischaemic necrosis of muscle due to: vascular damage (Tully, et al., 1996); 'compartmental syndrome' – where the muscle swells after excessive contraction; or 'crush syndrome' –due to trauma and oedema of the muscle following injury (Warren, et al., 2002), could possibly explain some of the lesions observed in birds subsequent to capture and handling. The possibility of underlying disease, such as bacterial or viral infection or environmental toxins (such as heavy metals), has not been fully investigated in birds. Windingstad et al. (1983)reported negative findings on cultures for bacterial and viral pathogens in a crane diagnosed with CM on necropsy. The presence of avian pox was not statistically linked to deaths due to CM

in partridges examined by Höfle et al. (2004), although with a significance level of *P*=0.08 perhaps would be worth investigating further. Concurrent coccidosis in one whooping crane and concurrent aspergillosis in two cases were considered additional, rather than contributory to CM (Hanley, et al., 2005).

1.5.4 Clinical Signs

Clinical signs of CM will vary according to the severity of the disease and any secondary effects. Common to all cases of muscle damage, indicators include muscle stiffness, pain and reduced muscle function (Guis, et al., 2005). In mammals the most common clinical presentations of capture myopathy include muscle stiffness, severe muscle pain, ataxia, paresis, torticollis, prostration and paralysis (Williams & Thorne, 1996; Paterson, 2007). In severe cases mammals typically become hyperthermic (Williams & Thorne, 1996), anorexic and unresponsive (Paterson, 2007). In wild birds, the most common reported findings are paresis, ataxia, inability to fly, anorexia and death (Rogers, et al., 2004; Smith, et al., 2005; Marco, et al., 2006; Businga, et al., 2007). In human medicine, clinical signs of rhabdomyolysis include muscle swelling, muscle fatigue and weakness, pain, cramping and reddish-brown discoloured urine due to the filtration of myoglobin by the kidneys (Guis, et al., 2005).

Clinical signs of myopathy appear to have an ill-defined timing of onset in birds. Records in the literature report immediate signs of myopathy and paresis after chase and capture in mammals (Basson & Hofmeyr, 1973) but appear to be rarely recorded in birds. Often CM is discovered only after birds are found dead, usually subsequent to capture and monitoring projects (Windingstad, et al., 1983; Nicholson, et al., 2000; Höfle, et al., 2004). Some reports suggest onset of signs can occur within the first few hours of capture. For instance: a male whooping crane developed signs of CM immediately following a handling period of 30 minutes (Hanley, et al., 2005); two red-legged partridge developed ataxia and flightlessness approx. 55 minutes after capture (Höfle, et al., 2004); several great knots were unable to walk or fly within hours of cannon-net capture (Rogers, et al., 2004); three little bustards showed difficulty in walking or flying at release following capture by leg nooses and a total restraint time of 25-55 minutes - these birds were found dead 5-8 days later (Marco, et al., 2006).

Other reports show clinical signs did not develop until hours to days after capture: Wobeser (1997) reported geese caught with rocket nets did not show any stiffness or inability to fly until 18 hours after capture; Businga et al. (2007) reported 3 of 56 cranes showed inability to stand 24 hours after capture and sedation. From the report it can be

presumed that the birds were left unattended overnight, therefore signs may have developed sooner but not observed. Smith et al. (2005) described CM occurrence in a rhea that developed severe leg lameness in 24 hours which progressed rapidly to an inability to stand within 48 hours.

Death due to the effects of CM can occur within hours, days or weeks after the capture event (Spraker, 1993). Acute renal failure due to precipitation of myoglobin within the kidney tissue has been documented in humans (Clarkson, 2007) and mammals (Herraez, et al., 2007). Other causes of death include severe shock, metabolic acidosis, electrolyte disturbances and/or cardiac failure (Spraker, 1993). In birds, death is commonly attributed to predation, but it is often unknown as to whether the bird was alive and predated or simply scavenged after death (Cox Jr. & Afton, 1998; Marco, et al., 2006). Mortality directly resulting from chronic capture myopathy has been reported in birds, but mainly in those birds under human care in rehabilitation settings (e.g. Carpenter, et al., 1991). In some cases of rehabilitation, euthanasia was elected due to an extremely poor prognosis (Tully, et al., 1996). In some projects, birds that were radio-monitored after release showed reduced movement prior to death. The investigators rapidly retrieved the carcases and reported the direct and indirect effects of CM to be the likely cause of death (Windingstad, et al., 1983). Some authors suggest that renal failure also occurs in birds (Wobeser, 1997) potentially due to myoglobin damage (Windingstad, et al., 1983). Myoglobin damage to kidneys and death due to renal failure are not well documented in birds and require further investigation.

Presumptively, animals with severe clinical signs are more likely to die than those with mild signs. However, correlation between severity of clinical signs and mortality has not been well documented and several reports have conflicting evidence. For instance, Rogers et al. (2004) described rapid recovery in some of the shorebirds that they were treating for CM. These birds went from sternal recumbency to standing and walking 'suddenly' after several days of no apparent improvement. On the other hand, mild signs of CM in birds observed at capture have been associated with high mortality following release (Höfle, et al., 2004).

1.5.5 Clinical syndromes

Several authors describe clinical 'syndromes' that attempt to categorise the range of clinical signs and disease progressions observed since myopathy was first described. Spraker (1993) described four syndromes: acute death syndrome, ataxic myoglobinuric syndrome, ruptured muscle syndrome and peracute death syndrome. These syndromes may be related to the pathophysiology that occurs during the muscle breakdown and the severity of metabolic derangements occurring within the body, described in more detail below. Potentially these syndromes could have correlates in birds. For example Hanley et al. (2005) classified 3 whooping cranes with CM as capture-shock syndrome, ruptured muscle syndrome and a combination of the latter and ataxic myoglobinuric syndrome. However, true correlation of these syndromes in birds has yet to be confirmed. Once more, it is likely that the actual CM process is a continuum and animals may present with a degree of overlap of the described syndromes (Paterson, 2007).

Capture shock syndrome (Acute Death Syndrome)

Soon after capture or even during the chase, the animal shows clinical signs including rapid breathing, increased heart rate, hyperthermia, depression, weak pulses and may die suddenly. Serum biochemistries will usually show elevations in creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Gross post-mortem often reveals congestion of the small intestine, liver and lungs and the intestinal contents may be bloody. Histological findings include small areas of necrosis & scattered micro-thrombi in various organs including skeletal and cardiac muscle, brain, liver, adrenal glands, lymph nodes, spleen, pancreas and renal tubules (Spraker, 1993). At the time of writing there were no published reports with clinical correlates to this syndrome in birds.

Ataxic myoglobinuric syndrome

This is the most commonly seen presentation in mammals. Clinical signs develop within hours to days post-capture and may be mild with subsequent recovery, to severe with consequent mortality. The animal may be ataxic, have reddish brown urine (myoglobinuria) and torticollis. Blood serum biochemistries show elevations in CK, AST, LDH and blood urea nitrogen (BUN). Post-mortem examination will normally reveal swollen, dark kidneys and myoglobinuria. Grossly, the skeletal muscles have scattered areas of pale, dry, necrotic tissue and splits may be present. Skeletal histological lesions consist of myocellular necrosis. Renal tubular lesions consistent with myoglobin damage are evident histologically (Spraker, 1993).

The ataxia, biochemical alterations and post-mortem findings described above are consistent with several reports of birds afflicted with CM (Windingstad, et al., 1983; Spraker, et al., 1987; Tully, et al., 1996; Hanley, et al., 2005; Businga, et al., 2007), other than myoglobinuria. One report of a post-mortem in an Emu that had severe CM showed pathological changes in the kidneys (Tully, et al., 1996), as did one whooping crane (Hanley, et al., 2005) and a greater sandhill crane, though the latter was likely due to dehydration seeing as urate crystal deposits were found (Windingstad, et al., 1983). Only the case report by Hanley (2005) showed a suggestion of renal cytoplasmic golden granules that could have been due to myoglobin deposition.

Ruptured muscle syndrome

This syndrome is characterised in mammals by marked dropping of the hind limbs or hock hyper-flexion and usually occurs within one to two days of capture. Extreme elevations of CK, AST and LDH are measured, with a normal to elevated BUN. The animal may die within days to weeks. On necropsy, massive subcutaneous haemorrhage may be found, with moderate to severe necrotic lesions in the leg muscles, often bilateral but not symmetrical. Many small to large ruptures may be present in the necrotic muscle. Histological lesions include severe necrosis of skeletal muscles, fibrosis and some evidence of muscle regeneration (Spraker, 1993). CM in birds potentially has a similar pathophysiology. Ataxia in birds can manifest as dropping at the hocks and "hock sitting" (pers. Obs.) or sternal recumbency (Hanley, et al., 2005) and haemorrhage has been a feature in the pathology of CM in birds described by Spraker (1987) and Tully (1996).

Delayed-peracute syndrome

Spraker (1993) describes this syndrome in wild caught animals that are kept captive for at least 24 hours. If suddenly disturbed or chased, the animal suddenly stops and stands or lies down briefly, before dying in a matter of minutes, usually due to ventricular fibrillation and cardiac arrest. CK, AST and LDH are elevated on the biochemistries, but there are few observable post-mortem lesions, other than a few pale necrotic areas within the skeletal muscles. These reveal focal areas of rhabdomyolysis histologically, especially in the hindlimbs. Spraker (1993) does not discuss whether this syndrome occurs in animals released back into the wild with no observable clinical signs. It seems likely that post-capture deaths in the field would be likely, given other stressors such as predators and human interference are common. As such, true mortality rates are likely higher than rates at release. There are no reports of any correlating syndrome occurring in birds.

1.5.6 Long term effects

There is a paucity of information in the literature regarding longer term effects of myopathy in either birds or mammals. It appears that CM can cause reduction of body condition either for the short or long-term, but whether this has significant effects on other life history events is largely unknown. For example, overall body condition was reduced significantly more in those bears repeatedly captured over several years as opposed to bears caught only once, with potential effects of survival and reproduction (Cattet, Boulanger, et al., 2008). Severe weight loss was reported in a post-mortem examination of a sandhill crane affected by CM (Windingstad, et al., 1983). A further three sandhill cranes successfully treated for CM showed weight loss over the rehabilitation period of 7.6% to 23%, with the greater loss being the male crane that was left to self-feed rather than being supplementary fed (Businga, et al., 2007), however longer term 2/3 of these birds went on to breed successfully. Persistent muscle damage may lead to other, more difficult to document effects, such as reduced range of movement (Windingstad, et al., 1983) or reduced ability of an animal to escape predation (Spraker, et al., 1987; Abbott, et al., 2005). Cardiac lesions have been observed in birds with CM and the authors suggested chronic heart failure or decreased exercise tolerance may result (Spraker, et al., 1987), which would largely depend on the severity of the lesions and individual requirements of the bird. Spraker et al.(1987) also speculated that turkeys may have recovered from mild skeletal and cardiac damage had the birds been released. Certainly skeletal muscle has the ability to regenerate with mild damage but cardiac muscle has little regenerative capability and tends to scar (Boudoulas & Hatzopoulos, 2009). While reports are few, it does seem possible that some animals diagnosed with CM go on to lead long, reproductively successful lives and this may largely depend on the severity of the disease and the opportunities the animal has for recovery. More studies of long term determinants such as fecundity, migration and survival following diagnosis and/or treatment of CM are required.

1.5.7 Pathophysiology

Myopathy simply means pathology (disease) of the muscles, whereas rhabdomyolysis literally means “dissolution of striped skeletal muscle” (Warren, et al., 2002). Exertional rhabdomyolysis may therefore be a better term for explaining the pathophysiology involved in capture myopathy. The full cascade of events following an exertional aetiology is incompletely understood (Zager, 1996; Guis, et al., 2005). However, the review by Zager (1996) suggests the initiating factor to be direct muscle cell damage, particularly due to eccentric contractions where tensile stress is exacerbated, such as tension against an

elongated muscle (Newham & Edwards, 1986). Myocellular damage is exacerbated by concurrent factors such increased heat production, reduced blood flow (due to continuous contraction), and energy depletion. Hypokalaemia and hypophosphataemia may also play a role (Zager, 1996; Guis, et al., 2005). Anaerobic metabolism in chase and capture, resulting in lactic acid build up, creates local and systemic metabolic acidosis which may contribute to increased permeability of cell membranes and cell lysis (Chalmers & Barrett, 1982). Stress and activation of the sympathetic nervous system and adrenal glands in the 'flight-or-fright' response will also play a role in animal cases (Spraker, 1993).

Spraker (1993) considered CM to be the result of failure of homeostasis in times of crisis – identical to severe shock – resulting in blood pooling, reduced cardiac output, tissue hypoxia, vascular collapse and death. For animals not killed immediately by the vascular crisis, longer term effects are seen such as ischaemic renal tubular necrosis, due to prolonged vasoconstriction by the sympathetic nervous system. This author believes myoglobinuria to be a secondary cause of renal failure. Muscle lesions are thought to develop due to tissue hypoxia and lactic acidosis, and where the animal survives for longer periods, progressive necrosis of damaged muscles ensues. Delayed-peracute syndrome is suggested to be a result of exacerbation of hyperkalaemia by a further stressful event, resulting in cardiac fibrillation and death. However, the author describes CM pathophysiology as "probably" (p. 486) identical to shock and had "theorized, based on observations, necropsy results and basic physiology" (p. 487).

Looking towards human medicine, where rhabdomyolysis has been extensively researched, yields different aspects to the disease pathophysiology. While shock may indeed by a cause of acute mammalian death, in human studies, shock has much less to do with the observed outcomes of myopathy. Zager (1996) describes the essential function of adenosine triphosphate (ATP) to muscle contraction and how derangements to aerobic or anaerobic energy production are central to all cases of muscle necrosis. Depletion of ATP and a damaged cell membrane (due to physical/exertional trauma) significantly disrupt calcium homeostasis, such that intracellular calcium quickly rises to toxic levels (Zager, 1996). Elevated intracellular calcium results in continual contraction of myofibrils (Elsayad & Reilly, 2010) and calcium-activated proteases and phospholipases cause destruction of proteins of the cell membrane, contractile fibres and cytoskeleton (Guis, et al., 2005). By-products from these reactions induce oxidative free radical damage and can cause direct lytic damage to the cell membrane. Cellular lysis results in leakage of toxic cellular products such as myoglobin and potassium into surrounding tissues and the bloodstream. Local vascular damage and infiltration of inflammatory cells can further

exacerbate ischaemic necrosis (Zager, 1996). The effects of resulting muscle necrosis can be far-reaching. Acute kidney damage can affect up to a quarter of all human patients affected with rhabdomyolysis (Elsayad & Reilly, 2010). While myoglobin can directly damage renal tubules, concurrent renal vasoconstriction, hypovolemia and tubular cast formation are essential factors (Zager, 1996). In contrast to Spraker (1993), Zager (1996) describes the causes of renal hypoperfusion to be due to several factors directly related to rhabdomyolysis: 1. Volume depletion due to third spacing of body fluids in response to muscle injury; 2. Activation of the endotoxin cascade (by unknown mechanisms); and 3. Scavenging of nitrous oxide (a potent renal vasodilator) as a result of myoglobinaemia. Severe hyperkalaemia due to massive cell lysis can result in cardiac arrest (Guis, et al., 2005). Death may also occur due to severe hyperthermia, metabolic acidosis, calcium or phosphate metabolic disturbances, or DIC (Guis, et al., 2005). Therefore it is argued that the range of severe effects from paralysis to renal failure or acute cardiac failure can all result from the direct and indirect effects of muscle necrosis.

Generally, birds could be expected to have a higher tolerance for exercise and the effects of hypoxia. Birds have a more efficient gas exchange system and a greater supply of blood to individual muscles cells due to a network of capillaries surrounding the muscle fibres, rather than a single straight capillary as mammals do (Powell, 2000). Additionally, birds with greater oxygen demands, such as high altitude dwellers and diving birds, have higher concentrations of myoglobin in the muscle cells. The role of myoglobin is to facilitate oxygen movement from the capillaries to the further reaches of the muscle cell and in birds this process is very efficient (Powell, 2000). While myoglobin still plays an undetermined role in consequences of myopathy in birds, those with higher concentrations (such as migratory birds) could potentially have a higher loading on the kidneys following muscle lysis. As such, further investigation of kidney histopathology of birds affected by CM is warranted.

This raises questions regarding the role of exertion and eccentric muscle contractions in the pathophysiology of CM in birds. Most birds are caught by nets rather than chased, so in most cases this is a sudden stop or capture of a bird rather than an increase in exercise-related exertion. Exertion may increase as the bird attempts to escape the net or its handler, however this usually involves struggle. It is therefore likely that muscle damage through eccentric contractions (such as resistance against a net) and the direct effects of trauma from the net and resistance to handling, that play a greater role in the pathogenesis of CM in birds.

1.5.8 Pathology

Muscle damage may be found grossly or histopathologically even in the absence of clinical signs (Spraker, et al., 1987). However, in most cases of clinically evident myopathy severe muscle changes have been found post-mortem.

Skeletal muscles

Pathological findings reveal muscle necrosis that is often in patches within muscles rather than entire muscle bellies and is often bilateral, but not symmetrical (Spraker, et al., 1987). The degree of change and progression of muscle injury and repair reflect differences in severity of the syndrome and the time since injury. Macroscopic lesions include streaks, large patches or diffuse pale discoloured muscle tissue in the legs, pectoral muscles and wings (Windingstad, et al., 1983; Spraker, et al., 1987; Tully, et al., 1996; Hanley, et al., 2005). The muscles may appear swollen and haemorrhagic, or have a dry, fibrous or friable texture (Rose, 2005). Some authors describe the necrotic muscle as having a "cooked" appearance (Höfle, et al., 2004, p. 346). Bruising and haemorrhage is often associated with gross muscle rupture of leg muscles (Spraker, et al., 1987; Tully, et al., 1996).

Myocellular damage looks similar histologically, regardless of cause. Milder changes include sarcolemmal (muscle cell membrane) basophilia and striations with hypertrophic nuclei. More obvious damage includes oedema, loss of striations and hyalinisation of muscle cells (Höfle, et al., 2004; Hanley, et al., 2005). Other degenerative changes include cellular fragmentation, granular or calcium deposits and areas of coagulative necrosis (Windingstad, et al., 1983; Tully, et al., 1996). Myocytes may be swollen with basophilic membranes, eosinophilic cytoplasm and pyknotic nuclei (Spraker, et al., 1987). Macrophages are observed engulfing the degraded tissue and there may be nuclear proliferation (Windingstad, et al., 1983) and increased inflammatory infiltrates surrounding the necrotic cells (Spraker, et al., 1987; Tully, et al., 1996). The degenerative changes may be observed in scattered single muscle fibres (Windingstad, et al., 1983; Hanley, et al., 2005), or affect entire muscle bundles (Windingstad, et al., 1983). Some muscles may show extensive areas of necrosis and interstitial fibrosis (Spraker, et al., 1987; Hanley, et al., 2005). In longer term cases, regeneration of muscle cells may be evident with chains of nuclei within narrow myocellular bands. Disorganised clusters of myocellular nuclei may be evident in sub-optimal regeneration (Hanley, et al., 2005).

Heart

Microscopically, myocardial lesions have consisted of small areas of coagulative necrosis found mainly in the left ventricles, along with "collapse of intercellular stroma and mild to moderate myocardial nuclear proliferation" and an absence of inflammatory cells (Spraker, et al., 1987, pp. 448-449). Small areas of fibrosis may be evident (Hanley, et al., 2005). Increased cardiac myocellular eosinophilia was the only change observed in an emu with CM (Tully, et al., 1996).

Kidneys

There are limited reports of renal pathological findings in birds. Windingstad et al. (1983) reported post-mortem findings of a greater sandhill crane – the kidneys were grossly pale and blotchy, with tubular nephrosis on histopathology and urate precipitate found in the distal tubules and collecting ducts. This suggested dehydration rather than myoglobin damage. Tully et al. (1996) found histopathological changes in the kidneys of an emu including: scattered mild cortical myxomatous degeneration and fibrosis; renal tubular dilation, with irregular mineralisation; occasional regenerating tubules; and rare organising venous thrombi. Only one report by Hanley et al. (2005), suggested possible myoglobin damage due to the presence of "golden cytoplasmic granules in renal tubular epithelial cells" (p.493). Areas of acute renal tubular necrosis and mineralisation were also evident. Other authors have found no evidence of renal damage in carcases with pronounced lesions of myopathy (Marco, et al., 2006). Further studies of kidney histopathology of birds affected by CM are needed to establish the contribution of renal damage to the disease process in these birds.

Other

Hanley et al. (2005) reported thymic haemorrhage and necrosis and bronchial haemorrhage (possibly trauma related) in post –mortem examinations of whooping cranes affected with CM. Several authors have examined sciatic and femoral nerves and found no abnormalities on histopathology (Spraker, et al., 1987; Hanley, et al., 2005).

1.5.9 Diagnosis

Diagnosis of capture myopathy is based on a combination of history, clinical signs, presence of myoglobinuria (in mammals), plasma or serum elevations in muscle enzymes and post-mortem findings. Fever and leukocytosis are common in people (Warren, et al., 2002). Elevated temperatures (Nicholson, et al., 2000) and leukocytosis (Smith, et al., 2005) have been mentioned in birds but are rarely investigated. In human medicine, quantification of urinary or plasma myoglobin levels, genetic testing, muscle biopsies and other muscle function tests are commonly performed to investigate further (Guis, et al., 2005).

Muscle cell lysis causes serum elevations of creatine kinase (CK), aspartate aminotransferase (AST), aldolase, lactate dehydrogenase (LDH) and hydroxybutyrate dehydrogenase. Other muscle cellular contents released into the bloodstream include myoglobin, creatine, potassium (K+) and urea (BUN). Calcium metabolism is altered, but can show either a hypo- or hyper- calcaemia, while serum phosphate can also be elevated (Warren, et al., 2002). Acid-base balance can also be affected, with blood gas measures showing metabolic (lactic) acidosis (Spraker, 1993; Williams & Thorne, 1996), although this has not been studied in birds. Some birds with CM have shown a persistent leukocytosis, even upon resolution of clinical signs (Smith, et al., 2005).

Creatine kinase (CK)

Creatine kinase is reported to be the most useful indicator of muscle damage in birds (Franson, et al., 1985) and mammals (Cardinet, 1989). Massive elevations of CK, sometimes over 200,000 IU/l, have been reported in birds affected by CM (Carpenter, et al., 1991; Abbott, et al., 2005; Smith, et al., 2005; Businga, et al., 2007). In comparison, normal (baseline) CK levels in birds usually number in the hundreds of units per litre (e.g. Bollinger, et al., 1989). CK is also used as an indicator of rhabdomyolysis in humans, with CK elevations of over five times normal levels considered diagnostic (Gabow, et al., 1982 as cited in Warren, et al., 2002) and CK levels over 100,000 IU/l have been linked to greater mortality (Elsayad & Reilly, 2010).

Mild CK rises have also been reported in birds due to the effects of strenuous exercise (Knuth & Chaplin, 1994), including recent migration (Guglielmo, et al., 2001). Unfortunately Guglielmo, et al. (2001) did not report the actual CK values, prohibiting comparison to CM affected shorebirds. However, peak values reported in untrained hawks of under 5000 IU/l (Knuth & Chaplin, 1994) are much lower than those reported for CM affected birds.

Several studies in birds have shown that CK begins to rise rapidly in the blood following muscle damage (Williams & Thorne, 1996). In birds, baseline levels of CK have been estimated by removal of blood as soon as possible after capture, such as within the first 10 minutes (Bailey, et al., 1997). Peak levels of CK are reached in approximately 24 hours in birds (Bailey, et al., 1997), although peaks levels at 2-3 days have also been described (Businga, et al., 2007). Delayed peak measures of CK are reportedly due to the long transit time of the CK molecule from the interstitial space, through the lymphatic system and into the blood (Friedel, et al., 1979), which does not explain why many studies do show rapid increases in CK. Studies in human triathlon athletes showed peak CK occurred 6-24 hours after the event, with declines beginning 48 hours after the event (Margaritis, et al., 1999). In CM affected birds, after its peak CK progressively declines to initial or baseline levels after approximately 10-12 days (Businga, et al., 2007), but in some cases may take more than 24 days (Smith, et al., 2005). Cardinet (1989) also stated that CK elevations in birds are maximal in 6-12 hours and return to normal in under 48 hours, unless continuing necrosis occurs. Pre-conditioning to the type of exercise also appears to reduce the amount of CK rise and muscle damage in humans (Siegel, et al., 1980) and in birds (Knuth & Chaplin, 1994). More studies with repeated CK measures are needed to establish the clinical picture of muscle damage in birds following capture and handling and for those with myopathy.

Additionally, the relationship between absolute CK values and clinical signs of myopathy or death is largely unknown (Bollinger, et al., 1989) and some authors argue that no correlation exists (Visweswaran & Guntupalli, 1999 as cited in Latham & Nichols, 2008). Some human studies have used CK levels to quantify the amount of skeletal (Apple & Rhodes, 1988) or cardiac (Sobel, et al., 1976 as cited in Apple & Rhodes, 1988) muscle damage. More recent studies showed that humans completing eccentric muscle exercises may have large increases in CK levels without any renal compromise (Clarkson, et al., 2006) and found no statistical linkages between muscle soreness or function tests and elevated CK levels following endurance races (Margaritis, et al., 1999). However these studies did not look at muscle biopsies to establish the degree of muscle injury at a microscopic level. Results from several bird studies also lack correlation between CK values and overt signs of myopathy or survival. For instance, houbara bustards caught, handled for blood sampling and transported minor distances (<5mins) showed elevations of 15 times baseline levels of CK (up to 4940 +/- 442 IU/l), suggesting that significant muscle damage had occurred (Bailey, et al., 1997). The birds in this study showed no clinical signs of myopathy, even though the authors found their CK levels to be similar to CM affected houbara bustards (Bailey, unpublished data, as cited in Bailey, et al., 1997).

Abbott, et al., (2005) found CK levels varied from a few thousand to greater than 300,000IU/l of wild-caught bobwhites. Although CK levels were arithmetically different, they did not find any clear statistical relationship between survival times of the birds and the magnitude of the CK level. Only one study could be found at the time of writing, where CK activity was significantly linked to mortality. Nicolson, et al., (2000) found that CK activity prior to release was significantly higher for turkeys that died within 14 days of capture compared to those that survived (CK x=4807 IU/l vs. x=1986 IU/l, $p<0.01$).

It therefore appears that CK is an excellent indicator of muscle damage but is not specific for capture myopathy and should be interpreted in combination with clinical signs and other indicators of muscle disease. Studies such as those by Bollinger, et al. (1989), which only measure blood biochemistries for an hour or two, may be missing the peak CK level and results should be interpreted with caution. Studies in which birds are left for a longer period after baseline CK measures would provide a greater degree of change and therefore potentially greater accuracy in assessing muscle damage. Additional longer-term studies of repeated measures of birds affected by CM could provide more information on the correlation between CK levels, clinical signs and survival.

Aspartate Aminotransferase (AST)

AST is also released from damaged skeletal and cardiac muscle tissue, but it is less specific to muscle injury than CK, as it can arise from other sources such as liver, kidney and red blood cells (Cardinet, 1989). Many studies have shown marked increases in AST along with CK in cases of CM in birds (e.g. Smith, et al., 2005; Businga, et al., 2007). However, AST appears to be a less immediate indicator of muscle damage as it is slower to rise and fall in the blood. For instance, AST peaked 4 days after capture in cranes with CM - a day later than CK peaks. AST was still 2-5 times elevated at the time of release following successful treatment, whereas CK had returned to reference levels (Businga, et al., 2007). AST usually has a lower quantitative value and relative change than CK (e.g. Bollinger, et al., 1989; Businga, et al., 2007). As such it may not show significant differences in the same time period as CK measures. For instance, Nicholson, et al. (2000) found increased CK activity (measured at capture) to be a significant predictor ($P<0.01$) for mortality in captured wild turkeys, whereas AST showed no significant difference ($P=0.49$). Due to the slower decline of AST, it could potentially be useful to show changes over longer time periods, or to show previous injury where clinical signs have resolved. Plasma concentrations of AST are routinely utilised to help differentiate liver damage from muscle damage, as CK will not be elevated with the former (Hochleithner, 1994). In conclusion, AST is not as specific an indicator for muscle damage, and is slower to show changes in the blood in the immediate

post-capture period. Nevertheless, AST is a useful indicator of muscle or liver damage that is commonly included in biochemistry panels for many bird and mammalian capture studies.

Lactate Dehydrogenase

Lactate Dehydrogenase (LDH) is also commonly used as a measure of muscle injury and Franson, et al. (1985) showed it had the second highest muscle activity after CK (AST had the third highest). Similar to AST, it is not specific to muscle as it can be released by damaged red blood cells and other internal organs, such as liver and kidney (Franson, et al., 1985). Several studies in birds have shown massive increases in LDH along with CK (e.g. Bailey, et al., 1997) and AST (e.g. Businga, et al. 2007), with peak levels occurring 24-48 hours after capture, a slight delay behind CK peaks. Peak levels of LDH are often much lower than CK absolute values (e.g. Bailey, et al., 1997). LDH also has slower declines than CK. In a study of muscle damage in bustards, LDH was still significantly elevated ($P=0.009$) when compared to baseline levels 14 days after repeated captures, whereas CK had fallen back within the normal range (Bailey, et al., 1997). A panel of all 3 enzymes, where funding allows, therefore would reveal the greatest clinical picture than any of the enzymes alone. It is also important to note that significant differences occur between species for the same enzymes (Franson, et al., 1985). Therefore normal or baseline values for a given species are required, and absolute values become less important than the relative changes over time.

Potassium

Potassium has a critical role in electrolyte homeostasis, with 98% of potassium actively stored intracellularly via potassium pumps in the cell membrane. Disease such as adrenal dysfunction, metabolic acidosis, kidney disease, haemolytic anaemia, massive tissue trauma or dehydration can all cause hyperkalaemia, while hypokalaemia can result from inadequate dietary intake, alkalosis or chronic fluid loss such as diarrhoea (Hochleithner, 1994). Low blood potassium has been described as a risk factor for CM in people (Warren, et al., 2002) and hyperkalaemia has been described in both mammals (Kock, Jessup, et al., 1987) and birds (e.g. Hanley, et al., 2005) with myopathy. Along with other cellular metabolites, potassium is released from damaged muscle cells into the blood stream and can rise rapidly with plasma biochemical analysis after muscle injury (Warren, et al., 2002). Many studies of capture physiology in birds lack results of potassium measures to date. Potassium can be difficult to accurately measure as it can be released by damaged red blood cells and requires timely processing to avoid false elevations or declines (Hochleithner, 1994). For instance, heparinised blood samples of chickens (*Gallus*

domesticas) and pigeons left to sit at room temperature for up to 24 hours showed significant decreases in potassium (*Columba livia domestica*)concentration in the first two hours (Lumeij, 1985). The role of potassium in the pathogenesis or diagnosis of CM in birds requires further investigation.

Uric Acid

While Blood Urea Nitrogen (BUN) is the main measure of kidney function in mammals (Finco, et al., 1989 as cited in Williams & Thorne, 1996), in birds the main measure is uric acid (UA) (Fairbrother, et al., 1990). Uric acid levels can be mildly, but significantly affected by reproductive status, age and sex, with greater levels seen in egg laying and very young birds (Fairbrother, et al., 1990). Uric acid levels are known to elevate due to renal failure and gout, severe dehydration, tissue damage, starvation, post-prandially and in liver disease (Hochleithner, 1994). The UA measure of a sandhill crane with myopathy was lower than the reference mean and range for that species (Carpenter, et al., 1991), which is the only CM related paper to have reported UA measures in birds. There is no information currently on UA measures in response to capture and handling, nor as a predictor of renal impairment in birds suffering from myopathy. Further information and reporting on plasma uric acid measurements in captured wild birds would be valuable.

1.5.10 Risk factors

There are few studies that look at risk factors for CM in birds and the studies are often significantly flawed. Admittedly, it can be difficult to assess for risk factors due to the complex interaction of events at the time of capture, making individual factors difficult to tease out. In the following section the identified and theorized risk factors will be further explored by category.

Capture Related Risk Factors

Cannon nets

Several authors agree that cannon-netting has greater chances of post-capture complications than other methods of capture. A paper evaluating 3 methods of capture in mallard ducks, conducted by Bollinger, et al., (1989) is frequently referenced in the CM literature, yet has several important flaws. The authors found a significantly higher average CK value for wild ducks caught by cannon-nets (and decoy traps) when compared to bait-trapped birds, yet the difference was only significant at the time of banding, and was not significantly different an hour later (though still arithmetically different). This is at the time that CK should still be rising in the blood if muscle injury had occurred, and a significant difference at the second sample would have given the data more substance. Additionally, the initial blood sample was taken at the time of banding and was anywhere from approximately 15 mins to over an hour after capture, with cannon-net captures having the longest interval between capture and first blood sample (Bollinger, et al., 1989). Previous studies have shown that CK begins to rise quickly in the blood (e.g. Bailey, et al., 1987), therefore observed CK differences between the capture methods may have been influenced by the difference in timing of sample collection. In the same study, a significantly higher CK level was found in mallards with clinical signs of myopathy (poor flight at release) when caught by bait traps, but not by other methods of capture (Bollinger, et al., 1989). Again, these differences were no longer significant an hour later, and the initial 'elevated' CK was lower in absolute value than birds with normal flight caught by other methods, bringing the relevance of these results into question.

A different study of little bustards by Ponjoan, et al., (2008) had much clearer results. A total of 151 little bustards were caught by 3 methods – leg nooses, cannon nets or funnel traps - handled, fitted with transmitters and released. Mobility alterations were measured subjectively and mortalities were also recorded. These authors concluded cannon-nets were a significant risk factor and increased the chance of developing mobility alterations (myopathy) by a factor of 10 times. Interestingly, this still held true despite the leg noose

method of capture having significantly greater handling and restraint times, which were also found to be significant risk factors in this study. While CM was not the proven cause of all of the deaths (n=10), 4/4 necropsies had histological evidence of myopathy. Unfortunately no blood samples were taken in this study. It would have been interesting to be able to correlate the clinical signs observed with muscle enzyme levels at the time of capture to give quantitative results.

Dabbert & Powell, (1993) also showed clearer results that suggest a true risk factor of cannon-net captures. These authors were able to show that mallard ducks caught by entanglement cannon-net had greater CK and AST values ($P<0.0001$) than both baseline values and following controlled handling for the same species. The CK and AST were also significantly higher ($P<0.001$) when compared to mallards caught by enveloping type rocket net, as recorded by Bollinger, et al., (1989). These authors thought the changes were likely related to an increase in time spent struggling and/or entangled by the net. Entanglement nets have a greater sized hole in the mesh meaning wings and necks can protrude through the holes and meaning greater difficulty in disentangling birds from the nets (Dabbert & Powell, 1993).

Drop nets

Drop-nets have also been recognised as a potential risk, at least for turkeys (Spraker, et al., 1987). Nearly half (13/27) of wild turkeys caught by drop net were found to have gross lesions of CM on necropsy, while birds caught by alpha chloralose or cage-traps in other years (0/14 and 0/19) did not have any gross pathology lesions. Microscopically, 40% of the drop-net birds had severe skeletal muscle lesions, 30% moderate and 30% mild. Comparatively, birds caught by the other two methods showed much milder lesions: none had severe lesions; 16% had moderate lesions; 73% had mild lesions, and 11% had no lesions (Spraker, et al., 1987). The authors rightly suggested that drop nets increase the severity of CM lesions compared with the other methods, although this data was not statistically analysed, the data was taken over 3 separate years (meaning greater chance of subjective discrepancy) and it was not stated whether the histopathology was performed by the same pathologists each time.

Mist nets

There are few studies that assess for muscle damage due to mist-net captures in birds. In an anecdotal report, Minton (1993) states that mist-netting caused proportionately more problems (i.e. injuries and deaths) than cannon netting in several catches of waders (red-necked avocet and bar-tailed godwits) in Western Australia and reported it as likely related to the length of time struggling in an uncovered or unmonitored net. This was a

speculative report that did not assess for muscle damage in any scientific context. Guglielmo, et al. (2001) found that mist-net captures of western sandpipers *Calidris mauri* resulted in CK levels up to tenfold higher 1-2 hours post-capture than at capture, indicating that mist nets can also cause muscle damage in birds. These results were not correlated to clinical signs of myopathy nor survival data, nor were the results compared to other methods of capture for this species. The studies by Spotswood, et al. (2012) and Recher et al. (1985) revealed that injuries and mortality due to mist-net captures do occur at low levels of 0.05% up to 2.8%, however the relationship of muscle damage as a causative factor was not established. Further robust studies of muscle damage due to mist-netting in birds are needed.

Struggling and handling time

Suggested risk factors for myopathy include: high chase speeds, prolonged exertion without rest, excessive handling, prolonged restraint, struggling due to restraint in unnatural positions, crating and transportation (Paterson, 2007). These could roughly be grouped into the same category as each of these factors results in greater exertion, struggling or handling time.

Several reports propose the risk of myopathy is greater the longer the time birds spend struggling within or beneath a net, or for total time of handling, such as for banding and measurements. For instance, Dabbert & Powell, (1993) compared groups of mallards caught by rocket net. They found that those under the net for the longest time, rather than those confined for the longest total period, had significantly greater CK elevations. A study of ring-billed gulls by Southern & Southern (1983) revealed that significantly less gulls were sighted post-release if they had been processed (removed from the net, banded and released) last. The first 100 birds to be processed had resighting rates of 87.5% compared to 60% ($P<0.01$) for birds 101-122. The authors suggested the difference to be related to an increased amount of time under the net and greater handling and injuries, as the birds removed last were more entangled. Similarly, Ponjoan, et al. (2008) found that handling time and restraint time were significant risk factors, not only for the presence of locomotory problems in little bustards, but also the degree of severity in these alterations i.e. the most severe clinical signs were associated with the longest handling times. While nearly half of these birds subsequently died, mortality was not significantly related to handling and restraint times under the same analysis, which is unusual seeing that the authors found that those birds with the worst mobility problems were more likely to die (83%) than those with mild signs (33%) and perhaps reflects a lack of numbers of birds in the mortality analysis or a discrepancy in the model, rather than lack of correlation.

Nicholson, et al. (2000) also found handling time to be a significant factor in predicting the risk of mortality in wild turkey hens caught with cannon-nets. Handling time was considered the time of capture to the point of release. Birds that died (20/130 – 15%) had a significantly greater handling time than those that survived ($P<0.01$) and the authors suggested keeping handling time to a minimum to improve chances of survival.

It is of interest that body temperature was found to be a risk factor in the study by Nicholson, et al. (2000) – where birds that died had a greater mean body temperature (42.4°C, SE 0.4) than birds that survived (41°C, SE 0.2, $p<0.01$). Body temperature elevation could be a complication of heat stress, struggling or over-exertion. This study indicates body temperature would be a good parameter to include in future studies.

The reasons that birds may develop myopathy due to these factors have not been fully investigated, yet it is presumed by many authors that a greater degree of injury and strain is incurred, especially when using leg motions and effort that is unnatural, such as entanglement or pushing upwards against a net (Green, 1980; Dabbert & Powell, 1993; Minton, 1993). Additionally, stress responses are reportedly greater in birds held in traps for longer periods (Lynn & Porter, 2008) which may exacerbate CM development. In light of evidence that eccentric muscle contractions cause greater degree of muscle injury and that depletion of ATP due to over-exertion is an inciting cause of myopathy, it stands to reason that struggling and handling times are risk factors for myopathy development in birds.

Height of holding cages

Anecdotally, Bainbridge (1976) and Green (1978) speculated that waders kept in low keeping cages whilst awaiting banding procedures may have developed CM due to prolonged periods of sitting on folded legs. This theory was not evaluated by blood testing or other scientific methods so remains speculative. Birds will naturally sit down on folded legs for prolonged periods with little ill effect (e.g. nesting) and theoretically blood supply should not be compromised by such action. Additionally, further anecdotal reports by Stanyard (1979) and Minton (1980) found myopathy occurred in caught waders (including curlews and bar-tailed godwits) despite adequately sized holding boxes.

Signalment

Some anecdotal reports suggest that females may be more predisposed to CM (Purchase & Minton, 1982). However, other peer-reviewed authors have shown that sex was not a risk factor for clinical signs of myopathy (e.g. Ponjoan, et al., 2008), degree of CK or AST

elevation (e.g. Dabbert & Powell, 1993), presence of gross lesions of myopathy (e.g. Spraker, et al., 1987) or self injury of radio-tagged birds (Hofle, et al., 2004). Logically, there should be no reason for females or males to differ in the degree of muscle damage, unless there were significant behavioural or physical differences in the response to capture. However, the literature reports are few and further investigations would be worthwhile.

The very old and very young have been suggested as the most susceptible to CM by (Ebedes, Van Rooyen & Du Toit, 2002, as cited in Paterson, 2007). Several scientific studies appear to give validity to these assumptions, at least for juvenile birds. In 27 post-mortem examinations of turkeys caught by drop-net, 48% had gross lesions of CM. Of these, 73% of birds were immature birds compared to 17% mature birds (Spraker, et al., 1987). They suggested that young juvenile turkeys may therefore be more susceptible than adults to CM, however statistical significance was not analysed. Similarly, Nicholson, et al. (2000) reported higher losses in subadult (4/19 - 21%). vs. adult (16/111 - 14%) eastern wild turkey hens that died following cannon-net captures, but these were not statistically significant, perhaps due to low numbers of birds in the subadult group. In a study of little bustard caught by two methods of capture (cannon-nets and leg nooses), significantly more juvenile birds had clinical signs of myopathy, compared to adults (31% vs. 10%: P=0.004). These authors produced a model that calculated juveniles were 5 times more likely to develop mobility problems when restraint and handling time were standardised (Ponjoan, et al., 2008). Although there is potential bias - juveniles in this study had mainly been caught by leg nooses and were predominantly males – the fact that cannon-net captures were a higher risk compared with leg nooses suggests age to be a true factor in this study. Additionally, the model only accounted for 41% of the potential risk factors, suggesting that several contributing factors were unidentified in this particular study (Ponjoan, et al., 2008). This highlights how differentiating true risk factors can be difficult: lack of independence in the variables and interaction effects, along with unidentified factors means we rarely get a clear picture of the determining factors for CM occurrence.

Environmental factors

Extremes in ambient temperature, rain and high humidity have been suggested as risk factors for CM (Paterson, 2007). Yet there are few good papers looking at environmental risk factors and some reports are conflicting. Nicholson, et al. (2000) produced an excellent study, where eastern wild turkey hens were monitored for 14 days post release after capture by cannon nets. At the time of capture, relative humidity levels and air temperatures were recorded, as well as CK activity, body temperatures and

handling time. The authors found that higher ambient temperatures and lower relative humidity levels were the best predictors of mortality (as well as CK activity), and these factors were significantly different between those birds that died vs. survived (both $p<0.01$). This is an interesting result in that temperatures were still low for those that died (average of 7 ºC vs. 0.8 ºC for survival group), much lower than average catching temperatures in New Zealand (pers. obs). Also the humidity level was not terribly low at 47% (died) vs. 66% (survived). Conversely, Höfle, et al. (2004) found that deaths of red-legged partridge appeared to be linked to lower mean temperatures ($p=0.03$) (actual temperatures not stated) and higher humidity levels ($P=0.05$). Perhaps these differences indicate acclimatisation, meaning that environmental risk factors may differ between avian species. In shorebirds, anecdotal reports from Purchase & Minton (1982) and Minton (1993) proposed high temperatures to be risk factors, but with no supportive evidence. Overall, these reports suggest that environmental factors could be contributing to CM occurrence and require further robust studies to determine their significance.

Species

As previously described, CM appears to affect some bird species more than others, with long-legged birds such as cranes, turkeys, ratites and shorebirds appearing most commonly in the literature. Yet there appears to be few studies of myopathy in many other species and groups of birds (e.g. parrots) and CM cannot be ruled out in these groups. Even species differences can occur within the same genus or family. For example, necropsies performed by Bailey, et al. (1996) found up to 63% of Kori bustards and 29% of houbara bustards died with capture myopathy, while none of the rufous-crested bustard (*Eupodotis ruficrista*) were found with any CM lesions at all. These authors concluded bustards to be particularly prone to capture myopathy and capture injuries and urged caution in their capture and handling (Bailey, et al., 1996; Ponjoan, et al., 2008). Yet no explanation was offered as to the possible species differences. Perhaps physiological or behavioural differences, capture methods or differences in handling could be evaluated in future to clarify risk factors for these species. More comparative studies, in which different species are caught under the same conditions and evaluated for muscle damage, could gather further information on species risk factors.

Other Diseases

Paterson (2007) relates that underlying disease such as external and internal parasitism may weaken the animal, predisposing them to CM. However, there have not been any

detailed papers or reviews on the prevalence, nor the relationship of pre-existing disease to the development of capture myopathy. Hypokalemia (low blood potassium) is known to be a risk factor for rhabdomyolysis in people via several mechanisms including depolarization of muscle membranes, reduced cardiovascular output, impaired glucose metabolism and reduced muscle blood flow (Warren, et al., 2002). Hypokalemia in birds could be caused by severe stress, where potassium is excreted by the kidneys in response to elevated serum proteins. Other causes of hypokalemia include low dietary intake, chronic diarrhoea, diuretic therapy, renal failure, liver disease, antifungal medication, or alkalosis (including iatrogenic following bicarbonate therapy) (Hochleithner, 1994), starvation, diabetes mellitus and vomiting (Steinohrt, 1999). Further investigation into underlying disease and the role of potassium is warranted in birds.

Nutrition

Nutritional myopathies are regarded to differ from CM due to substantially differing aetiologies (Williams & Thorne, 1996). Selenium and Vitamin E deficiencies may cause 'white muscle disease', while free radical damage from toxins in food can also cause muscle damage and death, such as in pelicans that died with signs of myopathy after being fed rancid fish (Giri, et al., 2007). Vitamin E/ Selenium deficiency is reportedly associated with CM in ratites (Stewart, 1994). However, 4 cases of CM in ratites were not associated with such deficiencies (Tully, et al., 1996).

Bobwhite quail were found to have significantly reduced mortality after capture, handling and transportation when injected with Vit E / selenium mixtures prior to release (Abbott, et al., 2005). The glaring omission in this report is whether there was a normal Vit E/ Se level in these birds prior to injection, although in wild birds this would be unexpected. Had there been a deficiency then supplementation would have served the birds well in recovering from muscle injury compared to deficient birds. These authors premised that Vit E/ Selenium injection would counteract muscle damage incurred through capture, a hypothesis that has yet not been fully explored. Additionally, the survival of birds was relatively poor even in the treatment group, with just over half surviving to 180 days even in the best year, suggesting that other factors could be involved in the poor survival of this species on release. However, the significant findings in this report do suggest that Vit E/ Se as a prevention or treatment for myopathy should be evaluated further. Ideally this would be conducted under controlled conditions, with more clinical laboratory investigations (such as repeated CK and AST measures) and ensuring pre-treatment baseline levels are evaluated.

1.5.11 Prevention

Research to date has shown that treatment of capture myopathy in birds and mammals is largely unrewarding and the focus has often been drawn to prevention (Spraker, 1993; Williams & Thorne, 1996; Paterson, 2007). Suggested preventative measures in the peer reviewed and grey literature include:

Reducing struggling, handling and/ or restraint time

Current recommendations by several authors include reducing the time the bird is left struggling within a net, and/ or being handled and restrained. Handling times of < 1 hour was suggested by Nicholson, et al. (2000), while Ponjoan, et al. (2008) recommend 20 mins or less handling time in bustards, as mobility problems were not seen in birds that were handled for a mean of 25 mins or less - it was not explained why 20 minutes was chosen as a guideline when their results were based on a mean of 25 minutes. Paterson (2007) included prevention measures such as minimising handling, using experienced personnel and ensuring transportation is as brief as possible, reducing transportation stress and ensuring the transport facilities are suited to the species. Several other authors recommend removal of birds from the nets as fast as possible after capture (Stanyard, 1979; Southern & Southern, 1983; Guglielmo, et al., 2001), limiting the numbers of birds caught simultaneously and having sufficient experienced extractors (Stanyard, 1979; Purchase & Minton, 1982; Minton, 1993). Minton (1993) also suggested covering birds with hessian as quickly as possible to minimise struggling and to extract and process susceptible species from the net first. Most of these recommendations are anecdotal and are based on common sense, but are useful recommendations based on the studies to date which suggest struggling/ handling times to be significant risk factors.

Capture Method

Several authors have suggested cannon-net captures cause greater muscle damage than other methods (Bollinger, et al., 1989; Dabbert & Powell, 1993; Ponjoan, et al., 2008). Drop nets (Spraker, et al., 1987) and mist nets (Guglielmo, et al., 2001) are also known to cause muscle damage. Despite clear indications that these capture methods, especially cannon-netting, are risk factors, no one has yet recommended to avoid cannon-net captures altogether and most authors focus on using smaller mesh nets (e.g. Guglielmo, et al., 2001) and retrieving birds from the nets as soon as possible (Southern & Southern, 1983). Southern and Southern (1983) also suggest that careful alignment of the nets and minimal use of projectiles can reduce injuries and mortalities for cannon-netting operations. Operator competency is not often mentioned in the literature, yet it can be expected that

experienced, proficient operators are likely to cause less harm than inexperienced, or over-confident, groups.

Environmental temperatures

Minton (1993) recommended avoiding captures when ambient temperatures were above 35ºC, however this was anecdotal and unsupported by any scientific data. Nicholson, et al. (2000) recommend capturing wild turkeys at temperatures below 10ºC, while Höfle, et al. (2004) found lower mean temperatures (actual temperatures not stated) were a risk for partridge succumbing to CM. As such, there are no guidelines that are appropriate across all species and countries and further work is needed in this area.

Improved handling techniques

Guglielmo, et al. (2001) recommend that eccentric muscle contraction be reduced by holding wings/ legs of the birds firmly and minimising extension of wings or legs against the effort of the bird. While it is true that eccentric muscle contractions cause greater amounts of muscle damage (Newham & Edwards, 1986) reducing eccentric contractions in birds would need to start at capture in order to avoid muscle damage.

Vitamin E/Selenium injections

The study by Abbott, et al. (2005) raised an interesting possibility as to the potential for Vitamin E/ Selenium injections to alleviate muscle damage following capture trauma. Some of their results were significant; however in the absence of baseline measures of Vitamin E/ Selenium we cannot know whether this was simply correcting a deficiency. Further studies are needed to assess the potential of this preventative measure. However, in human studies Vitamin E supplementation was not found to have any protective effects for muscle damage due to exercise (Beaton, et al., 2002).

Increase care with juveniles

Several authors suggest juveniles are more susceptible to CM than adults (Ponjoan, et al., 2008; Spraker, et al., 1987), however the recommendations to take extra care when capturing these birds often refer to reducing handling and restraint time (Ponjoan, et al., 2008).

Other than reducing handling and restraint time, many recommendations found in the literature are vague, unsubstantiated or species-specific. Further investigation into the physical, environmental and physiological factors that contribute to the occurrence of CM in avian species will help to establish recommended guidelines for prevention.

1.5.12 Treatment

Treatment of clinical cases in wild birds appears to be rarely successful, with only a few successful outcomes reported in the peer-reviewed literature. For those cases, there were normally significant costs and time invested (Rogers, et al., 2004; Businga, et al., 2007), such as the 28 days of intensive treatment for a rhea to recover from CM (Smith, et al., 2005).

There is an absolute lack of controlled studies for providing an evidence base for treatment recommendations. The literature is for the most part a series of anecdotal observations on low numbers of individuals and the treatment protocols vary widely. Successful treatment methods have included various combinations of fluid therapy, force feeding, physiotherapy, steroidal or non-steroidal anti-inflammatories, oral anxiolytics, muscle relaxants, Vitamin E/ Selenium and cage rest. The following section highlights the main treatment modalities utilised or suggested for the treatment of CM to date.

Fluid Therapy

Fluid therapy is important in all critically ill avian patients. Intravascular volume expansion is required to counteract the effects of hypovolaemia, to ensure adequate kidney perfusion and to treat dehydration (Steinohrt, 1999). In human medicine, diuresis is considered essential for treatment of most of the common complications of rhabdomyolysis, including hypercalcaemia, hyperkalaemia and myoglobinuria (Warren, et al., 2002). Oral fluid therapy was described in treatment of shorebirds affected with CM (Rogers, et al., 2004), although dose rates were not described and arbitrary amounts (whatever the birds would swallow) seem to have been given. Parental fluid therapy was a component for therapy in an emu (Tully, et al., 1996), a rhea (Smith, et al., 2005) and 3 sandhill cranes (Businga, et al., 2007) affected by CM. However, the doses given ranged from below maintenance requirements (Smith, et al., 2005) to greater than twice maintenance levels (Tully, et al., 1996), where maintenance requirements are suggested as 50ml/kg/day (Redig, 1984 as cited in Quesenberry & Hillyer, 1994). The bird that received the greatest amount of fluids (Tully, et al., 1996) was euthanised, while the other birds recovered. Hydration monitoring, such as packed cell volume (PCV) and total protein (TP) measures, were not described by these authors. A suggested fluid rate and monitoring of hydration status in CM affected birds would be beneficial for future treatment recommendations.

Sodium bicarbonate therapy has been used successfully in zebra with CM (Harthoor & Young, 1974) to correct acid-base imbalances, with a reduction in mortality. The role of

metabolic acidosis in the pathogenesis of CM in birds has not been described. Additionally, reference values for bicarbonate in most bird species are non-existent, meaning estimates are required (Harrison, 1986). Sodium bicarbonate was given intravenously (IV) for a "possible acidosis" in an Emu (Tully, et al., 1996, p. 97), but this was not diagnosed nor monitored via blood tests. In one case where blood gas analysis was performed, the bird was alkalotic, not acidotic (Hanley, et al., 2005). Moreover, in most cases, metabolic acidosis can be corrected with parenteral balanced electrolyte solutions such as lactated Ringers solution (Steinohrt, 1999).

Physiotherapy

Physiotherapy, including sling therapy, allows for temporary relief of constant hock sitting or sternal recumbency and supports the bird when attempting to stand (Smith, et al., 2005). Physiotherapy also aims for such effects as ensuring continued range of movement, pain relief, preventing contracture and disuse atrophy and to prevent secondary injuries (Veenman, 2006). All of the successful cases of CM treatment have included physiotherapy as part of the treatment regimen. Businga, et al. (2007) reported success in treatment of three greater sandhill cranes with a combination of physiotherapy initiatives including passive leg motion, assisted hock sitting, standing and walking. A rhea was successfully rehabilitated with the use of sling therapy to allow assisted standing (Smith, et al., 2005); a similar method was utilised for the successful treatment of shorebirds (Rogers, et al., 2004) and was suggested as a therapy anecdotally as far back as 1979 (Stanyard, 1979). Water therapy has been described as a new technique in an anecdotal report, where great blue herons with CM were successful rehabilitated by utilising water cages for the birds to immerse in at rest. Apparently some birds began to recover after 3-4 days (Hausermann, 2011). For long-legged wading birds this technique sounds promising and should be further investigated. However in severely affected birds that are unmonitored this technique could lead to drowning, and in land based species this would not be appropriate therapy.

Anti-inflammatories

Corticosteroids have been popular in the treatment of CM cases in birds, in both successful and unsuccessful cases (Carpenter, et al., 1991; Tully, et al., 1996; Hanley, et al., 2005; Smith, et al., 2005; Businga, et al., 2007). In most cases the steroids were used for their perceived anti-shock value (Businga, et al., 2007) or for anti-inflammatory properties (Smith, et al., 2005). The role of shock in birds has not been elucidated, nor the use of corticosteroids to counteract the effects. It appears that their use is extrapolated from mammalian therapy, but corticosteroids have a very different range of effects in birds,

including significant effects on the Hypothalamic-Pituitary-Adrenal (HPA) axis and liver (Hess, 2002). Corticosteroids are a controversial treatment for birds due to the high chance of severe immunosuppression, even after a single dose (Quesenberry & Hillyer, 1994). Additionally, corticosteroids can increase potassium retention in birds (Oglesbee, et al., 1997, as cited in Hess, 2002), which is worrying when hyperkalemia is known to be a complication of CM.

Non-steroidal anti-inflammatory therapy has not been described as a treatment for CM in birds but has potential due to pain-relieving and anti-inflammatory properties. They have minimal immunosuppressive effects as they are selective to the cyclooxygenase (COX) enzyme pathway (Hawkins, 2006). NSAID's are commonly used for pain relief in both mammals and birds but adequate kidney perfusion and hydration must first be ensured (Paterson, 2007). Older generation NSAID's that have been utilised in birds include ketoprofen,, flunixin meglumine and phenylbutazone (Machin, 2005), with newer generation NSAID's including carprofen, meloxicam and peroxicam more commonly used in current practice (Paul-Murphy, 2006). Potential side effects include gastrointestinal ulceration and kidney lesions (Machin, 2005; Pereira & Werther, 2007). Additionally, plasma pharmacokinetics appear to vary widely with each NSAID and with different avian species, with rapid elimination in some species. (Baert & De Backer, 2003), although plasma half-life may not be closely related to efficacy (Hawkins, 2006). Although further studies are needed for NSAID use in birds (Machin, 2005), they have potential as an adjunctive therapy for pain relief, provided that adequate hydration and kidney perfusion are ensured.

Benzodiazepines

Benzodiazepines (e.g. diazepam, midazolam and zolazepam) are pharmacological agents with the effects of anxiolysis, muscle relaxation and sedation. Diazepam (Valium™, Hoffmann-La Roche) has been shown to reduce the prevalence of myonecrosis in macropods when administered intramuscularly after capture (Shepherd, 1982). Subjective positive effects were reported anecdotally when oral diazepam was administered to red knots suffering from CM with apparent improvements in recovery (Piersma, et al., 1991). In contrast, Taylor (1994) found that diazepam trialled in three godwits with CM appeared to calm the birds but did not hasten recovery time. However, these were simply observations with no control group, small numbers of birds and there are currently no known indications as to 'normal' recovery time for CM in the literature. Further investigations of benzodiazepines in the treatment of CM under controlled conditions are necessary.

Other possible treatments

Methocarbamol, a muscle relaxant, and haloperidol, a sedative, were used in the successful treatment of a rhea (Smith, et al., 2005) but have not been utilised in any other cases to date. Dantrolene sodium is used to prevent and treat malignant hyperthermia in humans and exertional rhabdomyolysis in horses (McKenzie, et al., 2004). Its activity prevents the release of calcium from the sarcoplasmic reticulum (Krause, et al., 2004), helping to prevent the intracellular hypercalcaemia that precedes myocellular necrosis. Dantrolene has not been reported as a treatment for capture myopathy in birds. Vitamin E/ Selenium are common treatments utilised in mammalian medicine for the treatment of myopathies (Paterson, 2007), and are also recommended in some avian texts (Carpenter, 2005). Vit E/Se has been used as part of the treatment regimen in CM affected birds in both successful (Businga, et al. 2007) and unsuccessful (Hanley, et al., 2005) cases. A mode of action is presumably related to antioxidant properties, membrane stabilisation (Beaton, et al., 2002) or pre-existing deficiency (Chalmers & Barrett, 1982), but the effectiveness of Vit E/Se as a preventative for damage from eccentric muscle contraction has been refuted (Beaton et al 2002) and its use as a treatment is unknown (Businga, et al., 2007). Iron dextran and multivitamins have also been used as ancillary therapies (Hanley, et al., 2005), but have not been scientifically evaluated.

The treatment methods described above are varied, yet the few successful cases are encouraging. While prevention is preferred, successful treatment protocols are imperative for rare or threatened species, or individual animals of high value in a captive setting. It is also a welfare concern for any bird suffering from this debilitating condition. Euthanasia may be required where time, environmental (e.g. field stations) or financial restraints restrict treatment options (Hanley, et al., 2005). As Paterson (2007) suggests, the more that is known about the physiological events that trigger severe myopathy, the better can treatment protocols be developed. Continued publication of observations and treatments of affected birds will allow greater refinement of treatment protocols to a successful regimen.

1.5.13 Triage and Prognosis

As CM can have a prolonged recovery period with intensive treatment requirements, it would be very useful to have pre-identified prognostic indicators such as those commonly used in wild bird triage situations (e.g. body mass, PCV or CK level). That way, triage and/or euthanasia could be elected with the help of recommended guidelines. There is currently only one paper regarding prognostic indicators in birds. Hanley, et al. (2005) suggested that birds that are unable to stand, especially after prolonged periods (12 days was suggested), have a poor prognosis. Additional suggested factors included pressure sores, nerve dysfunction such as muscle spasms and knuckling, anorexia and depression (Hanley, et al., 2005). Unfortunately all of these suggestions are subjective or common clinical signs or complications of CM in birds. Another study found that elevated CK was a significant risk factor for capture-related death (Nicholson, et al., 2000) however, the mean CK of 4,807 IU/l in that study is much lower than avian cases of CM that have made a full recovery - e.g. a peak CK of >200,000 IU/l in a rhea (Smith, et al., 2005). Further investigations of this nature are required to determine the usefulness of CK or other definitive measures as prognostic indicators.

1.5.14 Post-treatment survival

Treatment success has usually been measured as recovery to standing and release and there is little information on the long term survival or breeding success for treated birds, especially in migratory birds. Businga, et al. (2007) found that two of three successfully rehabilitated greater sandhill cranes were frequently re-sighted (and reproduced) over the following seven years. The third crane (a juvenile) was only seen for 2 days post-release, but was assumed to have survived. Rogers, et al. (2004) released eight red and great knots after rehabilitation from CM. Seven of these were seen in the field in the following weeks. The following non-breeding season, four of the eight (50%) rehabilitated birds were seen again; this rate was similar to the resighting rate of non-CM birds color-banded at the same time (52%). In contrast, Ponjoan, et al. (2008) found 43% (10/23) of turkeys that showed ataxia subsequent to capture and handling died 2 –11 days later. It is likely that deaths following capture influence the re-sighting rate of birds affected by clinical, as well as sub-clinical (undetected) capture myopathy (Williams and Thorne, 1996). Further long-term studies that determine the fates of birds released after treatment for capture myopathy are needed.

1.6 RESEARCH NEEDS CITED IN LITERATURE

It is evident that there are several areas of needed research relating to capture myopathy in birds. The correlation between the severity of clinical signs, degree of muscle enzyme elevation (particularly of creatine kinase) and mortality is currently unknown, with several reports showing conflicting evidence and apparent species variation. In particular, comparative and repeated measures studies of CK after capture and handling or during treatment for CM could provide greater information on: 1) the clinical picture and significance of muscle damage in birds following capture by different methods, 2) the timing of peak values including the investigation of a biphasic CK response in birds, 3) the correlation between CK values at the time of capture and the development of signs of myopathy and thus the use of CK as a predictor of risk, and 4) the correlation between peak CK levels, clinical signs of CM, prognosis and survival. Repeated measures of other biochemical analytes, including potassium, sodium and uric acid are needed to assess the clinical relevance of these parameters in the pathogenesis, diagnosis and treatment of CM and its sequelae.

It is not clear why some birds succumb to CM while others do not under the same conditions and the factors that increase susceptibility. Further robust studies evaluating risk factors in birds are essential to understanding these factors. A particular area for investigation includes comparing more capture techniques for a given species, especially involving mist-net capture which is one of the most common methods for avian capture worldwide. Other potential risk factors worthy of investigating include body mass, signalment (age/sex), environmental risk factors, comparative studies in which different bird species are caught under the same conditions, and underlying disease. As many of the current recommendations for prevention of CM are vague or unsubstantiated, results of risk factor studies could be utilised to provide recommendations for prevention.

The literature contains only a few reports of successful treatment of CM in birds, and only one contains long term results where birds have bred successfully for several years. Recommended areas for investigation of treatment methods include physiotherapy, fluid therapy recommendations, hydration monitoring (such as PCV/TP measures), use of NSAID's and investigation of benzodiazepines in the treatment of CM under controlled conditions. The usefulness of CK or other definitive measures as prognostic indicators and the long-term fates of birds released after treatment for CM are needed, as determined by such factors as breeding success, migration and survival.

1.7 SHOREBIRD SPECIES IN THIS STUDY

The shorebirds studied in this thesis include bar-tailed godwits, red knots and great knots, all of which are long-distance migratory birds which 'over-winter' in New Zealand and Australia. These birds traverse the circuit known as the East Asian-Australasian (EAA) Flyway on an annual basis (Bamford, et al., 2008). The following gives a brief description of each study species, its basic biology, estimated population size and migration routes.

1.7.1 Bar-tailed Godwits (*Limosa lapponica*)

Bar-tailed godwits, maori name "Kuaka", are a wading bird of the order Charadriiformes (the sandpipers) (**Figure 3**). The genus *Limosa* contains 4 species, the black-tailed godwit (*Limosa limosa)*, the Hudsonian godwit (*Limosa haemastica*), marbled godwit (*Limosa fedoa*) and the bar-tailed godwit (*Limosa lapponica*), the latter of which has five known sub-species (Woodley, 2009). All godwit species migrate annually, breeding in the northern hemisphere (including Scandinavia, Siberia and Alaska). They migrate southwards to 'overwinter' in Europe, Africa, South America, the Pacific Islands and Australasia (Heather & Robertson, 1996) - an annual migration cycle of over 29,000 km (Woodley, 2009). Bar-tailed godwits, *L.l. baueri* and *L.l. menzbieri* have the greatest southbound journey – over 11,000km, predominantly from Alaska and eastern Siberia, to New Zealand and Australia, respectively (McCaffery & Gill Jr., 2001). Bar-tailed godwits are the most common Arctic migrant seen in New Zealand, with birds arriving from September and leaving again in March (Battley, 1997), stopping over at the Yellow Sea feeding grounds, to arrive in the breeding grounds in late May to early June (Higgins & Davies, 1996). Godwits are gregarious birds found in large flocks and feed on polychaete worms and molluscs in tidal mudflats and estuaries (Battley, 1996).

Godwits have a slim-to medium build, with long legs, a small head and a long, slim, bill for probing sand. They have speckled brown to grey and white plumage with russet coloured chests in the breeding season (Woodley, 2009). Bar-tailed godwits are sexually dimorphic with females being significantly larger in size measurements, including tarsal, wing and bill lengths, and heavier than males (Battley & Piersma, 2005). The average non-breeding body mass for females is approximately 330 grams, and males roughly 280grams (Conklin, et al. 2011). In the immediate pre-migratory period, body mass of female godwits increases dramatically to 550g (range 487-624g) and males to almost 450g (rage 377-503g), with fat loading of up to 45% of body mass in both sexes (Battley & Piersma, 2005).

Although bar-tailed godwits are considered to be a species of concern to some groups (PRBO Conservation Science, 2008) they are listed as "least concern" on the IUCN Red List (IUCN, 2012). Estimates of godwit numbers have been elucidated by several authors, yet there are discrepancies between different groups. Bamford et al. (2008) estimated a stable godwit population of approximately 325,000 (155,000 *L.l. baueri* and 170,000 *L.l. menzbieri*) in the EAA flyway. However, there is mounting evidence that godwit numbers are less than this and declining. A study by Sagar et al. (1999) estimated a NZ population of approximately 102,000 birds. Only 71,000 *L.l.baueri* were recording in the NZ national census in 2008 (Woodley, 2009) and a decrease of approx. 20,000 birds was found in censuses from 1994-2005 (Melville & Battley, 2006). Morrison et al. (2006) also suggested a declining Alaskan breeding population of approximately 90,000 *L.l. baueri,* a reduction from 120,000 estimated in the late 1990's. Similarly, Gill & McCaffery (1999) reported a post-breeding Alaskan population size of *L.l. baueri* of approx. 94,000 birds. Significant declines in bird counts have also been found for bar-tailed godwits recorded in Japan over 10, 20 and 30 year datasets (Amano, et al., 2010). Estimates of *L.l. menzbieri* are of 170,000 birds, with the majority in the northwest of Western Australia (Barter, 2002). A proportion of godwits stay over the NZ winter, mainly juveniles (Heather & Robertson, 1996), with numbers averaging 10,000-17,000 birds (Sagar, et al., 1999).

Figure 3. A bar-tailed godwit male (*Limosa lapponica*). *Photo Phil Battley*

1.7.2 Red Knots (*Calidris canutus*)

Red knots also known as "Huahou" (Maori) or "lesser knot" are the second most numerous arctic migrant to overwinter in New Zealand (Heather & Robertson, 1996). Two of the six subspecies occur in the EAA Flyway: *C.c. rogersi,* which nests in far-eastern Siberia; and *C.c. piersmai,* which nests on the New Siberian Islands (Tomkovich, 2001). It is thought that *C.c. piersmai* is found largely in Western Australia in the non-breeding season, with *C.c. rogersi* migrating to eastern Australia and New Zealand, though they do overlap (Rogers, et al., 2010) and *C. c. piersmai* reaches New Zealand. *C.c. rogersi* tend to arrive in September and depart in March/ April (Battley, 1997), while the western Australian counterparts do not leave until May (Battley, et al., 2005). Their migration route takes them via the Yellow Sea, to arrive in Siberia in June (Tomkovich & Soloviev, 1996), a journey of over 10,000km in a month (Battley, et al., 2005). Similar to the godwits, a small proportion of birds (7-15%) will stay throughout the southern hemisphere winter (Heather & Robertson, 1996).

In New Zealand, red knots are found in large flocks, often with bar-tailed godwits, in inlets, harbours and estuaries of coastal New Zealand (Heather & Robertson, 1996). A relatively small shorebird of 23-26cm height (Pizzey, 1997), males and females are not sexually dimorphic. Red knots have southern hemisphere arrival weights of approximately 88 grams (Barter, 1992 as cited in Battley, 1997), fattening to approximately 185 grams at departure for northward migration (Battley & Piersma, 1997). A plain speckled grey colour, with a short black bill (3cm) and short greenish legs, the face and body turn a rich chestnut colour when in breeding plumage (Pizzey, 1997). This species feeds on bivalves, molluscs and gastropods (Battley, 1996; Heather & Robertson, 1996), as well as worms and crabs found in the tidal mudflats (Tulp & de Goeij, 1994).

Bamford et al. (2008) gave an estimated stable EAAF population of 220,000 red knots. Similarly, Watkins (1993) estimated approximately 88,000 red knots migrate annually to NZ out of a total population of approximately 255,000 birds. However, more recent estimates of approx 42,000 (27,300 - 66,500) birds in NZ (Southey, 2009) and totals of approx. 105,000 birds in the EAAF may represent a significant population decline since the 1980's (Rogers, et al., 2010). Other subspecies found in North America also appear to have undergone drastic population declines, noted in many over-wintering sites (e.g. Morrison, et al., 2006). Interestingly, a study conducted in Japan showed small but significant increases in numbers of red knots observed over a 10 and 30 year period to 2008 (Amano, et al., 2010).

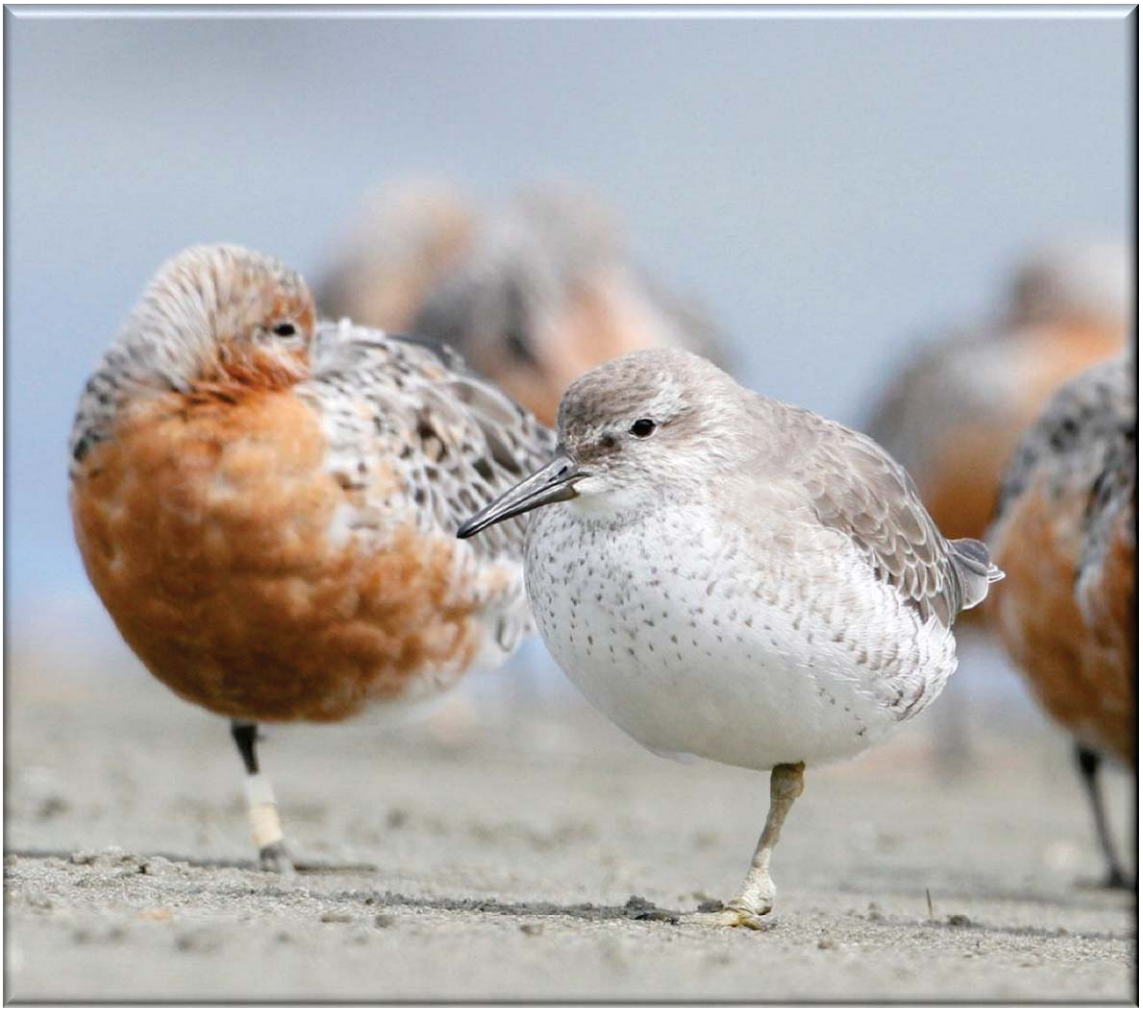

Figure 4. Red knots (*Calidris canutus*). *Photo Phil Battley*

1.7.3 Great Knots (*Calidris tenuirostris)*

Great Knots are another long-haul migrant sandpiper, which breed in north-east Siberia and Russia from late May to early August and migrate southwards from late June to early September annually (Tomkovich, 1997). Their destinations include India, Asia, Indonesia and Australia; predominantly the Northern Territory and Western Australia, although they also stopover in other parts of coastal Australia and Tasmania (Pizzey, 1997). Immature birds do not migrate back to the breeding grounds, remaining in the southern hemisphere for up to 4 years. (Battley, et al., 2004).They are a rare stray to New Zealand, often found roosting or feeding with large flocks of red knots (Heather & Robertson, 1996) on tidal mudflats, estuaries, shoreline and wetlands (Pizzey, 1997). Great knots feed predominantly on bivalves, but also will eat gastropods, worms, crabs, shrimp and sea cucumbers (Tulp & de Goeij, 1994).

They are slightly larger (26-28cm) than the red knot (Pizzey, 1997), with a 4cm black bill (Heather & Robertson, 1996), a grey- brown plumage with dark speckles and pale underparts. Females are moderately (approximately 20 grams) larger and heavier than males (Battley, et al., 2004). Similar to the other two species above, birds develop a reddish breeding plumage on the chest (Pizzey, 1997). The birds fatten from a mean non-breeding weight of approximately 140grams (Barter & Minton, 1998) to approximately 250grams prior to migration (Higgins & Davies, 1996).

Watkins, (1993) estimated a world population of approximately 319,000 great knots, while 380,000 birds were estimated by Bamford et al. (2008). In contrast, Barter et al. (2002) estimate the global population to total 290,000 great knots – these were from count data conducted in the Yellow Sea and the authors conceded that counts may have been underestimates. However, the global great knot population has almost certainly declined dramatically since 2006 due to the Saemangeum reclamation project in South Korea, which destroyed the most important staging site for great knots. Up to 90,000 great knots were displaced by this single reclamation project, and local and nationwide surveys in Korea ondicate these birds likely perished rather than relocated to other sites (Moores, et al., 2008)

It has been reported that many shorebird species are in population declines worldwide. For example, a study of 35 species of shorebird showed declines in 80% of the species (Morrison, et al., 2001). Population declines in migrant species represent a real-time example of wildlife as sentinels for environmental change. One of the major threats to shorebird survival is habitat loss and degradation (Rogers, et al., 2010). Particularly of

concern are reclamation projects occurring in the Yellow Sea region of China, an area that that currently supports large populations of northbound migrating shorebirds on the East Asian-Australasian Flyway (Rogers, et al., 2010). Climate change may also contribute to the decline of some species of migratory birds (Both & Visser, 2001). While some of the important habitats have been protected under the RAMSAR Convention (Woodley, 2009), in order to ensure the survival of migratory shorebirds it is of utmost importance to identify and protect their routine and emergency staging grounds (Rogers, et al., 2010).

While there are many studies investigating shorebird biology and ecology, there are very few studies that assess shorebird health or disease and even fewer that give quantitative results for shorebird responses to capture. Evidence to date suggests that several shorebird species are susceptible to capture myopathy. This research provides an opportunity to further investigate shorebird responses to capture and provide recommendations for prevention or treatment of capture myopathy.

Figure 5. A great knot (*Calidris tenuirostris*). *Photo Craig Steed.*

1.8 RESEARCH OBJECTIVES

The aims of this research are to provide further information on capture myopathy in shorebirds, by:

1a) Investigating the use of haematology and plasma biochemical parameters including creatine kinase (CK), aspartate aminotransferase (AST), uric acid (UA) and potassium (K+) to detect clinical and subclinical effects of capture techniques in shorebirds.

1b) Investigating risk factors for capture myopathy by comparative analysis of biochemical indicators of muscle damage for:

I. three species of shorebirds (bar-tailed godwits, red knots and great knots)

II. birds captured using mist-netting and cannon-netting

III. birds captured in differing environmental conditions and over-wintering sites (e.g. Miranda and Foxton Beach, New Zealand and Broome, Australia) (**Figure 6**), and

IV. birds at different stages of their life history (e.g. overwintering arrival and departure)

2) Investigating treatment methods for any birds that develop clinical signs of capture myopathy for the duration of the research period.

I. Investigate the use of midazolam, a benzodiazepine, as an ancillary treatment for capture myopathy

II. Determine prognostic indicators for survival to release and investigate long-term indicators of success for those birds, such as survival (re-sightings) migration, and breeding success

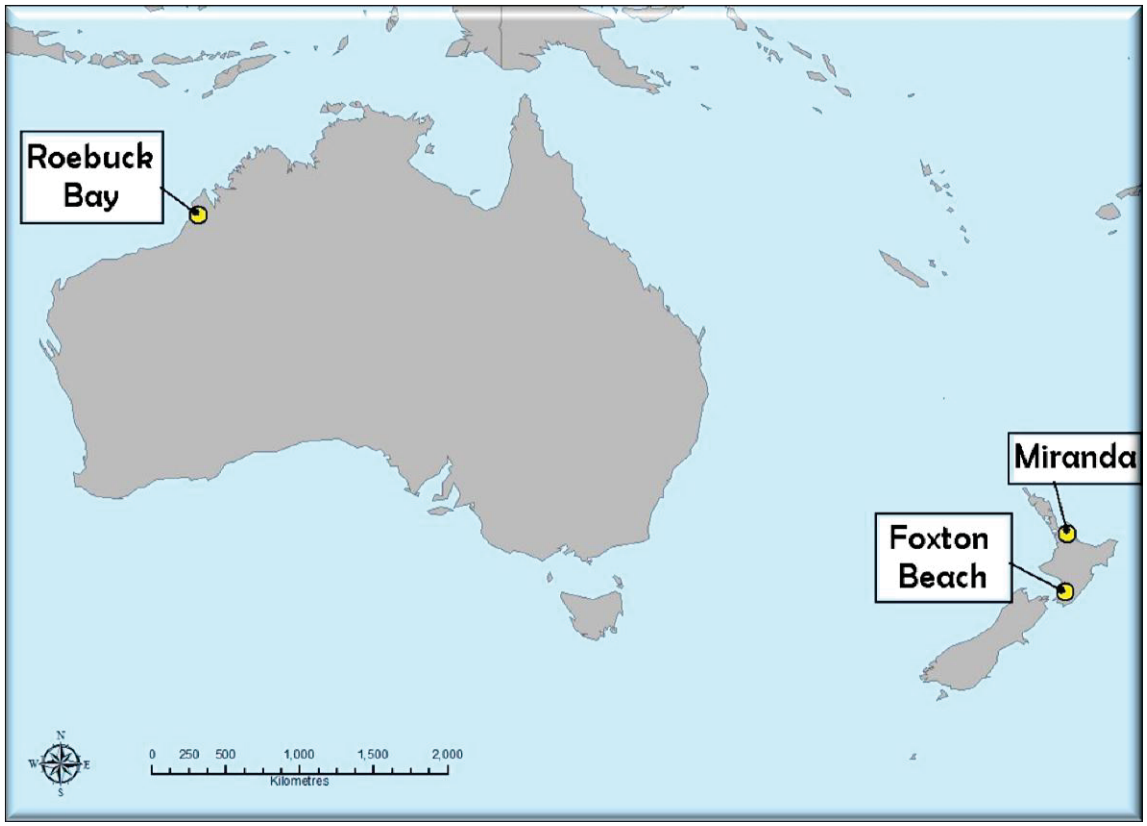

Figure 6. Map of Australia and New Zealand showing the proposed catching locations: Miranda and Foxton Beach, New Zealand; and Roebuck Bay, near Broome, Western Australia.)

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Chapter Two

RISK FACTORS FOR CAPTURE MYOPATHY IN SHOREBIRDS

Risk Factors for Capture Myopathy in Shorebirds
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Chapter Two: *Risk Factors for Capture Myopathy in Shorebirds*

ABSTRACT:

Risk factors for the occurrence of capture-myopathy and capture-related physiological changes were investigated in bar-tailed godwits (*Limosa lapponica*), red knots (*Calidris canutus*) and great knots (*C. tenuirostris*). Serial blood samples, taken immediately post-capture and prior to release, were examined for changes in the plasma concentrations of creatine kinase (CK), aspartate aminotransferase (AST), uric acid (UA) and potassium (K+). Comparisons were made for two capture methods: mist-netting and cannon-netting. Environmental factors were investigated by comparing cannon-net captures in 3 locations with differing ambient temperatures. We found marked increases in plasma concentrations of CK in godwits and great knots following capture and banding, with cannon-net captures causing significantly greater elevations than mist-net captures in godwits. In godwits, sex or body mass differences did not affect the changes in plasma CK concentration. Godwits caught in high environmental temperatures did not show greater elevations in CK concentration than cooler locations, but did show significantly elevated plasma concentrations of AST. This suggests that heat stress did not exacerbate muscle damage but lead to greater generalized tissue or organ damage. Plasma concentrations of uric acid showed a significant decline in the post-capture period that may relate to interruption of digestion due to acute stress. Both knot species showed greater initial UA concentrations than godwits that may reflect species variations in prey digestion or protein metabolism. Plasma concentrations of potassium increased in godwits after capture suggesting release from damaged cells or metabolic acidosis. However, potassium results were significantly influenced by the timing of sampling. During the research period, 11/107 (14%) of godwits and 3/35 (8.6%) of red knots showed mild to severe signs of paresis indicating the susceptibility of both of these species to capture myopathy. The majority of these birds (71.4%) were caught by cannon net. Our results imply that common capture techniques have significant effects on the muscular, digestive and homeostatic physiology of shorebirds. Based on this study, we recommend the use of light, fine mesh nets for cannon net capture and to avoid the use of entanglement nets. It would be preferable to use mist-nets where possible for susceptible shorebirds.

Key Words: Aspartate aminotransferase, bar-tailed godwit, *Calidris spp.,* cannon-net, capture, capture myopathy, creatine kinase, electrolyte, great knot, *Limosa lapponica*, mist-net, physiology, potassium, red knot, rhabdomyolysis, shorebird, uric acid.

2.1 INTRODUCTION

Capture of wild birds occurs commonly throughout the world. The reasons for capture often relate to scientific purposes such as research, ornithology, disease screening and conservation practices, such as translocations. Whilst commonplace, many wild bird capture techniques are far from innocuous. Reported complications include severe stress (Lynn & Porter, 2008), injuries (Spotswood, et al., 2012) and deaths (Cox Jr. & Afton, 1998). Of all the potential concerns, capture myopathy is regarded as one of the most important (Williams & Thorne, 1996). Clinical signs of capture myopathy include depression, anorexia, inability to fly and paresis, or complete paralysis (Smith, et al., 2005; Businga, et al., 2007), with high levels of mortality following in days to weeks following capture (Carpenter, et al., 1991; Höfle, et al., 2004; Marco, et al., 2006). Mortality is typically due to debilitating muscle necrosis, metabolic disturbances, and kidney or heart failure (Spraker, 1993). Secondary predation is common in affected birds, subsequent to physical impairment (Cox Jr. & Afton, 1998; Ponjoan, et al., 2008). Worryingly, mortality has been attributed to capture myopathy in birds released without any clinical signs (Windingstad, et al., 1983) and subclinical myopathy may be occurring in more cases than previously recognized (Spraker, et al., 1987).

While it is postulated that any species could develop capture myopathy (CM) (Williams & Thorne, 1996), long-legged bird species such as cranes (Businga, et al., 2007), ratites (Smith, et al., 2005) and bustards (Marco, et al., 2006) are more commonly reported in the literature. Other affected species include turkeys (Spraker, et al., 1987), waterfowl (Cox Jr. & Afton, 1998), partridge (Höfle, et al., 2004) and shorebird species such as red knots (*Calidris canutus*), great knots (*C. tenuirostris*), bar-tailed godwits (*Limosa lapponica*) (Rogers, et al., 2004) and curlews (*Numenius spp.*) (Stanyard, 1979).

The pathological process that leads to muscle cell breakdown is triggered by energy (ATP) depletion and direct or indirect damage to the muscle cell membrane (sarcolemma). This interrupts calcium metabolism, leading to toxic intracellular calcium accumulation and myocellular death (Zager, 1996). Cellular metabolites including creatine kinase (CK), aspartate aminotransferase (AST) and potassium (K+) are released into the blood stream (Zager, 1996). CK and AST are thus useful as indicators of muscle damage due to capture (Dabbert & Powell, 1993; Ponjoan, et al., 2008), with CK reported to be the most specific and sensitive indicator of muscle damage in birds (Franson, et al., 1985). Studies have shown that plasma concentrations of CK begin to rise quickly in birds following capture (Bailey, et al., 1997) with peak levels occurring from twelve (Cardinet, 1989) to twenty-four hours (Bailey, et al., 1997) in the absence of ongoing necrosis (Cardinet, 1989). Avian

cases of CM have had delayed CK peaks of 24 hours (e.g. Ward, et al., 2011) up to three days after capture with AST peaks following approximately 24 hours later (e.g. Businga, et al., 2007). Both metabolites slowly decrease over time (e.g. Smith, et al., 2005), but the correlation between enzyme levels and the degree of muscle damage (Bollinger, et al., 1989) or potential for recovery is still unclear .

High blood potassium (hyperkalaemia) has been described in both mammals (Kock, et al., 1987) and birds (Hanley, et al., 2005) with capture myopathy. Additionally, severe hyperkalaemia due to massive cell lysis can result in cardiac arrest (Guis, et al., 2005). The role of potassium in the pathogenesis or diagnosis of CM deserves further investigation. Uric acid (UA) is the main metabolite of nitrogen metabolism and a measure of renal function in birds (Fairbrother, et al., 1990). There is currently little information regarding changes in plasma UA concentrations in birds subsequent to capture and handling. Acute renal failure as a sequelae to myopathy has been recorded in humans (Clarkson, 2007) and other mammals (Herraez, et al., 2007) and is thought to be due to a combination of renal hypo-perfusion, cast formation and myoglobin damage (Zager, 1996). Renal pathology has rarely been described in birds (Windingstad, et al., 1983; Tully, et al., 1996), with only one case showing histological changes suggestive of myoglobin damage (Hanley, et al., 2005).

Treatment of CM is seldom successful, requiring intensive therapy for up to several weeks (Rogers, et al., 2004; Smith, et al., 2005; Businga, et al., 2007; Ward, et al., 2011) and there is little known about the long-term effects on health and reproduction. Prevention of CM is preferred; however there is a paucity of information regarding risk factors. Additionally, it can be difficult to isolate aetiological factors due to the complex interaction of events at the time of capture. Cannon-net capture has been proposed as a risk factor for bustards (Ponjoan, et al., 2008) and mallards (Dabbert & Powell, 1993) and drop-net capture for turkeys (Spraker, et al., 1987). Juveniles may be more susceptible than adult birds (Spraker, et al., 1987; Ponjoan, et al., 2008). Prolonged handling and restraint times have been reported as significant risk factors in several species (Southern & Southern, 1983; Bollinger, et al., 1989; Nicholson, et al., 2000; Ponjoan, et al., 2008). Environmental factors such as temperature and humidity may also be risk factors for CM (Nicholson, et al., 2000; Höfle, et al., 2004), yet studies of environmental variables to date have conflicting results and further research is required.

This study aimed to:

1) Investigate the effects of mist-netting and cannon-netting on the changes in plasma concentrations of CK, AST, K+ and UA in bar-tailed godwits, red knots and great knots.

2) Use these parameters to evaluate risk factors for capture myopathy including capture method (mist vs. cannon nets), species (godwits vs. red or great knots), sex, body mass and location differences (as a measure of environmental conditions).

2.2 MATERIALS AND METHODS

This study took place in New Zealand (NZ) and Australia on several capture events from 01 September 2008 to 27 September 2009. Rather than targeted captures, our blood sampling studies were appended to ongoing capture and banding studies by the Australasian Wader Study Group (AWSG), the Ornithological Society of New Zealand (OSNZ) and the Massey University Ecology Group (NZ). Details of the capture locations and environmental temperatures are presented in **Table 1**. Results were pooled for captures by the same method, in the same locations. Bar-tailed godwits (*L.l. baueri*) and red knots were caught in New Zealand, whilst bar-tailed godwits (*L.l. menzbieri*) and great knots were caught in Australia. Relevant permits were obtained from the NZ Department of Conservation, NZ Ministry Department of Primary Industries, Australian Department of Environment, Water, Heritage and the Arts, Western Australian Department of Environment and Conservation and the Massey University Animal Ethics Committee (NZ).

Mist-net captures were conducted at dusk, with fine mesh nets erected over roosting ponds. Two to three nets were erected and constantly monitored. Birds that flew into the net were promptly removed, placed in holding boxes and brought over a short distance (approx. 100m) to the field bleeding station for sampling. The first blood sample was taken as soon as possible after capture to provide baseline (reference) levels of the analytes and the second sample was used to compare changes to the analytes over time. The time to the first sample was recorded as the time from the birds encountering the net until sampling, using individual stop-watches for each bird. Birds were then placed back into holding boxes to wait for processing and banding. Cannon-net captures occurred in the daytime in all locations with mid to late morning firing, using enveloping type cannon-nets. However, for the Foxton capture a combination of enveloping (fine mesh) and entanglement (larger mesh) nets were used. The time to first blood sample was recorded as the time from the firing of the cannon net until sampling. Birds extracted from beneath the cannon-net first were preferentially sent for blood sampling, due to the requirement for rapid first samples. Once retrieved from beneath the net, birds were treated similarly to the mist-netted birds.

Individual birds were identified by temporary numbered leg-tags which were matched to banding combinations prior to removing the leg-tag. Sex was determined through morphometric measurements and body mass (Conklin, et al., 2011). Once processed and banded, most birds were placed in holding boxes for short periods prior to the second sample to allow recovery from banding, or to wait in a queue when numbers were high. The second blood sample was taken just prior to release and the time recorded. Birds

were monitored at release for signs of physical impairment, such as poor flight, staggering walk or inability to walk/fly and findings were recorded.

Blood samples were taken from the brachial/ulnar veins of either wing, or the right jugular vein, using a 1 ml syringe and 25 gauge needle. Between 0.2 ml and 0.8 ml was collected from each bird. The blood was transferred to lithium heparin microtainers (Becton Dickenson Vacutainer Systems, Preanalytical Solutions, Franklin Lakes, NJ 07417) and stored chilled until it could be processed after the capture event. Between 2-10 hours after collection, the blood was separated via centrifugation and the plasma transferred to plain microtainers and immediately frozen to -20°C. Biochemistry analysis was performed at New Zealand Veterinary Pathology (NZVP, IVABS Building, Massey University, Palmerston North, New Zealand) from 4 days - 4.5 weeks (Broome samples only) after sampling. The biochemical parameters measured included creatine kinase (CK), aspartate aminotransferase (AST), potassium (K+) and uric acid (UA). Data were analysed only if there were repeat measures for each bird: Interval 1 (first blood sample) and Interval 2 (second blood sample). Haemolysed samples were not included in the dataset for CK or K+ due to possible artefactual elevations. Due to predicted rapid increases of biochemical parameters in the blood, any Interval 1 samples greater than 40 minutes after capture were not included in the analyses. All Interval 2 samples were included in the analyses, unless they were unpaired.

Statistical methods

Statistical analyses were performed using SPSS v20 (IBM-SPSS Inc.). All results are presented as means ± one standard error (SE) unless otherwise stated. For godwits and red knots, all repeated measures of CK, AST, K+ and UA were assessed for significant differences over time and between groups using a multivariate or univariate repeated measures ANOVA. The time to first and second sample was included as a covariate in the ANOVA to evaluate for the effects of the variance in the timing of sampling. For the great knot analyses, we used an ANOVA within and between sample intervals to check for changes in biochemical parameters over time. If there was a significant covariate effect of time to sampling, then no further analysis was possible. A significance level of $P<0.05$ was assumed. The frequency of occurrence of CM between species was compared by Chi-squared analysis.

Table 1: Location, capture method, dates and temperatures associated with the capture of bar-tailed godwits (*Limosa lapponica*), great knots (*Calidris tenuirostris*) and red knots (*Calidris canutus*) in New Zealand and Australia 2008-2009

LOCATION	CAPTURE METHOD	DATE	TEMPERATURE			
			MIN DAILY in °Celcius	MAX DAILY in °Celcius	NORMAL MONTHLY MEAN (Min-Max) [6]	CATCH TEMP (approx) in °Celcius[4,5]
Miranda, NZ[1] (-37.18°S, 175.32°E)	Mist-net	1 Sept 2008	7	16.5	12.6 (8.7-16.5)	9.1-11.0
	Mist-net	2 Sept 2008	8.5	17	12.6 (8.7-16.5)	9.3-11.8
	Mist-net	3 Sept 2008	8.5	18	12.6 (8.7-16.5)	9.6-11.9
	Mist-net	3 Oct 2008	10	19	14.4 (10-6-18.3)	12.7- 13.2
	Mist-net	4 Oct 2008	13	17	14.4 (10-6-18.3)	12.6- 13.5
	Mist-net	23 Aug 2009	8	18	11.2 (7.3-15.1)	10.5-11.8
	Mist-net	8 Sept 2009	3.5	16	12.6 (8.7-16.5)	5.0-10.3
	Mist-net	25 Sept 2009	14	19.1	12.6 (8.7-16.5)	7.9-11.6
	Mist-net	26 Sept 2009	13	18.5	12.6 (8.7-16.5)	11.0-12.0
	Mist-net	27 Sept 2009	9	17	12.6 (8.7-16.5)	11.3-11.8
	Cannon-net	29 Jan 2009	14.4	24.8	19.7 (15-24.3)	19.1-22.7
Foxton Beach, NZ[2] (-40.47°S, 175.22°E)	Cannon-net	30 Oct 2008	6	16.7	12.6 (8.8-16.4)	No hourly temps avail. Was during warmest hours of the day (pers. obs.).
Roebuck Bay, Western Australia[3] (-18.00°S, 122.37°E)	Cannon-net	22 Feb 2009	24.1	33.2	(26.0-32.9)	32-34
	Cannon-net	23 Feb 2009	25.5	35.1	(26.0-32.9)	33-34
	Cannon-net	24 Feb 2009	29.1	34.2	(26.0-32.9)	34-35
	Cannon-net	25 Feb 2009	29.1	33.2	(26.0-32.9)	32-33

1 Temperature data derived from National Institute for Water and Atmospheric Research (NIWA) climatic database - Thames 2 station 20.6km from capture site. http://cliflo.niwa.co.nz/

2 Temperatures data derived from National Institute for Water and Atmospheric Research (NIWA) climatic database. Levin AWS station 17.2km from capture site.

3 Tempearture data derived from Commonwealth of Australia 2012, Bureau of Meteorology. Broome Airport weather station, approx 10-14km from capture sites. Prepared using Climate Data Online, Bureau of Meteorology http://www.bom.gov.au/climate/data.

4 New Zealand hourly temperature data from Pukekohe AWS
Station 40.4 km from capture site

5 Australian catch temperatures measured at site with digital thermometers (x 2) in the shaded processing area and inside bird boxes.

6 Normal monthly mean derived from National Institute for Water and Atmospheric Research (NIWA) climatic database (NZ), average monthly temperature for 1971-2000; and Commonwealth of Australia 2012, Bureau of Meteorology (Broome WA 1939-2012) http://www.bom.gov.au/climate/averages/tables/cw_003003.shtml

2.3 RESULTS

Bar-tailed godwits and red knots were caught by both capture methods in New Zealand, while great knots were only caught by cannon-net in Australia. Banding and handling occurred from 30 mins up to 4.5 hours after capture, depending on the numbers of birds caught simultaneously. Time to the first blood samples (Interval one) included in the dataset were from a minimum of two minutes to a maximum of 39 minutes post-capture, with the time to second blood samples (Interval two) taken from a minimum of one hour and maximum of five hours from capture.

2.3.1 Bar-tailed godwits

A total of 107 godwits were captured over the research period, of which 39 paired results were suitable for analysis for capture by cannon-netting and 29 paired results for mist-netting. Six of 107 (5.6%) godwits developed mild signs of myopathy including unsteady or ataxic gait when released. Four of these were Miranda mist-net captures and two were Broome cannon-net captures. Nine of 107 (8.4%) godwits developed severe signs of myopathy including inability to walk or fly. They were all from the Foxton cannon-net capture. These nine and a further eleven godwits from the same catch (but not included in this dataset) were hospitalized for treatment (see Ward, et al., 2011).

Creatine Kinase

Scatter plots of plasma CK concentration taken in interval one, prior to removing unpaired and late samples, are shown in Figure 1. Plasma concentrations of CK start to become elevated compared to earlier samples after approximately 40 minutes (**Figure 1a**). Plasma CK concentrations in the second interval have greater variation and larger peaks (**Figure 1b**). The plasma concentration of CK showed a significant increase over time for all locations ($F_{1,2,34}$ =4.44, p=0.043) and by both methods of capture ($F_{1,1,64}$ =9.67, p=0.003). The peak concentration of plasma CK ranged from 422 to 26,353 IU/l. There were no significant differences in this pattern of plasma CK elevation due to sex ($F_{1,1,39}$ =1.04, p=0.313) or body mass ($F_{1,1,39}$ =0.03, p=0.874) in godwits. For cannon-netting, there was no effect of location ($F_{1,2,34}$ =0.005, p=0.995) for the degree of CK elevation (**Figure 2a**). Therefore, location results could be pooled in order to compare capture methods. Significantly greater CK levels were associated with cannon-netting than mist-netting for the second sample only ($F_{1,1,64}$ =6.75, p=0.012) (**Figure 2b**). There were interaction effects between time and capture method ($F_{1,1,64}$ =8.94, p=0.004), due to the significant effect of time to sampling for the first sample ($F_{1,1,65}$ =8.39, p=0.005), but the results were unaffected by the timing of the second sample ($F_{1,1,65}$ =1.31, p=0.257).

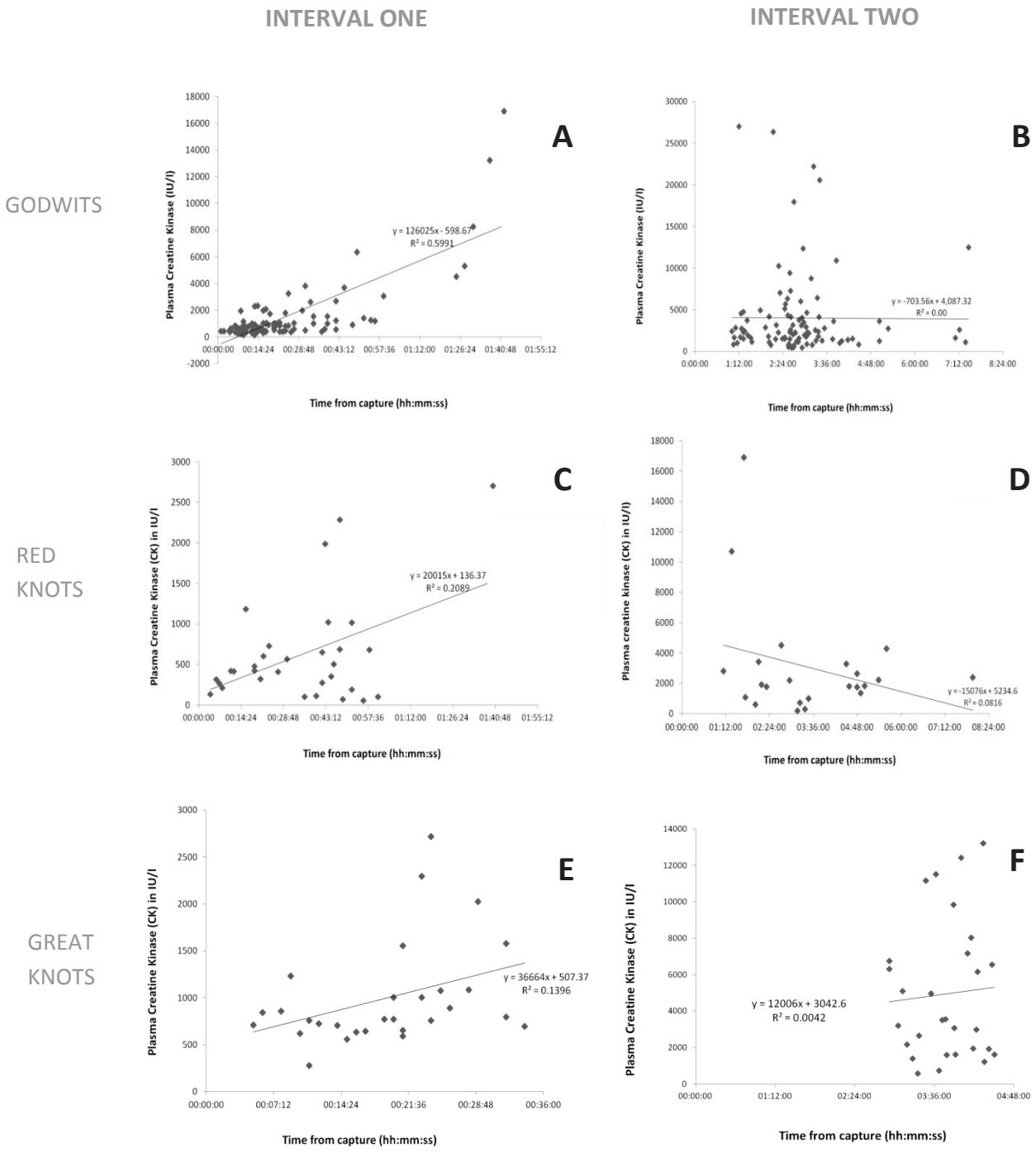

Figure 1. Scatter plots of the plasma concentration of creatine kinase (CK) against the time from capture in bar-tailed godwits (*Limosa lapponica*) **(1A, B),** red knots (*Calidris canutus*) **(1C, D)** and great knots (*Calidris tenuirostris*) **(1E, F)** caught by cannon and mist-nets in New Zealand and Australia 2008-2009. The first blood samples (Interval 1) are shown in graphs **1A, C, E** and the second blood samples (Interval 2) are shown in graphs **1B, D, F.**

Aspartate Aminotransferase

For godwits caught by cannon-netting, there were significant differences in plasma AST concentration changes between locations ($F_{1,2,41}$ =3.30, p=0.047). However this was complicated by interaction effects of time and location ($F_{1,2,41}$ =4.93, p=0.012). *Posthoc* analysis revealed that location was not important for the first sample ($F_{2,42}$ =0.105, p=0.900), but that samples collected from birds in Broome showed a greater elevation of plasma concentration of AST in the second blood sample ($F_{2,42}$ =5.26, p=0.009, **Figure 2c**). Due to location differences, the effect of capture methods were only compared for birds captured at a single location, Miranda. There was no significant effect of time ($F_{1,1,43}$ =1.92, p=0.173) or capture method ($F_{1,1,43}$ =0.04, p=0.848) on the changes in plasma concentration of AST (**Figure 2d**). Additionally the AST results were significantly influenced by the variability in timing of the sample collection, with the timing of collection for the first ($F_{1,1,43}$ =8.90, p=0.005) and second ($F_{1,1,43}$ =8.44, p=0.006) blood samples confounding the results.

Uric Acid

There was a decline in the plasma concentrations of uric acid over time ($F_{1,1,39}$ =10.113, p=0.003) in godwits (**Figure 2e**). The time from capture to first sample had a significant effect ($F_{1,40}$ =6.51, p=0.015) but not for the second sample ($F_{1,40}$ =0.533, p=0.469). For cannon-net caught godwits, the location had a significant effect on the plasma concentrations of UA ($F_{1,2,38}$ =4.92, p=0.013), but confounding factors included the time taken to gain the first blood sample ($F_{1,38}$ =8.69, p=0.005) and the Foxton location ($F_{2,39}$ =6.43, p=0.004), both of which had significant effects the first sample. In contrast, the second sample was not affected by the location ($F_{2,39}$ =2.65, p=0.084), or by the sampling interval ($F_{1,39}$ =0.04, p=0.845). Due to potential location differences, the effect of capture methods were only compared for birds captured at a single location, Miranda. Capture method affected changes in plasma concentrations of UA, with mist-netting associated with a significantly greater decline in UA concentration than cannon-netting ($F_{1,40}$ =5.54, p=0.024) (**Figure 2f**).

Potassium

There was a significant effect of time ($F_{1,2,54}$ =73.27, p<0.001) and capture location on the plasma concentrations of potassium ($F_{1,2,54}$ =17.264, p<0.001) (**Figure 2g**). However, the results were confounded by the significant effect of time to first sample ($F_{1,2,54}$ =14.736, p<0.001). Potential location difference meant that capture method was only compared at the Miranda location. There was a significant increase in the plasma concentration of potassium over time ($F_{1,1,28}$ =9.55, p=0.004) that was not (quite) significantly affected by

capture method ($F_{1,1,28}$ =3.55, p=0.070) (**Figure 2h**). However, there was a significant effect of time to sampling on the first blood sample ($F_{1,1,28}$ =10.77 p=0.003), therefore comparisons between Interval 1 and 2 were confounded. Additionally, there was a significant interaction between time and capture method ($F_{1,1,28}$ =19.79, p<0.001).

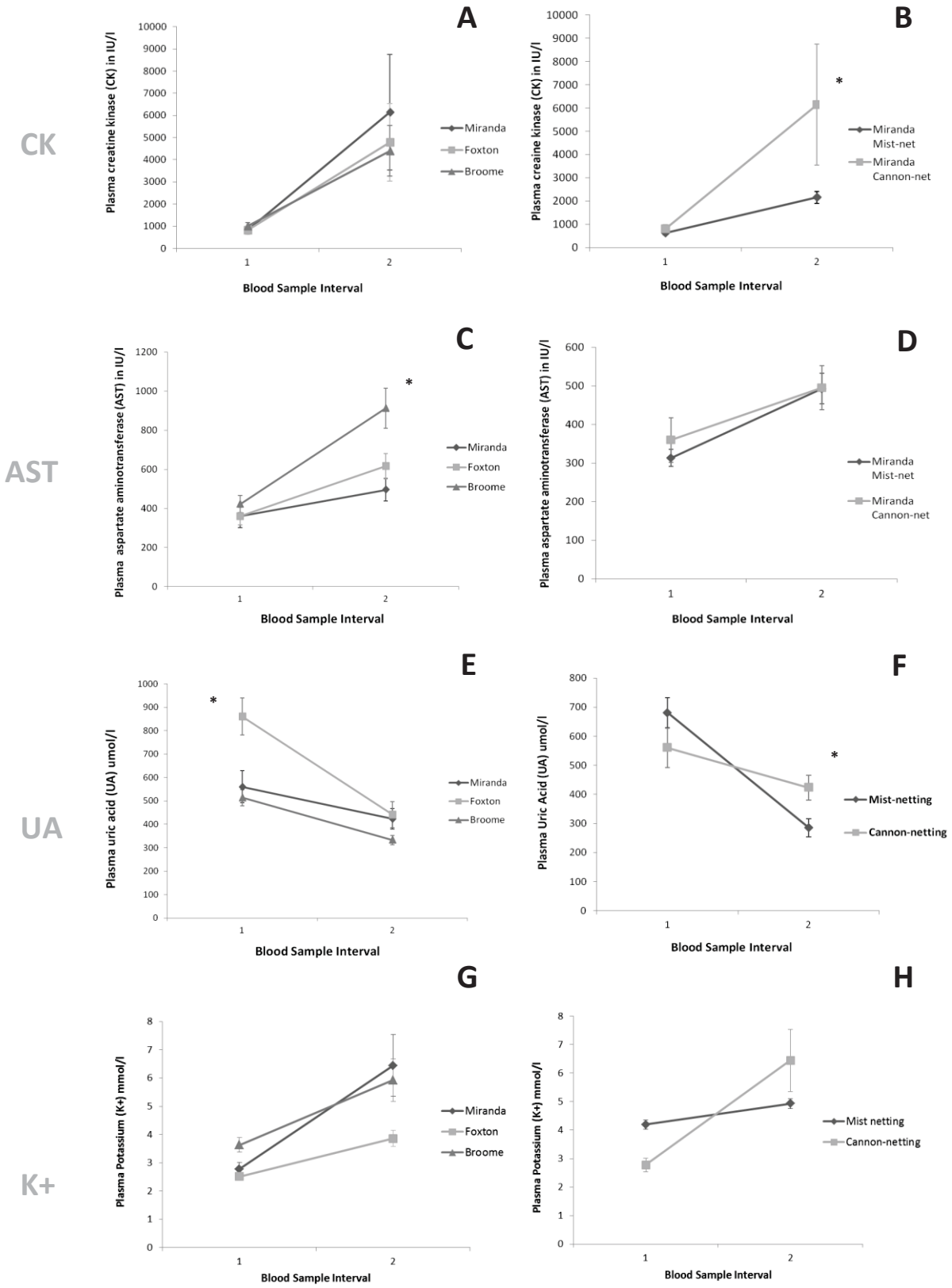

Figure 2. The change in plasma concentration of creatine kinase **(2A,B)**, aspartate aminotransferase **(2C,D)**, uric acid **(2E,F)** and potassium **(2G,H)** for serial blood samples of bar-tailed godwits (*Limosa lapponica*) captured in New Zealand and Australia 2008-2009. Graphs **2A,C,E and G** show comparisons of godwits captured by cannon-net in three locations. Graphs **2B,D,F and H** show comparisons of godwits caught by mist-nets vs. cannon-nets. Asterisks denote significantly different results.

2.3.2 Red Knots

A total of 35 red knots were caught over the research period, for which only six paired samples were suitable for analysis from birds caught by mist-netting and eight paired samples from birds caught by cannon-netting. Three red knots (8.6%) showed moderate to severe clinical signs of myopathy following the Foxton cannon-net catch.

Creatine Kinase

Scatter plots of plasma CK concentration over Interval 1 and 2 are shown in **Figure 1**. Peak plasma concentrations of CK in red knots following capture ranged from 176 to 4510 IU/l. There was no significant change in the plasma concentrations of CK over time ($f_{1,1,9}$ =0.47, p=0.512). There was also no effect of location for birds caught by cannon-net ($f_{1,1,9}$=0.00, p=0.993, **Figure 3a**), allowing results to be pooled for capture method analysis. There was a higher plasma concentration of CK on the second sample in birds caught by mist-nets ($F_{1,1,9}$ =7.41, p=0.022) (**Figure 3b**). However, there was a significant confounding effect of the timing of blood collection for the second samples ($F_{1,1,9}$ =5.05, p=0.048), with second samples for mist-netted birds taken from 1 hr 8 mins – 2 hrs 43 mins post-capture, compared to 3 hrs 10 mins – 5 hrs 23 mins post-capture for cannon-netted birds

Aspartate Aminotransferase

There was no significant change over time ($F_{1,1,9}$ =4.06, p=0.075) or effect of capture location ($F_{1,1,9}$ =1.85, p=0.206) on the plasma concentrations of AST in red knots caught by cannon-net (**Figure 3c**). Cannon-net data were then pooled to assess the effects of capture method. While there was an effect of capture method ($F_{1,1,9}$ =7.59, p=0.022) (**Figure 3d**), this was confounded by a significant covariate effect of time to first sample ($F_{1,1,9}$ =11.2, p=0.009). Within subjects there were also interactions between time and capture method ($F_{1,1,9}$ =9.81, p=0.012) and time to second sampling ($F_{1,1,9}$ =10.49, p=0.010). Therefore no conclusions can be drawn from the data.

Uric Acid

There were no effects of time ($F_{1,1,6}$ =0.25, p=0.638), location ($F_{1,1,6}$ =1.91, p=0.216) or capture method ($F_{1,1,6}$ =0.50, p=0.508) on the plasma concentration of UA in red knots (**Figure 3e,f**).

Potassium

There was no significant change over time ($F_{1,1,9}$ =0.03, p=0.864), effect of location ($F_{1,1,9}$ =0.75, p=0.409) or capture method ($F_{1,1,9}$ =0.34, p=0.573) on the plasma concentrations of potassium in red knots (**Figure 3g,h**).

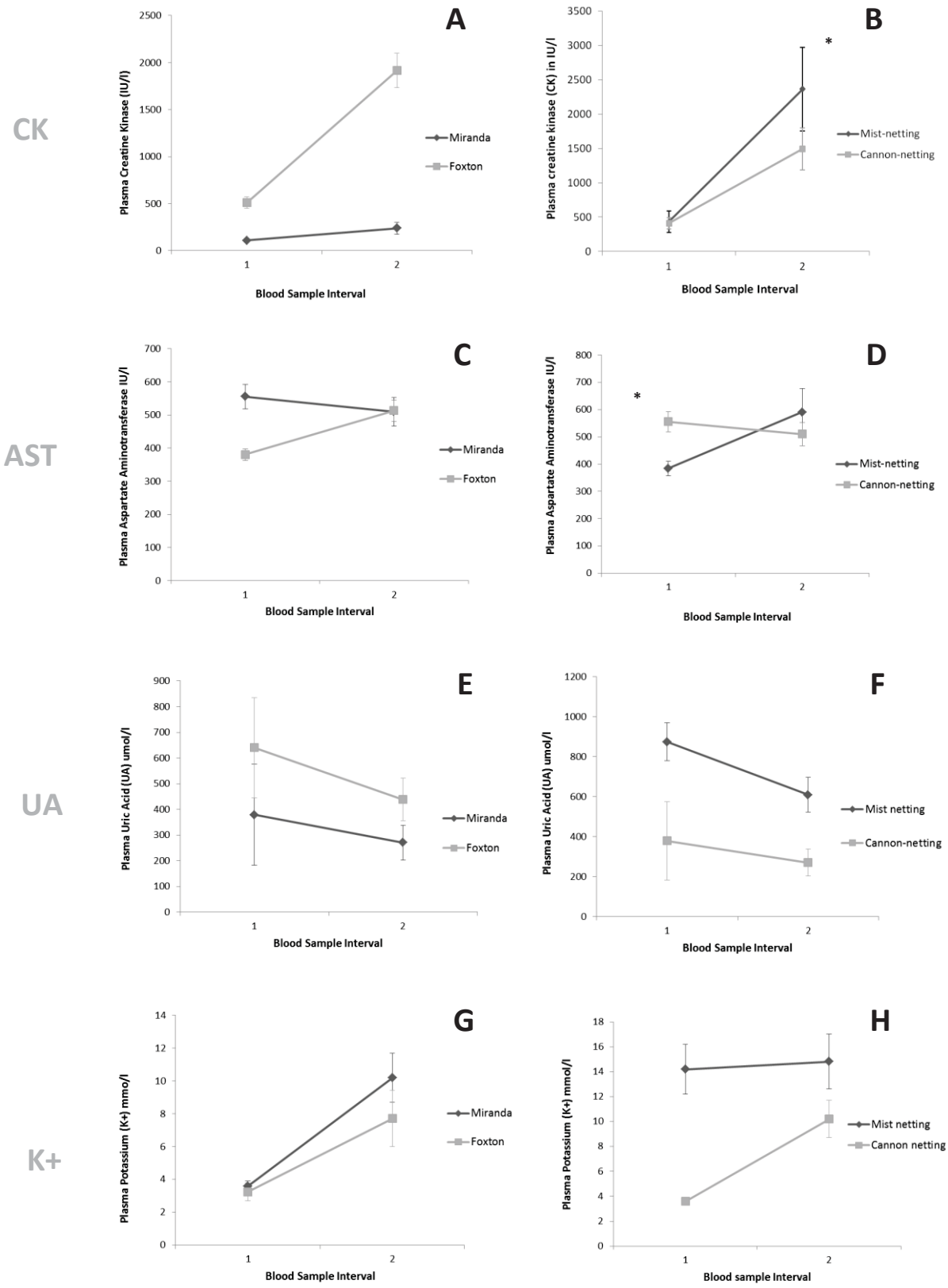

Figure 3. The change in concentration of plasma creatine kinase **(3A,B)**, aspartate aminotransferase **(3C,D)**, uric acid **(3E,F)** and potassium **(3G,H)** for serial blood samples of red knots (*Calidris canutus*), caught in two locations in New Zealand 2008-2009. Graphs **3A,C,E and G** show comparisons of red knots captured by cannon-net in two locations in New Zealand. Graphs **3B,D,F and H** show comparisons of red knots caught by mist-nets vs. cannon-nets. Asterisks denote significantly different results.

2.3.3 Great Knots

Great knots were only captured by cannon-net in Broome; therefore comparisons were only possible for changes in analytes over time and for species comparisons. There were 30 great knots caught, of which 21 paired blood samples were suitable for analysis. Peak CK measures for great knots were in the range of 586-12,424 IU/l. There were significant increases over time for the plasma concentration of CK ($F_{1,20}$ =24.513, p=<0.001), and AST ($F_{1,20}$ =12.268, p=0.002). However, there was no significant change for plasma UA concentration ($F_{1,16}$ =1.204, p=0.289) and potassium results were confounded by a significant covariate effect of time to first sample ($F_{1,15}$ =15.369, p=0.001), prohibiting further analysis.

2.3.4 Species comparisons

For red knots and godwits affected by capture myopathy, there was no difference in the incidence of the disease between species (chi square = 0.707, df = 1, p=0.4). Plasma biochemistry results for godwits and red knots caught by mist-net at Miranda and by cannon-net at Foxton have been analysed separately to exclude the previously identified effects of capture location and method on the results. For birds caught at Foxton by cannon-net there was no difference between godwits and red knots for the patterns of change of plasma concentrations of CK ($F_{1,1,15}$ =1.10, p=0.311), AST ($F_{1,1,15}$ =0.222, p=0.664) or Uric Acid ($F_{1,1,13}$ =1.00, p=0.336) (**Figure 4b,d,f**). However, there was a higher plasma concentration of potassium in red knots ($F_{1,1,15}$ =12.51, p=0.003) (**Figure 4h**). For birds caught at Miranda by mist-net, there were no differences between godwits and red knots for the patterns of change of plasma concentrations of CK ($F_{1,1,33}$ =0.00, p=0.998) or AST ($F_{1,1,24}$ =0.194, p=0.663). The plasma concentrations of UA were significantly greater for red knots ($F_{1,1,32}$ =7.98, p=0.008) and plasma concentrations of potassium were also significantly higher ($F_{1,1,27}$ =128.56, p<0.001) (**Figure 5**). For birds caught by cannon-net at Broome, Australia, there were no significant differences between great knots and godwits in the patterns of change in plasma concentrations of CK ($F_{1,1,35}$ =0.15, p=0.703) or AST ($F_{1,1,43}$ =3.53, p=0.067) (**Figure 4a,c**). However, there were higher overall plasma concentrations of UA ($F_{1,1,37}$ =46.63, p<0.001) and potassium ($F_{1,1,32}$ =12.38, p=0.001) for great knots when compared with godwits captured at the same location (**Figure 4e,g**).

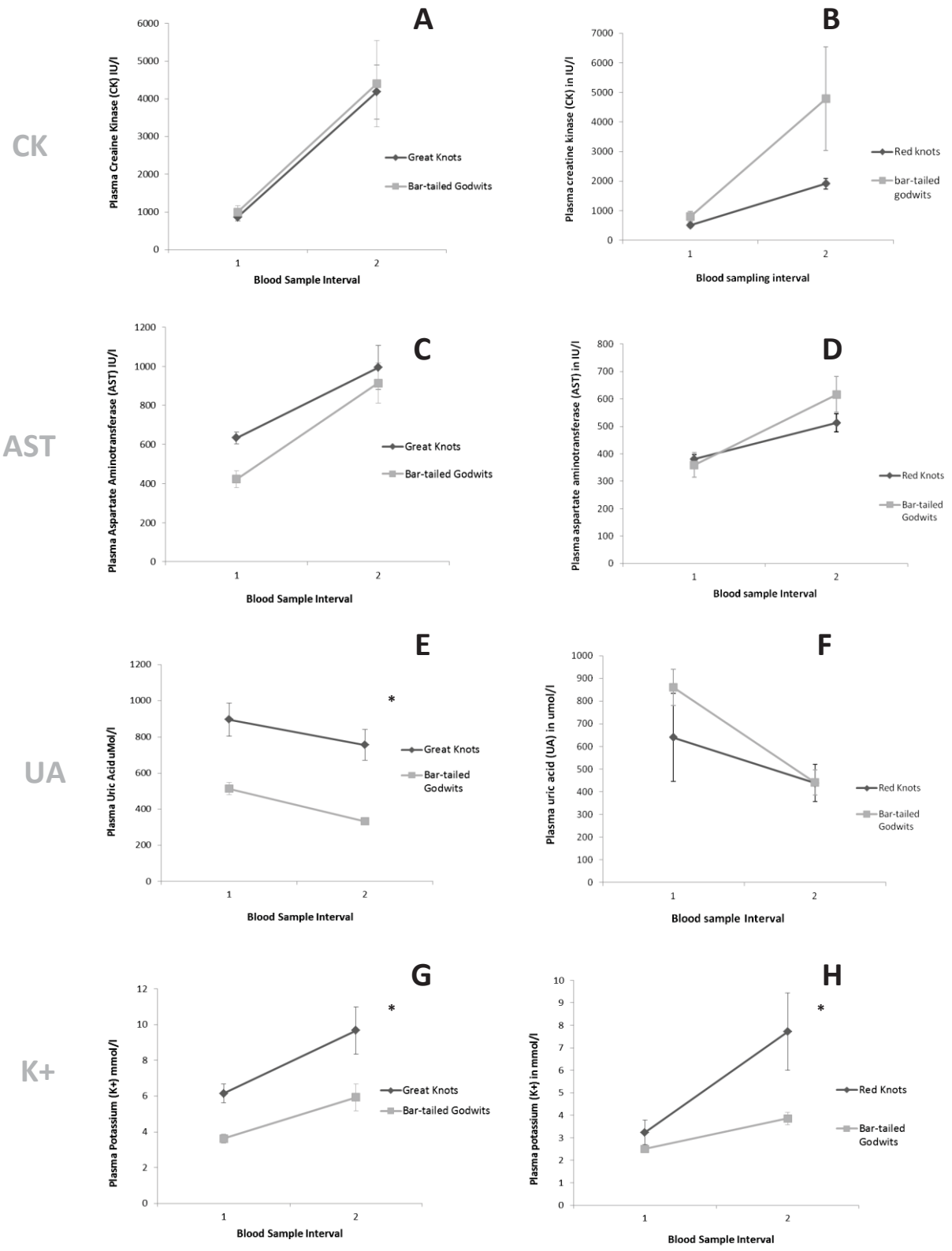

Figure 4. Species comparisons of the change in concentration of plasma creatine kinase **(4A,B),** aspartate aminotransferase**, (4C,D),** uric acid **(4E,F)** and potassium **(4G,H)** for serial blood samples of great knots (*Calidris tenuirostris)***(4A,C,E,G)** or red knots (*Calidris canutus*) **(4B,D,F,H)** compared with bar-tailed godwits (*Limosa lapponica*) caught by cannon-nets 2008-2009. Asterisks denote significantly different results.

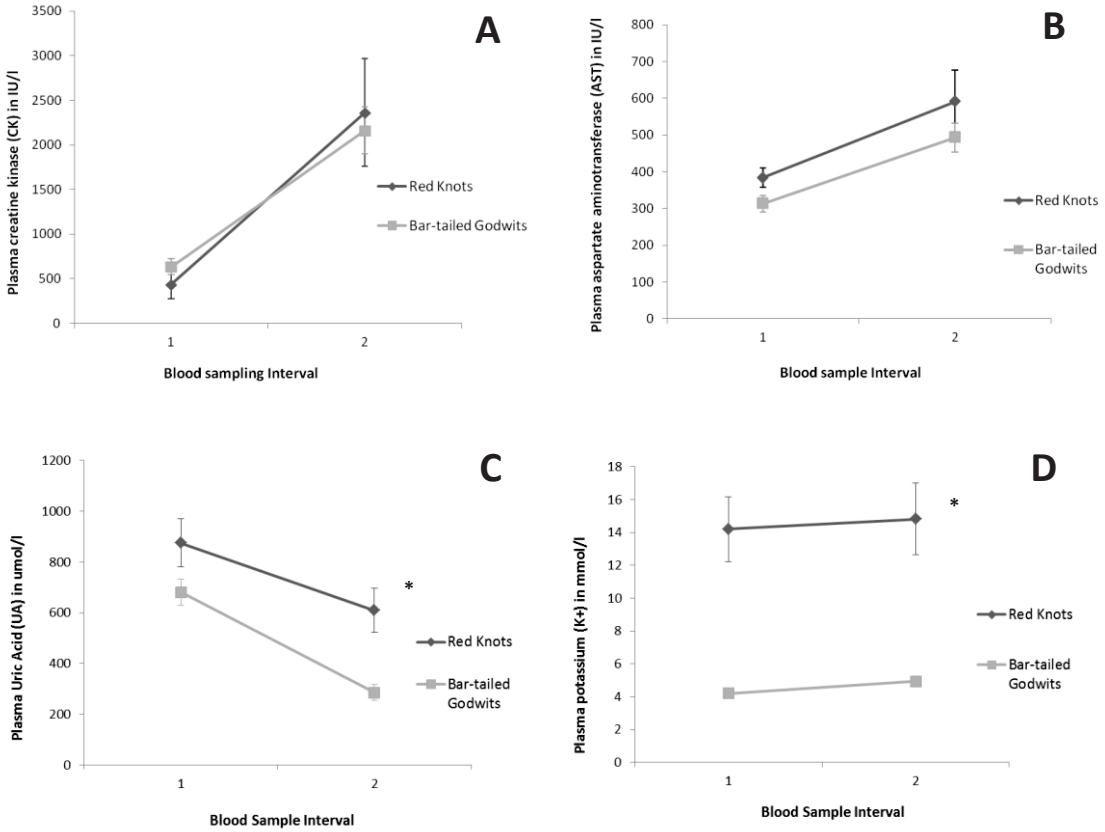

Figure 5. Species comparisons of the change in concentration of plasma creatine kinase **(5A),** aspartate aminotransferase**, (5B),** uric acid **(5C)** and potassium **(5D)** for serial blood samples of red knots (*Calidris canutus*) compared with bar-tailed godwits (*Limosa lapponica*) caught by mist-net in Miranda, New Zealand 2008-2009. Asterisks denote significantly different results.

2.4 DISCUSSION

Wild animal capture can have significant detrimental effects to the study animal, including severe stress (Lynn & Porter, 2008), injuries (Spotswood, et al., 2012), capture myopathy or death (Williams & Thorne, 1996). In fact, wild animal studies often have a defined post-capture censoring period to allow for capture-associated effects (Quinn, et al., 2012). Few studies have addressed the more subtle effects of capture on the metabolic and homeostatic mechanisms of birds. In this study, our results demonstrate significant effects of capture and handling on wild bird physiology, including alterations in biochemical parameters associated with muscle damage, electrolyte homeostasis and digestive function.

The indicators of muscle damage that we examined included biochemical parameters creatine kinase (CK) and aspartate aminotransferase (AST) and assessment of clinical signs of capture myopathy. We found marked increases in the plasma concentration of CK in godwits and great knots following capture, banding and handling. The time taken to the first blood sample was an important influence of the plasma concentrations of CK, as it quickly began to rise following capture, similar to the findings of Bailey et al. (1997). There were significant elevations of CK in godwits that were caught by cannon-nets or mist-nets, suggesting that some level of muscle damage was caused by both methods of capture. However, the greater change with cannon-net capture implies that this method caused greater muscle damage in godwits. What's more, the first birds to be extracted from beneath the cannon-net were preferentially sampled in order to gain rapid first samples for the repeated measures. This means we potentially missed more severely affected birds. Cannon-netting may induce greater muscle trauma due to excessive struggling, direct injuries and/or strains due to entanglement (Dabbert & Powell, 1993). Increased eccentric contractions, when birds struggle beneath the net, may be a contributing factor. Eccentric muscle contractions, where the muscle must lengthen during contraction (e.g. to slow movements or when external forces are greater than the muscle forces), are known to cause greater muscle damage and CK elevations than concentric (muscle shortening) contractions (Newham & Edwards, 1986; Zager, 1996; Proske & Morgan, 2001).

Red knots showed greater elevations in plasma CK concentration with mist-net captures compared to cannon-net captures. However, differences in the timing of the second blood sample collection between the groups had a significant effect on the results and prohibited clear conclusions. Interestingly, for species comparisons, the red knots also showed a lower average CK elevation with cannon-netting (Figure **4b**), though this difference was

not significant. However, the trend seen for greater CK elevation with mist-netting, which is the opposite of that seen in godwits, raises some interesting possibilities. The difference could relate to a sparing effect of small birds when multiple species are caught with cannon-nets, where larger species may bear the brunt of the forces. Alternatively, this could represent a true effect of mist-netting on knots, which are more likely to become entangled in mist-nets (godwits simply fall into a shelf 'pocket'; P. Battley, pers. obs.). Another possibility is that our results are inaccurate due to low sample sizes and variability in the time to sampling. While mist-net caught red knots in our study averaged elevations of five times baseline levels, plasma CK elevations of up to ten times have been observed in western sandpipers *Calidris mauri* caught by this method (Guglielmo, et al., 2001). These results imply that mist-net captures are not innocuous and capture effects on red knots deserve further investigation.

There was a surprising lack of difference in the patterns of CK change between the different locations studied. Conditions in Broome were very hot compared to other locations and also had higher than average temperatures at the time of capture (**Table 1**). It was expected that shorebirds caught in these conditions would suffer more seriously from capture myopathy. Other studies of environmental risk factors have shown conflicting results, with both higher temperatures and lower humidity (Nicholson, et al., 2000), or lower temperatures and higher humidity (Höfle, et al., 2004) being implicated in CM occurrence. In our study high temperatures did not appear to exacerbate CM occurrence or induce greater muscle damage. The reasons for this are unclear but perhaps reflect species differences and acclimatisation to environmental factors. If birds do become acclimatised to local environmental conditions, it may be more meaningful to look at relative changes in local temperatures and humidity for effects on CM incidence and severity. Additionally, other factors that differed between capture locations such as the speed of extraction and the gentleness of handling methods are likely to be important. Struggling and handling times are known to be risk factors for CM (Dabbert & Powell, 1993; Nicholson, et al., 2000).

Plasma concentrations of AST increased significantly over time for godwits and great knots with no appreciable difference between these two species. In contrast to our CK results, there was no effect of capture method on plasma concentrations of AST in godwits. As reported by Franson, et al.(1985), CK is a more specific and sensitive measure of muscle damage and although marked increases in AST have been recorded in cases of CM in birds, AST is slower to rise and fall in the blood (e.g. Businga, et al., 2007). Interestingly, plasma AST concentrations were significantly elevated in Broome-caught godwits (hot

temperatures) when compared to cooler New Zealand locations for cannon-netting. This was unrelated to differences in the timing of collection as there was a clear association of location with no interaction effects. Although plasma CK concentration also rose after capture, it did not have comparatively high measures compared with the other locations. AST is a non-specific analyte that can be found in near equal amounts in skeletal muscle, liver, kidney, brain, heart (Lumeij & Westerhof, 1987) as well as red blood cells (Cardinet, 1989). A possible explanation is that thermal stress caused significantly greater AST elevations due to generalised tissue damage, which has been shown to occur in heat-stressed rats (Hubbard, et al., 1979) and monkeys (Heaps & Constable, 1994). Alternatively, AST elevations may have been due to other organ involvement, such as liver damage (Lumeij & Westerhof, 1987).

Sex and body mass differences (Purchase & Minton, 1982) have been hypothesized as possible risk factors for CM. Sex differences were not evident in our CK results, which is similar to the findings of other authors where sex was not a risk factor for clinical signs of myopathy (Ponjoan, et al., 2008), degree of CK or AST elevation (Dabbert & Powell, 1993) or presence of gross lesions of myopathy (Spraker, et al., 1987). Neither was body mass a risk factor for the godwits we studied, although godwits in the latter stages of fattening for migration were not captured in this study. Some of the September birds were very thin, with evidence of recently completing their annual migration (pers. obs.), but this did not appear to affect their susceptibility to muscle damage.

Both red knots and godwits captured in New Zealand showed clinical signs consistent with capture myopathy revealing the susceptibility of these species to capture-related muscle damage. All three (100%) of the red knots and 11/15 (73%) of the godwits were caught by cannon net, with the majority (9/11 godwits and 3/3 red knots) caught at Foxton estuary where entanglement nets were used and prolonged extraction times from the net were problematic (see Ward, et al., 2011). Unfortunately, there were insufficient numbers to compare the muscle enzyme changes between CM-affected birds and unaffected birds. Additionally, the time frame of our study means we did not observe the maximum peak of plasma CK concentrations in our results. In reported cases of CM to date, peak CK measures were reached at 24 hours to 4 days post-capture (Smith, et al., 2005; Businga, et al., 2007; Ward, et al., 2011). This must be taken into account when investigating myopathy in birds. However, in most cases it is impractical and undesirable to keep birds for such prolonged periods following capture events. Prolonged holding periods have also been associated with long-term consequences, such as reduced reproductive success (Legagneux, et al., 2012).

Plasma uric acid concentrations reflect a very different aspect of capture effects on birds. Uric Acid is the main measure of nitrogen metabolism in birds (Fairbrother, et al., 1990) and elevations are commonly seen as with normal post-prandial change or with disease such as severe dehydration or renal disease (Hochleithner, 1994). In this study, we observed a decline in plasma concentrations of UA over time in godwits, with red knots (mist-netting only) and great knots (cannon-netting only) having higher plasma concentrations. Plasma concentrations of UA began to decrease rapidly following capture, but were unaffected by timing of the second sample. These declines do not reflect dehydration or renal dysfunction as both of these disorders cause elevations in UA over time (Hochleithner, 1994). The simplest explanation is that the birds initially had post-prandial UA elevations associated with physiologically normal nitrogen metabolism. Either this declined naturally over the holding period, or 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 (ter Steege & Kolkman, 2012). Knots primarily feed on bivalves, whereas godwits feed on polychaete worms, crabs and molluscs (Battley, 1996), therefore species differences in the results may reflect variation in prey digestion or nitrogen metabolism. Significant species variation in plasma UA concentrations under similar sampling conditions has been found in other avian species comparisons (Lumeij, 1987). Interestingly, godwits caught in mist-nets showed a greater decline in plasma concentrations of UA than those caught by cannon-net. Both methods of capture occurred at roosting time, when the birds were in a post-feeding state. However, birds captured by mist-net were caught at dusk and later into the night, while birds captured by cannon-net were caught in the daytime, so potentially circadian influences could have affected the results. Daily variation in fasting UA plasma concentrations has been shown in red-legged partridge, with significantly higher UA concentrations observed in the middle of the night (Rodríguez, et al., 2006).

Plasma potassium levels are an indication of electrolyte homeostasis and elevated levels (hyperkalaemia) are known to be an important contributor to capture complications in mammals (Kock, et al., 1987), but have rarely been analysed in bird capture studies. Potassium is found in high concentrations in muscle cells, red blood cells, liver and brain. Likely causes of hyperkalaemia secondary to capture include tissue damage, metabolic acidosis or dehydration (Woerpel & Rosskopf, 1984, as cited in Hochleithner, 1994). In our study, plasma concentrations of potassium significantly increased over time in godwits, by both capture methods. Unfortunately, the results were heavily confounded by the timing of sampling, limiting our interpretation of these changes. It is also of concern to the

validity of our results that red and great knots had excessively high plasma concentrations of potassium. Plasma concentrations *in vivo* of this magnitude should have been associated with clinical signs of hyperkalaemic toxicity resulting in cardiac arrhythmias or death, indicating post-sampling artifacts. Elevated potassium may be seen with haemolysis (Koseoglu, et al., 2011), potentially due to blood collection problems such as traumatic draws, incorrect needle size or sample handling, but can also be seen with stress and anxiety (Stankovic & Smith, 2004). Prior avian studies have shown significant species variations in potassium concentration. For example, heparanised blood samples from chickens (*Gallus domesticus*) and pigeons *(Columba livia domestica)* showed significant declines in plasma potassium concentration when left to stand prior to centrifugation (Lumeij, 1985), while macaws showed significant increases in potassium from heparanised blood samples stored at 4°C, thought to be due to leakage of potassium from stored erythrocytes (Harr, 2002). While rapid separation of collected blood is advocated (Lumeij, 1985; Harr, 2002), in the field situation this is not always possible. The species differences we observed may reflect a similar effect of stress, collection technique, or heparin on potassium metabolism in stored erythrocytes prior to centrifugation. Despite the lack of clear results, it is of interest to note that all species showed some derangements of potassium following capture, and the use of plasma potassium as a diagnostic tool is worthy of further evaluation. To investigate this phenomenon further we suggest that the use of rapid tests at the time of capture such as i-STAT® (Abbott Point of Care Inc., Abbott Park, IL, USA) machines, to counter for any anticoagulants and cell degradation.

In conclusion, all birds showed physiological effects of capture. Plasma concentrations of CK were the most useful measure of immediate muscle damage in shorebirds. Time to first sampling is important for plasma concentrations of CK, AST, UA and K+, due to rapid changes in the blood after capture. Observed declines in plasma UA concentrations after capture may have been due to interruption of digestion via the sympathetic nervous system. Cannon-net captures were associated with greater muscle trauma in godwits and occurrence of myopathy in godwits and red knots. Great knots suffered similar amounts of muscle damage as godwits when captured by cannon-nets. Hot ambient temperatures did not appear to contribute to the development of muscle damage in godwits, but the birds did show plasma AST elevations that may reflect heat stress or other organ involvement. Individual factors including sex and body mass do not appear to have a significant effect on vulnerability to muscle damage in godwits on capture, although birds with fat, pre-migratory body condition were not assessed in this study. Godwits and red knots are susceptible to capture myopathy and much care is needed with their capture and handling. We recommend the use of light, fine mesh nets if cannon-net capture is the only option,

and to preferentially use mist-nets where possible. We urge the avian community to avoid entanglement (large mesh) nets for these species.

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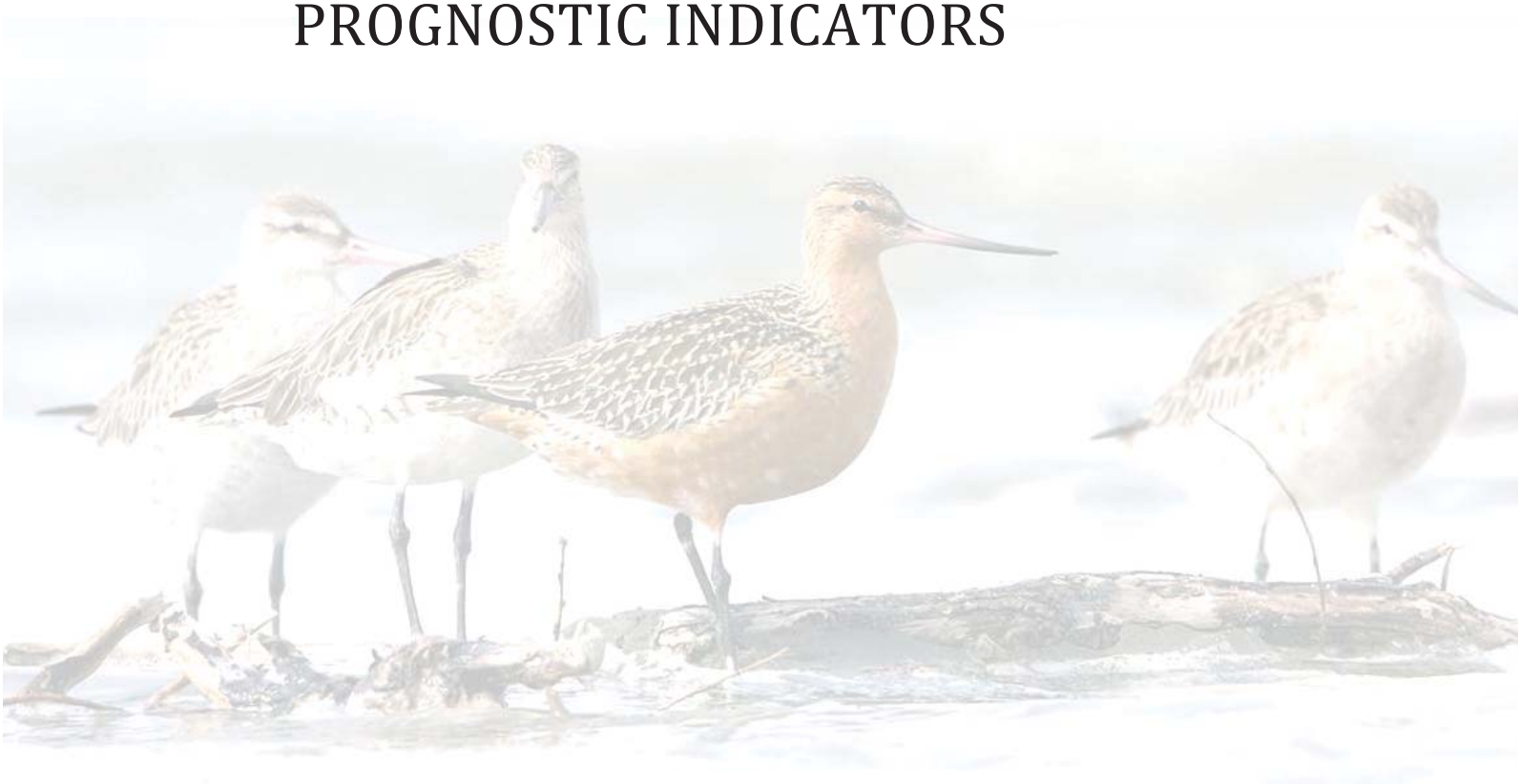
Chapter Three

MIDAZOLAM AS AN ADJUNCTIVE THERAPY FOR CAPTURE MYOPATHY IN BAR-TAILED GODWITS *(LIMOSA LAPPONICA BAUERI),* WITH PROGNOSTIC INDICATORS



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.

Photo: Phil Battley

Chapter Three: *Midazolam as an adjunctive therapy for capture myopathy in bar-tailed godwits, with prognostic indicators*

ABSTRACT:

Capture myopathy is a syndrome seen as a complication of capture and handling in many species of birds and mammals. Muscular necrosis leads to ataxia, paralysis and pain, while metabolic disturbances can result in death of the animal. This study reports on an opportunistic clinical trial on 16 bar-tailed godwits (*Limosa lapponica* subspecies *baueri*) that developed capture myopathy after a cannon-net capture in New Zealand in October 2008. The aim of this study was to assess the potential beneficial effects of midazolam, a benzodiazepine with the effects of anxiolysis, muscle relaxation and sedation, in the adjunctive treatment of capture myopathy. Physical and biochemical parameters were also analysed retrospectively for their potential as prognostic indicators for survival of shorebirds to the point of release. All birds (n=16) were treated with subcutaneous fluid therapy, a non-steroidal anti-inflammatory (meloxicam), gavage feeding, and sling therapy twice daily. The treatment group (n=8) were additionally treated twice daily with intramuscular midazolam injections at 1.5mg/kg. Surviving godwits were released over a period of one to nine days, with 6/8 birds in the treatment group surviving to release compared to 3/8 of the control group. Inability to counteract weight loss in captivity was the most significant problem encountered in the treatment of both groups of birds. Lack of waterproofing and predation were contributing causes to death of at least two godwits subsequent to release. Birds treated with midazolam showed subjective benefits including improved tolerance of handling and sling therapy. Clinical parameters (change in body mass, packed cell volume (PCV), plasma creatine kinase (CK), aspartate aminotransferase (AST), total protein (TP) and uric acid (UA) over time) were not statistically different between the two groups, though peak average values for CK, AST and UA were lower in the treatment group. As prognostic indicators we found decline in body mass (%), PCV, final plasma UA, and peak plasma CK to be the most useful parameters. Midazolam shows potential as an ancillary treatment for capture myopathy in birds and is worthy of continued study and use.

Key Words: Bar-tailed godwit, benzodiazepine, cannon-net, capture myopathy, *Limosa lapponica*, midazolam, prognostic indicators, treatment.

3.1 INTRODUCTION

Capture myopathy (CM), also known as exertional myopathy (Williams and Thorne, 1996), is a syndrome seen as a complication of capture and handling in many species of wild mammals and birds. The stress, struggling, and extreme exertion due to pursuit and capture is thought to create a physiological cascade with hyperthermia, anaerobic glycolysis, metabolic acidosis, reduced tissue perfusion and hypoxia (Spraker, 1993), resulting in myocellular necrosis in cardiac and skeletal muscles (Williams and Thorne, 1996). Reduced muscle function leads to ataxia, weakness, and partial or complete paralysis. Renal failure, circulatory collapse and death may occur in severe cases (Williams and Thorne, 1996). Some animals appear to recover, only to die suddenly days or weeks later following a further stressful or exertive event (Spraker, 1993).

Leakage of cellular enzymes from damaged muscle tissue into the blood stream causes serum analytes to become elevated, particularly creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), which are thus used as indicators of myocellular damage related to capture myopathy (Bollinger et al., 1989). Creatine kinase is reported to be the most useful indicator of muscle damage in birds (Franson et al., 1985) and mammals (Cardinet, 1989); however, the correlation between these enzyme values and overt clinical signs or death is largely unknown (Bollinger et al., 1989).

Wild and captive bird species reported to be susceptible to capture myopathy include wild turkeys (*Meleagris gallopavo*), flamingoes (*Phoenicopterus spp.),* ostriches (*Struthio camelus)* and African crowned cranes (*Balearica rugelorum)*, amongst others (as cited in Bollinger et al., 1989). Long-legged species appear to be over-represented in the literature as being susceptible to CM, although it is thought that any species could potentially develop the disease (Williams and Thorne, 1996). Shorebirds such as bar-tailed godwits (*Limosa lapponica*) (Purchase and Minton, 1982); Eurasian curlews (*Numenius arquata*), (Green, 1978); and red and great knots (*Calidris canutus* and *C. tenuirostris)* (Rogers et al., 2004) have been observed to develop clinical signs consistent with CM after mist-net and cannon-net captures and after leg banding.

There are few published reports of successful treatment of CM in wild birds (e.g. Rogers et al., 2004; Smith et al., 2005; Businga et al., 2007), and the focus remains on prevention (Williams and Thorne, 1996). However, for threatened or endangered species, or those of high value in a zoological setting, successful treatment of CM is imperative.

Fear, stress and over-exertion have been identified as etiological factors in the development of CM (Chalmers and Barrett, 1982). Benzodiazepines are pharmacological agents with the effects of anxiolysis, muscle relaxation and sedation. Subjective positive effects were reported anecdotally when oral diazepam, a benzodiazepine, was administered to red knots with suspected CM (Piersma et al., 1991). However, diazepam may cause circulatory disturbances with intravenous injection and has an unreliable uptake and a long half-life with subcutaneous or intramuscular administration (Hawkins and Pascoe, 2007). Midazolam is a benzodiazepine with excellent uptake by the intramuscular or subcutaneous route (Hawkins and Pascoe, 2007), a rapid onset of action, relatively short half-life (77 minutes in dogs) and has minimal cardiovascular depressive effects (Plumb, 2005). There have been no published records on the use of midazolam as an adjunctive therapy in the treatment of capture myopathy in birds.

This paper outlines an opportunistic clinical trial in which 16 bar-tailed godwits (subspecies *baueri*) that developed CM after a cannon-net capture were treated at the New Zealand Wildlife Health Centre. The aim of this study was to assess the potential beneficial subjective and objective effects of midazolam in the adjunctive treatment of capture myopathy. Physical and biochemical parameters were also analysed retrospectively for their potential as prognostic indicators for survival of shorebirds to the point of release.

3.2 MATERIALS AND METHODS

In October 2008 an attempt was made to capture a flock of 230 bar-tailed godwits, by cannon-net, at the Manawatu River estuary, New Zealand (40.47°S, 175.22°E) on a fine day with a maximum ambient temperature of 16.7C (The National Climate Database [2008], 2011). Two nets were used side by side – a small-mesh net and a larger-mesh net. In total, 166 bar-tailed godwits were captured, the majority of which were caught in the large-mesh net. Additionally, it was a "wet catch", in which the leading edge of the net reached the water. Many of the birds became wet and entangled and despite having a team of experienced bird handlers, it took approximately 75 minutes to extricate all of the birds from the nets. Twenty godwits developed clinical signs consistent with capture myopathy within 3 hours of capture. The primary clinical signs included one or more of the following: inability to stand; knuckling of the toes; paresis or paralysis; ataxia; hock sitting; or sternal recumbency. Other clinical signs included: increased respiratory rate (tachypnoea); wings held outstretched for balance; trembling; depression; and distressed vocalisation if disturbed.

The 20 birds were taken to the wildlife hospital at the New Zealand Wildlife Health Centre (NZWHC, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand) for treatment. The birds were allocated to either control (C) or treatment (T) groups by random draw and were given leg tags for identification. Four birds (two from each group) were released in less than 24 hours, such that each group totaled 8 birds. All birds were treated with fluid therapy, calculated as 50ml/kg/day maintenance plus an estimated 5% dehydration divided over 48 hours, to give a dose of approximately 10ml 0.9% NaCl subcutaneously in the inguinal fold twice daily. The birds were also treated with meloxicam (Metacam oral solution 1.5 mg/ml, Boehringer Ingelheim, Auckland, New Zealand), a non-steroidal anti-inflammatory, at 0.1 mg/kg per os (PO) once daily; nutritional support in the form of Wombaroo insectivore mix (protein 52 %, fat 12 %, fiber 5 %; Wombaroo Food Products, Adelaide, Australia), 9-10 ml gavaged as a slurry twice daily via crop tube; and twice-daily sling therapy (**Figure 1**) of duration ranging from 15 minutes to 1.5 hours. Sling therapy is a form of physiotherapy for paralysed birds that has been utilised in the successful treatment of capture myopathy in other species (e.g. Smith et al., 2005) and is described by Rogers et al. (2004). Slings were made using disposable surgical hats with holes cut for legs and threaded over a wooden broom handle, which was placed on top of a plastic tub lined with towels. It was ensured that all birds had similar times spent in a sling by rotating the groups on a per treatment basis and the duration was determined by the length of time it took to treat all of the birds. Additionally, the treatment group was injected with midazolam (DBL® Midazolam Injection BP, Hospira NZ Ltd, Wellington, New Zealand) intramuscularly in the pectoral muscles (alternating sides each time) at a dosage of 1.5 mg/kg (0.06 – 0.08ml) at the start of each treatment. All treatments were initiated on the first afternoon after admission, other than the slinging which began the following day.

The birds were left alone to recover between treatments with minimal disturbance in a large-dimension (2.0 x 4.0 m) room, with the temperature maintained between 24 and 26°C day and night. Soft, fenestrated rubber matting and towels were used as flooring to prevent complications such as slipping or pododermatitis (bumblefoot). Ad libitum water, insectivore slurry, mealworms and earthworms and strips of ox-heart were offered in two trays at either side of the room; however, most birds did not eat voluntarily. Small shallow water trays were made available for bathing, but they were not seen to be used.

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Figure 1. Bar-tailed godwits (*Limosa lapponica baueri*) undergoing sling therapy as part of a treatment regimen for capture myopathy (October-November 2008). Sling therapy allows the bird to have its weight supported while attempting to stand and relieves pressure on the limbs

Blood samples were taken of each bird on arrival; thereafter, the birds were weighed and blood samples were taken once daily in the morning. All blood samples were collected from either of the ulnar veins or the right jugular vein with a 25 gauge needle and 1 ml syringe. Each blood sample totaled no more than 0.3–0.8 ml (0.1–0.5 % of bodyweight); however, blood sampling was halted on the seventh day of the trial due to the severity of anemia developing in some of the birds. The blood was kept chilled at 4°C and then centrifuged to separate the plasma within 2 hours of sampling. Packed cell volume (PCV) was measured in house via capillary tube centrifugation. The plasma was frozen at -20°C and batch-analysed at New Zealand Veterinary Pathology (NZVP, IVABS Building, Massey University, Palmerston North, New Zealand) within 10 days of sampling. The biochemical parameters measured included creatine kinase (CK), aspartate aminotransferase (AST), total protein (TP) and uric acid (UA).

Birds were considered releasable once they were standing and walking unassisted and showing foraging and preening behavior. They were released singly or in small groups at the capture site, near to the remaining flock of godwits, in dry weather conditions and at low tide so that they could immediately forage. We attempted to release birds out of view of likely predators; however, southern black- backed gulls (*Larus dominicanus*) were sometimes present and were on occasion seen to attack newly released birds.

Statistical methods

Statistical analyses were performed using Systat for Windows 7.0 (1997 SPSS Inc.). All results are presented as means ± one standard error (SE) unless otherwise stated. All repeated measures of PCV, CK, AST and UA were assessed for significant differences over time and between the treatment and control groups using a multivariate repeated measures ANOVA. Because this analysis does not accept missing values, only the birds present for all of the first six days of the trial were included in the repeated measure analysis. Power analysis was carried out using G*Power 3.1 (Heinrich-Heine-University, Düsseldorf, Germany) using the methods outlined in Baguley (2004). To further explore the data, comparison of mean percentage weight loss, peak plasma AST, peak plasma CK and final plasma UA levels were conducted using a 2 factor ANOVA with treatment group and outcome (survival to release vs. died) as factors. Peak CK and AST values were log-transformed to satisfy the assumptions of normality and homogeneity of variances. Survival to release categorical data were analysed by Fisher's exact test.

3.3 RESULTS

3.3.1 Survival

Thirteen of the 20 birds affected by clinical capture myopathy survived to release (Table 1). Four of these were released less than 24 hours post-capture, so the sample of the trial totaled 16 birds. Nine of these 16 birds survived to release. Of the seven birds that died, one (from the treatment group) was euthanised due to severity of clinical signs and severe anemia. Six of eight (75%) birds in the treatment group survived to release compared with three of eight birds (37.5%) in the control group. The difference between the groups for survival to release was not significant (P=0.157). However, the power of our study is 0.74 and given that these proportions and effect size remain constant, it would take a sample size of 70 to achieve a power of 0.95. Nine of the 13 released birds (including the four released on the first day) had some form of identification allowing for post-release monitoring**.** Two of the birds from the treatment group apparently died shortly after release due to factors relating to poor body fitness (predation and drowning) (see Table 1).

3.3.2 Effect of Midazolam

The apparent onset of effect of the midazolam varied from 30 seconds to 2 minutes. Onset was characterised by sedation and relaxation of the bird. Resistance to handling ceased and the head drooped with relaxation of the neck muscles. Most birds remained alert, but some birds closed their eyes and apparently slept during treatments or whilst in the slings. These birds were easily roused when handled. The treated birds sat in the slings quietly, as compared to the non-treated group, some of which struggled and attempted to escape from the slings, and occasionally were entangled. Duration of effect was subjectively assessed as one to three hours, but the exact end-point of effect was difficult to quantify.

3.3.3 Body mass

All birds lost body mass (P= 0.019) of 10 – 35% during captivity (**Figure 2**). There was no effect of treatment group on total percentage of body mass loss (T: 19.7% ± 3.1 c.f. C: 23.6% ± 2.8, P=0.95); However, the birds that died lost more weight than the birds that were released (27.8% ± 3.3 c.f. 17.4% ± 1.6, P=0.014). Only three godwits showed slight pre-release mass gains. These gains were associated with self-feeding behavior, which was only observed once birds were ambulatory.

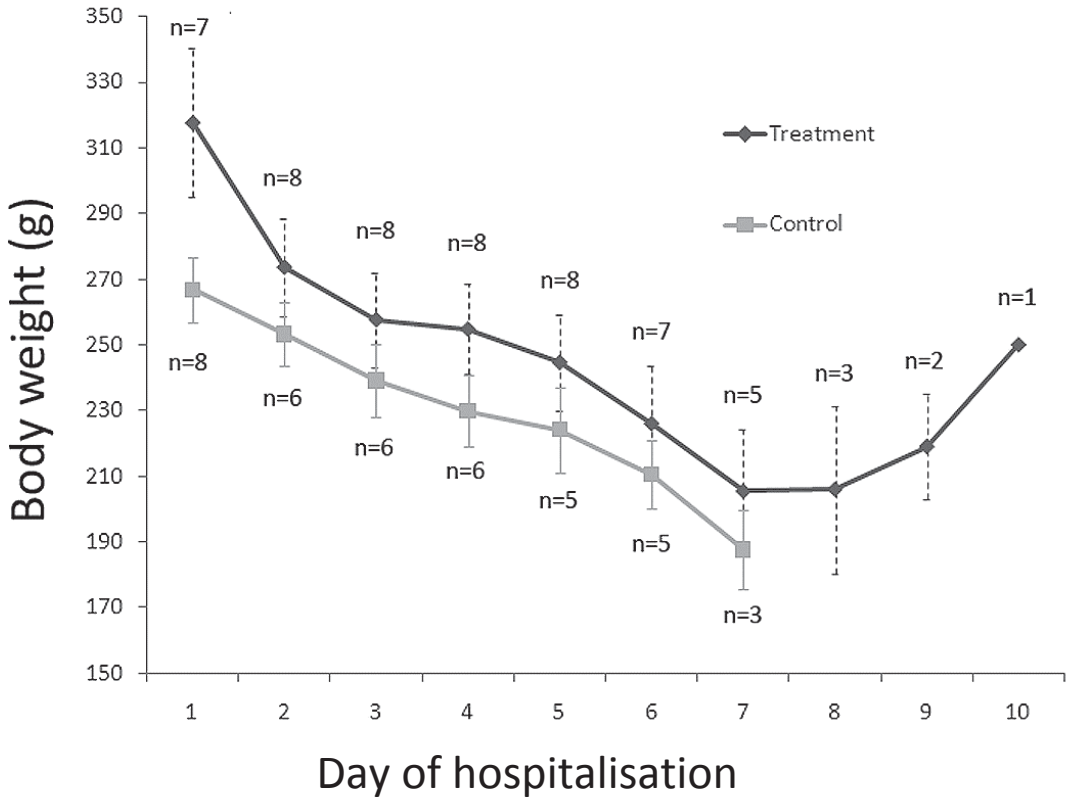

Figure 2: Mean +/- standard error of body mass (grams) over the period of hospitalisation for bar-tailed godwits (*Limosa lapponica baueri*) treated for capture myopathy October-November 2008. Birds in the Control group (C) were treated with fluid therapy (10mls 0.9%NaCl subcutaneously twice daily), non-steroidal anti-inflammatories (meloxicam 0.1mg/kg per os once daily), twice daily sling therapy and supplemental nutrition (10 mls insectivore slurry per os twice daily). Birds in the Treatment (T) group were additionally treated with intramuscular midazolam at 1.5mg/kg twice daily.

3.3.4 Clinical Pathology

Packed cell volume

Packed Cell Volume declined (P<0.001) over the period of hospitalisation (Fig. 3, A) However the pattern of decline did not differ between treatment and control groups (P=0.50). When the final PCV value (day 6) was evaluated, there was no statistical difference between the treatment vs. control groups (32.6% ± 5.1 c.f. 30% ± 7.4, P=0.68), or between the birds that were released vs. died (34.6% ± 3.1 c.f. 26.7% ± 9.7, P=0.30). There was a significant interaction effect (P=0.049) between outcome and treatment group. However, these analyses are heavily levered by an outlying control bird (C2) that died after two days with an increasing PCV. When this bird is excluded from the analysis the birds that died had a significantly lower final PCV than those that were released (18.4% ± 6.2 c.f. 34.5% ± 3.2, P=0.023). There was still no difference between treatment and control groups (P=0.713) and the interaction effect between treatment group and outcome was still significant (P=0.043). All birds with a PCV that dropped below 20% died (n=3) or were euthanized (n=1). These four birds (two birds from each group) also had the lowest body weights at death.

Biochemical parameters

There was an increase, followed by a decline, in the concentrations of plasma CK (P=0.003; Fig. 3, C) and AST (P<0.001; Fig. 3, D) over the period of hospitalisation. This decline did not differ significantly between the control and treatment groups for CK (P=0.35) or AST (P=0.69). Peak mean plasma AST did not differ between treatment and control groups (4238 IU/L ± 569 c.f. 4871 IU/L ± 966, P=0.93) nor between birds that were released or died (3876 IU/L ± 545 c.f. 5814 IU/L ± 1110, P=0.13). The observed difference in mean peak plasma CK for the groups (Fig. 3, C) was not significantly different between treatment and control groups (14992 IU/L ± 9763 c.f. 25354 IU/L ± 1532, P=0.683). The difference in peak CK between birds that were released vs. died also lacked statistical significance (13,455 ± 1746 c.f. 32,650 IU/L ± 13,070, P=0.059); however, there was a significant interaction effect (P=0.049) between treatment group and outcome. Godwits were released according to resolution of clinical signs and this clinical recovery corresponded to a drop in the CK level to below 775 IU/L for seven of nine birds and < 1450 IU/L for two birds.

There was an increase (*P*=0.027) in plasma uric acid (uMol/L) over the period of hospitalization (Fig. 3, B), but no difference (*P*=0.64) in the change in UA over time between the treatment and control groups**.** The final UA concentration was not significantly higher in control birds compared to treatment birds (1029 uMol/L ± 219 c.f. 528 uMol/L ± 100, *P*=0.096), but was significantly higher in birds that died compared to those that were released (1207 uMol/L ± 183 c.f. 465 uMol/L ± 86, *P*=0.005). The rise in UA did not correspond to a rise in PCV, or total protein, for birds within either of the groups, meaning that dehydration was unlikely to be primarily responsible for the hyperuricaemia.

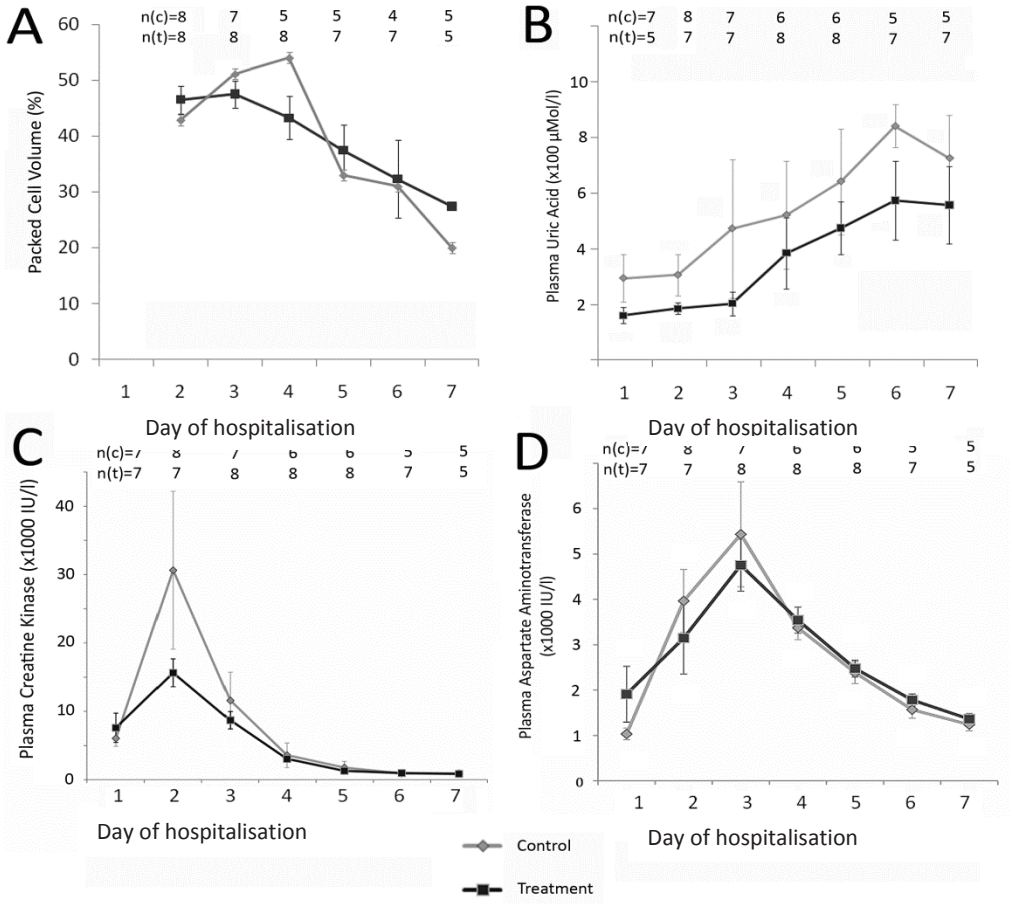

Figure 3. Mean +/- standard error of (A) packed cell volume (%), (B) plasma uric acid (uMol/L), (C) plasma creatine kinase (IU/L), and (D) plasma aspartate aminotransferase (IU/L) over 7 days of hospitalisation for bar-tailed godwits (*Limosa lapponica baueri*) treated for capture myopathy October-November 2008. Birds in the Control group (grey lines and bars) were treated with fluid therapy (10mls 0.9%NaCl subcutaneously twice daily), non-steroidal anti-inflammatories (meloxicam 0.1mg/kg per os once daily), twice daily sling therapy and supplemental nutrition (10 mls insectivore slurry per os twice daily). Birds in the Treatment group (black lines and bars) were additionally treated with intramuscular midazolam at 1.5mg/kg twice daily.

3.4 DISCUSSION

3.4.1 Effects of Midazolam

Successful treatment of capture myopathy remains a challenge. Midazolam showed subjective beneficial effects for the adjunctive treatment of capture myopathy, demonstrated by relaxation and reduced struggling of the birds during treatments. Fear and over-exertion have been identified as etiological factors in the development of CM (Chalmers and Barrett, 1982). Ongoing struggling and exertion with handling during the treatment periods may continue to exhaust or damage already fatigued muscles and inhibit recovery. The sedative, anxiolytic and muscle relaxant properties of benzodiazepines such as midazolam provide a means to counteract some of these effects.

Despite the fact that 75% of the treatment group survived to release compared with 37.5% in the control group, objective data analyses lacked apparent significance, suggesting that midazolam was ineffective in preventing mortality in this group of birds. However, there was low power in the analyses, partially attributable to small initial group sizes and ever-reducing group numbers, due to release or death of the birds. An objective determination of the effect of midazolam on treatment success was also complicated by significant interaction effects between treatment group and outcome. Clinical parameters affected by interaction effects included packed cell volume, and peak plasma CK activity. Despite the lack of significant findings, the group of birds treated with midazolam consistently showed improved results in almost all of the parameters measured, such as lower average peak PCV, CK, AST and UA levels. While not conclusive, our study suggests that midazolam shows great promise as an ancillary treatment for capture myopathy and is worthy of continuing study and use. In this study, both groups were treated with meloxicam for its analgesic and anti-inflammatory properties, however this adjunctive therapy for CM also requires evaluation.

3.4.2 Nutrition

Our findings suggest the most urgent research need is to investigate supplementary nutritional support for affected shorebirds. During the trial, the rate of weight loss between groups was not significantly affected by the use of midazolam. This suggests that inadequate nutritional support has a greater effect on body mass than stress due to handling and treatment. Emaciation has been reported as a result of chronic CM in some animals (Williams and Thorne, 1996). Godwits and other shorebirds also tend to do poorly in captivity, with severe weight loss and anorexia (pers. obs.), perhaps due to stress and suboptimal captive diets. The initial wide range of body weights seen at capture is

partially explained by substantial sexual dimorphism of bar-tailed godwits (Battley & Piersma, 2005); however, the majority of the birds were of moderate to low body condition at capture, having recently (one to eight weeks earlier) arrived from their 11,000km migration from Alaska. A lack of significant fat deposits and poor muscle mass would have prevented the birds from coping with a period of malnutrition. The amount of insectivore mix we could gavage was limited by gastric volume and supplementation with a higher caloric food such as small animal critical care formulas or blended fish slurries may have been beneficial.

3.4.3 Clinical parameters as prognostic indicators

We found that percentage of bodyweight lost, PCV, final plasma UA, and peak plasma CK were the most valuable prognostic indicators.

Weight loss

Percentage of body mass lost whilst in captivity was a useful prognostic indicator. Our study suggests that any shorebird losing greater than 20 – 25 % of its body mass may be considered a poor candidate for survival, but this would largely depend on the body mass of the animal at capture, as birds with large fat deposits may be able to tolerate greater loss over the same time period (see for example Figure 2 in Rogers et al. 2004, for pre-migratory knots treated for CM).

Packed cell volume

Packed Cell Volume could be a useful prognostic indicator for birds under treatment for capture myopathy. The observed declines in PCV were not adequately explained by blood sampling alone, as several godwits had no obvious anemia over the same sampling period. Each blood sample represented 5% or less of blood volume and it is generally accepted that a healthy bird can tolerate up to 10% of blood volume being removed for serial samples (Samour, 2006). The anemia and weight loss appear to be related as the four birds that died with severe anemia were also the most emaciated birds at death. Starvation-related anemia due to a decreased production of red blood cells, increased loss through muscular and/or internal hemorrhage, on top of serial blood sampling could explain the dramatic drop in PCV seen in several of these shorebirds.

Biochemical parameters

Plasma CK and AST are useful diagnostic indicators of myopathy. Although there are no published reference ranges for CK in godwits, Franson et al (1985) showed normal plasma CK in black-crowned night herons (*Nycticorax nycticorax*) of 365 – 420 IU/L. Similar to Hanley et al (2005) we found elevated plasma CK and AST concentrations correlated to clinical signs of myopathy. Approximately 24 hours post-capture, the godwits in treatment and control groups in this trial showed significant peak elevations of CK, with AST values peaking a day later. Resolution of clinical signs of myopathy correlated to a significant gradual decline of CK (Fig. 3, C), which is similar to that seen in greater sandhill cranes (*G. c. tabida*) treated successfully for capture myopathy (Businga et al., 2007). However declining CK and AST levels were also apparent in the majority of godwits that died, which is similar to the findings of Hanley et al (2005) in whooping cranes (*Grus americana*). This may suggest a poor correlation between resolution of clinical signs and declining CK and AST, or more likely, reflect the fatal complications of CM and the inherent difficulties in rehabilitation of wild birds, such as severe stress, anorexia and anemia.

Peak plasma CK has potential as a prognostic indicator, despite our study lacking the necessary sample size to show a clear statistical difference. However, the absolute CK value appears to have limited value in detecting severity of clinical signs or predicting recovery, especially between species (Franson et al., 1985). For example, Dabbert and Powell (1993) found relatively high CK values of 12,035 IU/l in mallards (*Anas platyrhynchos*) caught by entanglement net, yet these birds showed no clinical signs of CM. In our trial, the two godwits with the highest peak CK levels (>30,000IU/L) died, while other godwits with very high CK levels (>20,000 but < 30,000 IU/L) were subsequently released. The highest level of 109,220IU/L in one godwit that died in this trial is approximately half of that (208,400IU/L) observed in a rhea (*Rhea americana*) that was successfully rehabilitated (Smith et al., 2005). In hindsight, CK levels in the treatment group may have been iatrogenically increased by IM injections (Aktas et al. 1995) of midazolam. Although the injected volume was small (0.06–0.08 ml), injecting the control group with intramuscular saline might have reduced bias in the results. Alternatively, intranasal midazolam could have been used, as this has been shown to be efficacious in other species (Vesal & Eskandari, 2006).

Increasing plasma uric acid concentrations despite fluid therapy could be useful as a prognostic indicator. There are no published records for UA measures in godwits, however normal UA levels of 357–630 uMol/L were reported in Canada geese (Carpenter, 2005). Plasma UA increased significantly over time for all godwits in this trial and UA was

significantly higher in those birds that died (mean 1207± 183 uMol/l). Causes for hyperuricaemia include renal disease, post-prandial increases, high dietary levels of protein, protein catabolism in starvation or severe dehydration (Echols, 2006). Blood samples were collected prior to feeding, and dehydration was unlikely due to parental fluid therapy. Elevated plasma uric acid in these shorebirds is likely to reflect renal damage due to shock-related ischemia and myoglobin release from damaged muscles (Williams and Thorne, 1996) or ongoing muscle protein catabolism (Cohen et al., 2007).

3.4.4 Survival

Although 13 of the original 20 godwits were released back to the wild, this represents a relatively poor outcome, related to severe clinical disease, inadequate nutritional support, significant and worsening anemia and the ongoing stress of hospitalisation. Additionally, two of the released godwits from the treatment group were presumed dead (Table 1) due to drowning and predation shortly after release. The two adult color-banded birds (C7 and T2) not sighted since release we believe to be deceased, as adult godwits are extremely site-faithful, re-sighting effort is high at this location, and there have been no other missing color-banded adults in a 3-year study at the site (JRC, unpubl. data). Therefore, it is our view that a "successful" outcome in the treatment of capture myopathy cannot be determined at the time of release, as post-release mortality may be significant. Fatal complications of CM may include severe muscular dysfunction, renal or cardiac failure (Williams and Thorne, 1996) or indirect effects such as starvation and increased risk of predation (Spraker et al., 1987). Had we been able to provide suitable supplementary nutrition for this species, we could have been more stringent in our release criteria. As per Rogers et al (2004) we strongly recommend encouraging preening and bathing behavior in shorebirds prior to release.

As of February 2010, two of the marked released godwits (one treatment bird and one control bird) have been recaptured. One adult female (C1; Table 1) showed feather wear and breeding plumage consistent with having made the full annual migration to Alaska and back. There is scant published data on the post-release survival of birds following treatment of CM. Businga et al (2007) found that two of three successfully rehabilitated greater sandhill cranes were frequently re-sighted (and reproduced) over the following seven years. The third crane (a juvenile) was only seen for 2 days post-release, but was assumed to have survived. Rogers et al. (2004) released eight red and great knots after rehabilitation from CM. The following non-breeding season, four of these birds were seen again; this rate was similar to the re-sighting rate of non-CM birds color-banded at the same time (52%). In comparison, Ponjoan et al (2008) found 43% (10/23) of turkeys that

showed ataxia subsequent to capture and handling died 2 –11 days later. It is likely that deaths following capture influence the re-sighting rate of birds affected by clinical, as well as sub-clinical (undetected) capture myopathy (Williams and Thorne, 1996) and caution should be exercised in reporting unseen birds as alive.

Midazolam showed subjective beneficial effects in the adjunctive treatment of capture myopathy and is worthy of continued study. Treatment of CM can be costly and time-consuming, requiring intensive therapy to increase chances of survival on release. Prevention remains better than cure. However capture of wild animals for various purposes is likely to continue in the near future. Finding successful treatment protocols for this devastating disease presents a medical challenge for veterinary and wildlife professionals that demands further investigation.

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Chapter Four

GENERAL DISCUSSION

Photo: Phil Battley

Chapter Four: *General Discussion*

Capture myopathy is a mystifying and frustrating disease. Treatment is rarely successful, yet prevention relies upon a better understanding of the aetiological factors that increase risk. Wild animal captures are complex events with many interacting variables, and the reasons why individual animals vary greatly in their propensity to succumb to the disease are also difficult to evaluate. Adequate prevention strategies are reliant on: better understanding of individual susceptibility; the external factors under our control that may be contributing; and the environmental factors that must be taken into account when planning capture events. Until such risk factors are defined, developing effective treatment methods is essential to increasing our understanding of the disease and improving outcomes. This study focused on identifying possible risk factors and improving treatment regimens for shorebirds susceptible to capture myopathy.

4.1 INVESTIGATION OF RISK FACTORS

Individual factors that might influence susceptibility to CM include the species, age, sex, life history stage and body condition of the bird. The results of this study confirm that bar-tailed godwits and red knots are susceptible to capture myopathy. Although great knots did not develop CM in the course of this study, previous reports have shown their susceptibility (Rogers, et al. 2004). Additionally, comparisons of CK between species in this study showed great knots had similar muscle damage as godwits with cannon-net captures. Neither sex, nor body mass appeared to have had an effect on susceptibility to muscle damage in godwits. However, the majority of godwits that developed capture myopathy at Foxton estuary were in moderately poor body condition. This may have increased their susceptibility to CM, but certainly inhibited their ability to withstand prolonged captivity during recovery. We did not measure birds in the pre-migratory fattening stage, so cannot comment on the risk of high fat deposits for capture myopathy, but this would be an avenue for further research. Capture location was intimately related to life history stage, as Foxton birds were caught in October, Miranda birds in September and January, and Broome birds in February. As such, absence of location differences also reflects the lack of life history influence, at least for the over-wintering stages. However, this study did not evaluate birds during other times in their annual cycle, such as during migration, or on their breeding grounds in Alaska, and it is possible that their susceptibility to CM and stress may vary seasonally. For instance, seasonal variations in the stress responsiveness of birds are seen in other migratory species (Done, Gow, & Stutchbury, 2011; Li, et al., 2008).

External risk factors that are under our control include the capture method, the numbers and training of personnel at the capture event, the time spent in handling, processing and banding and provision of facilities at the capture site, such as shade and shelter. The only factor that we investigated quantitatively was the capture method. While some muscle damage was evident by both methods of capture, cannon-net captures showed greater evidence of muscle damage in godwits and a greater occurrence of capture myopathy in godwits and red knots. Entanglement nets were especially risky, associated with the most problematic capture and greater number of CM cases. This is corroborated by findings in other avian species, where cannon-nets (Ponjoan, et al., 2008), and particularly entanglement nets (Dabbert & Powell, 1993) have been associated with greater muscle damage and mortality.

Potential environmental risk factors include temperature and humidity variations. Interestingly, heat-stress did not appear to cause greater risk of muscle damage in godwits, perhaps reflecting species acclimatisation to hot conditions in Australia. Yet an observed elevation in plasma AST concentration in our results suggests that hot conditions may induce other capture complications, such as mild generalised tissue damage. These results add to the currently limited data on the influence of environmental factors on capture complications in birds. Previous studies by Höfle, et al. (2004) and Nicholson, et al. (2000) had conflicting results regarding the influence of temperature and humidity for risk of CM development in partridge and turkeys, respectively. The evidence so far suggests that assessing the impact of environmental factors is complicated by species susceptibility, environmental acclimatisation and deviation from normal environmental temperature and humidity. Further studies on the influence of environmental factors are needed for species prone to capture myopathy.

Guglielmo, et al. (2001) hypothesised that muscle damage is reduced in long-distance flights by migratory birds via superior morphological or physiological systems. If muscle damage is occurring following capture, then it makes sense that the damage is occurring in a way that is vastly different from what occurs routinely during flight. Dabbert & Powell (1993) attributed the increase in muscle damage to the larger holes of entanglement nets, increasing the chance of necks and wings getting caught, promoting further struggling and possibly unnatural positions of the limbs. Current knowledge of capture myopathy in birds and mammals indicates that overexertion, traumatic injuries and extreme eccentric muscle contractions are responsible for the initiating damage, leading to a physiological cascade of muscle degeneration that is difficult to reverse. Depletion of adenosine triphosphate (ATP) – the cellular currency for energy exchange - is also central to the

pathogenesis of all cases of rhabdomyolysis (Zager, 1996). Therefore, reducing injuries, entanglement and time spent in chase, handling or struggling are likely to be the most important factors for minimising CM occurrence.

4.2 TREATMENT SUMMARY

Treatment of capture myopathy is challenging due to the severity of the disease, the long time course of healing and the high potential for complications. Midazolam showed subjective beneficial effects in the ancillary treatment of capture myopathy and is worthy of continued study. Physiotherapy - in the form of standing sling therapy - was an important part of the treatment regimen and was well tolerated by the godwits in this study. Fluid therapy is an important component of treatment, in order to flush toxic metabolites from the body, to counteract dehydration and ensure kidney perfusion (Steinohrt, 1999). In fact, parental fluid therapy is considered essential in the treatment of rhabdomyolysis and its complications in people (Warren, Blumbergs, & Thompson, 2002). In our study we were unable to use intravenous (IV) fluid therapy due to logistical constraints, but ideally IV fluid therapy would be instituted in all cases of avian capture myopathy. Non-steroidal anti-inflammatory treatment would be preferred over corticosteroids, due to the high chances of immunosuppression with the latter (Quesenberry & Hillyer, 1994). Non-steroidal therapy was utilised in this study, but the specific benefits and potential side effects warrant further investigation. Additionally, this study demonstrated that inadequate knowledge of the nutritional requirements and inattention to waterproofing can impede recovery and prohibit survival. Attention to optimum nutrition could potentially increase survival in birds with low body condition. It would be ideal to keep rehabilitated birds in captivity until they demonstrate self-feeding behavior, the ability to walk, run and fly and are waterproof.

Several of the parameters we measured in godwits with CM showed promise as prognostic indicators which could provide a means for deciding when to euthanise, or for triage where resources are limited. A godwit with weight loss of greater than 20%, packed cell volume (PCV) of less than 20%, uric acid (UA) levels surpassing 1000umol/l and/or peak CK activity greater than approximately 33,000 IU/l could be expected to have a poor chance of survival. It is also apparent that treatment success cannot be determined by releasing birds back into the wild, as post-release mortality may be significant. Success could be interpreted as birds that are released which go on to live reproductively successful lives. Only one report has proof of successful reproduction by CM survivors

(Businga, et al., 2007), yet we have subjective evidence that one of the godwits we released had subsequently completed the full annual migration cycle and other authors have reported sightings of birds that were rehabilitated and release (Rogers, et. al.2004). More reports which include follow up monitoring of birds treated for CM would be useful in order to build on our current knowledge for successful treatment regimens.

4.3 PLASMA BIOCHEMISTRIES

Serial blood samples of shorebirds after capture are useful to show changes in biochemical parameters due to the capture event. The results of our study showed that capture had significant effects on biochemical parameters associated with muscle damage, electrolyte homeostasis and nitrogen metabolism. As described by Cardinet (1989) & Bailey, et al. (1997), it was imperative that the first samples were taken promptly after capture, as the first measures of CK, AST, UA and K+ were often significantly affected by time to sampling.

Creatine kinase increased significantly over time after capture and also in shorebirds affected with capture myopathy. However, peak CK values for godwits in the capture comparisons (Chapter 2) were much lower than the peak CK values found in CM affected godwits (Chapter 3). Because the maximum time interval of 6 hours for repeated CK measures was well below the 24 hour peak seen in CM affected birds, we potentially missed peak plasma CK concentrations in the field sampling. This means that we observed a snapshot in time of the CK rise that may, or may not, be an accurate measure of true muscle damage. If the progression of CK rise is linear across all individuals, then the earlier sampling may be representative of the peak value. Additionally, species variations may occur in CK measures after capture, and with rhabdomyolysis. Species specific studies, with regular controlled intervals of blood sampling for 48 hours after capture, may be able to delineate the CK progression over time.

Despite these limitations, CK remains the best biochemical indicator for muscle damage and diagnosis of capture myopathy in the live bird. To date, there have been few studies that correlate the degree of CK activity with clinical signs of disease, mortality (Bollinger, Wobeser, et al., 1989), or recovery. In this study we have shown that very large CK increases are correlated to clinical signs of disease, with reduction of these analytes as the bird recovers. This is similar to the reports by Hanley, et al. (2005), Smith, et al. (2005) and Businga, et al. (2007). Yet we also found moderate to large increases in plasma CK concentration in clinically normal birds at capture. Either this indicates that CK is not representative of muscle damage, or more likely, that birds are suffering considerable

injuries and muscle trauma due to capture that are not always obvious externally. These results imply that the capture-related muscle damage is likely to be having detrimental subclinical or delayed effects on some apparently healthy birds released back into the wild. Spraker (1987) confirmed this in captured wild turkeys which showed histological evidence of muscle damage in the absence of clinical sings. Likewise, mammalian studies have shown positive correlations of plasma CK concentrations with quantifiable amounts of muscle damage as proven by histological measurements (e.g. Ferré, et al., 2001). Additionally, large variation amongst individuals in the CK concentrations may show that not all birds respond in the same manner to capture, which may reflect a variety of types of muscle injuries or responses to injury occurring. Potentially, CM is a continuum of muscle damage, where mild cases do not outwardly show any signs at all and only severe cases present with clinical signs. Interpretation of the significance of this may differ - depending on your point of view. Perhaps where one observer sees a single paralysed bird as an aberrant case, from a medical point of view this single bird represents a 'tip of the iceberg' phenomenon, where one clinical case likely represents many birds sustaining sub-clinical muscle damage in the same capture event.

What CK measures do not tell us is whether the muscle damage is repairable, versus irreparable (Apple & Rhodes, 1988). Even if the damage is reversible, how much time would be required in order for that to occur? Worryingly, in mammalian studies eccentric exercise-induced muscle damage is associated with reduced strength and function for up to 10 days or more (Clarkson & Sayers, 1999). Impairment of flight for this amount of time could have severe consequences for a bird, such as impeding escape from predators (e.g. Cox Jr. & Afton, 1998). The rate at which a bird may recover from muscle injuries is likely to depend on other factors such as individual fitness, further stressors (e.g. presence of people), additional exertion (such as escape from a predator), or opportunity for healing in the wild. Further studies that monitor survival and behaviour of individual birds following capture and release would be beneficial.

Overall, we found that CK was a more useful measure for evaluating muscle damage in the immediate post-capture period, as AST peaks were delayed following muscular injury and also slower to decline over time, presumably due to the delayed release and longer half-life of AST metabolism (Boyd, 1983). AST therefore may be more useful to show previous muscle damage when blood samples cannot be taken immediately. This is corroborated by the findings of previous authors who found CK to be more specific to muscle (Cardinet, 1989; Franson, et al., 1985). Logically, this implies that analytes that quickly rise in the blood are more useful for rapid detection of muscle damage. Myoglobin is faster to rise in

the blood than CK (Warren, et al., 2002), as CK is a large molecule that must first enter the lymphatic system (Friedel, et al., 1979), whereas myoglobin is a smaller protein that may directly enter the blood stream following muscle trauma (Mair, 1999). Myoglobin was found to be a greater prognostic indicator of renal failure in humans affected by rhabdomyolysis (Mikkelsen & Toft, 2005) and serum or urinary myoglobin levels are often used for early diagnosis of muscle damage in people (Köppel, 1989). Additionally, myoglobin is found in greater concentrations in myoctyes of birds compared to mammals (Powell, 2000). This would be an excellent avenue of investigation for future CM research in birds.

4.4 LIMITATIONS

Throughout this study, the welfare of the birds was a primary consideration and it was imperative to ensure the birds were safely removed from nets following capture, which sometimes held up the blood sampling. It was also decided that late, unpaired or haemolysed samples would not be included in the analyses, meaning we were unable to include all of the data we collected. This mainly affected the red knot data, where less than half (14/35) of the birds we caught could be utilised in the study. This resulted in low power in the analyses of red knot data. We were unable to control the timing of the sampling as there was often only one person sampling and speed of sampling was determined by factors such as operator and handler efficiency, ensuring haemostasis and environmental factors such as heat and humidity. We attempted to counteract for timing differences by using the multivariate analysis of co-variance methods for statistical analysis, with Interval 1 and 2 as covariates. The need for prompt blood sampling after capture meant that we did not sample cannon-net caught birds that had been under the net for longer periods, potentially biasing the results. This may explain why the Foxton (entanglement net capture) birds did not show elevations compared to other locations. Differences in handling and processing of the samples were mainly influenced by time out in the field, or until the samples could be spun down. The Broome samples were handled differently, as once frozen at -20°C they had to be transported back to NZ during which time they likely thawed several times in-between transport. They also had the greatest time frozen compared to other plasma samples. Studies of hoof-stock have shown that up to 4 months freezing has no impact on CK measures and up to 7% loss of AST activity (Jones, 1985a,b, as cited in Nicholson, et al., 2000), so our maximum of 4.5 weeks should not have greatly affected the analytes, unless bird blood is significantly different. A lack of difference between the CK samples for cannon-net locations reveals that CK was not

greatly affected by the sample handling. The plasma concentration of potassium also appeared to be greatly affected by species differences and post-collection artifact. While rapid separation of collected blood is advocated (Harr, 2002), in our field situation this was not possible. Despite these limitations, we found many interesting and significant results that made this research a worthwhile undertaking.

4.5 ETHICS OF CANNON-NETTING

Capture myopathy is a welfare issue and impinges on two of the five freedoms that are central to animal welfare legislation – freedom from pain, injury or disease and freedom from fear and distress (Farm Animal Welfare Council, 2009). Obviously, for any wild animal it is impossible to prevent fear or distress in any form of capture operation, so the only welfare concern that is under our control is freedom from pain and injury. It is up to the operator - whether bird bander, veterinarian or researcher - to prevent pain or injury where possible. In this study, the majority of cases of capture myopathy, injuries and mortalities were due to cannon-net capture. The main differences between Australia and New Zealand captures, where the former had very few fatalities and no cases of CM, appeared to be the precise organisation of the Australian team, with greater proportions of trained personnel, where the NZ captures used relatively larger amounts of untrained volunteers; and environmental factors including temperature and substrate differences.

It is concerning that one of the cannon-net captures I attended was for training of in-experienced birder's and targeted species known to be susceptible to CM: this capture incurred two fatalities, plus one case of fatal capture myopathy and several traumatic injuries (pers. obs.). Over the course of this study I witnessed some horrific injuries to shorebirds caught by cannon-net including lower bill traumatic amputation, large skin and muscle lacerations, fractured wings and legs, internal bleeding, hip dislocation, carpal bruising and laceration, nerve damage (wing droop), and death. The huge explosion which goes along with a cannon-net firing may potentially have auditory effects and also warrants investigation. Potentially, I was witness to above average injury rates, as many cannon-net captures apparently incur few injuries or fatalities (P. Battley, pers. comm). However, even in the most efficient and seemingly perfect captures, the occasional shorebird would die whilst in the holding boxes with no apparent external injuries. Shorebirds are quiet species with little in the way of defense mechanisms once captured. Combined with the natural preservation reflexes of birds (where ill health is concealed) and the results seen in this study, I believe that many other mild to moderate injuries are

likely to be occurring that are not observed externally. More observations, blood-work and post-mortem evaluations of mortalities subsequent to cannon-net captures would be useful, yet there is no doubt that cannon-netting can be a cause of great harm and distress to some birds.

Therefore, the knowledge of potential harm must be weighed against the potential benefits (Ponjoan, et al., 2008) of this method of capture. The main benefit of cannon-netting other over methods appears to be the numbers of birds that can be caught at once, and in some environments it may be the only viable method of capture for shorebirds (P. Battley, pers. comm.). Other benefits mainly relate to shorebird studies in general, which have been central to a number of important discoveries and conservation measures. For instance, through information gleaned by banding and re-sightings of shorebirds, wader study groups postulated that godwits must be migrating from Alaska straight to NZ (Gill Jr., et al., 2005). This led to a study with radio-transmitters that led to the first proof that Godwits migrated in a non-stop flight from Alaska to NZ (Gauldin, 2007). Wader study groups are often the first to notice population declines (e.g. Buchanan, 2006), and leg banding studies have provided important information about the biology and movements of red knots and godwits in Australia and New Zealand (Riegen, 1995, as cited in Battley, 1997). Additionally, wading bird enthusiasts have been integral to increased inter-country co-operation in the East Asian-Australasian Flyway (EAAF) and have been influential in conservation efforts for protecting migratory shorebird habitat in parts of Asia (Vaughn, 2010). On the other hand, banded bird recoveries may be sporadic, with long time periods between re-sightings and very small proportions of recoveries to numbers of birds banded (Woodley, 2009). Baillie (1995) describes how only approximately 2% of banded birds are recovered. It also appears that much data gathered from banding and recoveries over the years may not have been collated, analysed or reported (Baillie, 1995; Wilson & Barter, 1998). In which case many of the reasons for capturing the birds in the first place become pointless and valuable information is lost to filing cabinets and hard drives.

It is undisputed therefore that shorebird capture and banding is an important tool for knowledge and conservation. Rather than prohibit capture, I suggest we modify the methods and improve on practices in order to prevent undue harm and to improve the welfare of the animals in our care. I believe cannon-netting impinges on animal welfare and should be avoided in species known to be susceptible to CM. Where there is no other choice and the proposed captures have scientific or conservation value, then attention should be brought to personnel and type of nets. Great priority should be given to having

a majority of trained, efficient personnel at cannon-net captures that can rapidly and safely extract the birds from beneath the nets and that minimise struggling during the capture and handling process. In my opinion, capture practice and training should be reserved for hardy and common species only, and training events should avoid cannon-net capture of species known to be prone to capture stress or myopathy. The net mesh size needs to be modified to suit not only the target species, but also those species likely to be caught at the same time. Enveloping (small mesh) nets appear to cause less damage and should be utilised over entanglement nets in all species known to be susceptible to capture myopathy. The ongoing occurrence of capture myopathy presents an opportunity to modify or devise new methods or net types for mass shorebird captures that minimise harm to the birds. As Fitzwater (1970) states, the methods of capture utilised by people today are not far removed from those used by our cave-dwelling ancestors and perhaps it is time to think up some ingenious new methods that reflect our progress as a humane species.

4.6 REPORTING

Theoretically, the requirement for training and permits suggest that wild bird capture should not be undertaken lightly, yet there is no formal reporting required in New Zealand for injuries or mortalities sustained from capture operations. While reporting is encouraged in training courses and may be required by species recovery groups, this is still largely voluntary (G. Taylor, pers. comm.). What's more, permits are issued to the person, rather than to an event. In New Zealand, banding operators may be supplied permits lasting up to 10 years during which time they can catch as frequently as they want, as long as it is targeting the species in the permit. From my personal experience and subjective interpretation of captures in New Zealand and Australia, dramatic losses of birds are considered "bad catches" and seem to be taken very seriously by the banding groups or individuals, with retrospective analysis of what went wrong and how to avoid similar problems recurring in the future. As such these groups, who obviously care very much for the birds' welfare, are essentially self-regulating. However, occasional individual losses are considered an unfortunate, but unavoidable 'normal' incidence by some groups and regardless of the quality of a group in general, there are always individuals that can cause problems. While serious infringements may cause revocation of permits, or could result in prosecutions under the Wildlife Act 1953 (G. Taylor, pers. Comm.), this is reliant upon reporting by other banders, or the honesty of the person in question. The NZ Department of Conservation co-ordinates targeted surveys and reports of birds caught

accidentally in the fishing industry, which includes identification and post-mortem evaluation of every bird that is handed in (Thompson, 2011), yet wild bird capture operators are not required to report injuries or mortalities, or conduct any post-mortem examinations. Wild bird capture is essentially a poorly regulated process, both in New Zealand and overseas. It would be advantageous if formal reporting was compulsory, especially for endemic, threatened or declining species. A database of such events would be simple to create and would provide a way to compare groups nationally and internationally. Reporting of these events would keep personnel and regulating bodies more aware of the potential problems occurring and provide incentive to modify methods, investigate further or undertake further training when necessary. If certain groups or individuals were showing poor track records, then supervision, re-training, or temporary or permanent banning could be employed. Of course this still involves full co-operation and honest reporting by a given group. Additionally, a database of complications due to capture would provide a resource for illuminating species, methods or other factors that are risk factors for CM occurrence and enable further protective measures to be employed.

4.7 COLLABORATIVE RESEARCH

Another potential way to minimise harm is to look at greater collaboration between researchers and other groups to gain as much information as possible per catch. This study was a collaborative project involving the Massey University Ecology group, the Ornithological Society of New Zealand, the United States Geological Survey (USGS) and Broome Bird Observatory/ Australasian Wader Studies Group. We did not capture birds specifically for the project, but "piggy-backed" on top of other research projects or capture operations. Greater collaboration would involve coordinated captures where many samples are gained, such that many groups benefit from the one capture. For instance, if each shorebird capture involved getting a representative number of blood smears, whole blood samples, faecal samples, cloacal swabs, feathers for DNA testing, etc., then many groups could benefit, including universities, government institutions, non-government research organizations and ornithologists. Limitations to this idea include potential problems with permits and where to store samples if they could not be processed immediately. Ideally, a centralised web-based database, notice board or wildlife network for New Zealand would be a way to co-ordinate such projects.

4.8 SUGGESTIONS FOR FUTURE STUDIES

From the literature and the results of this study, it is evident there are several areas of needed research relating to capture myopathy in birds. There is evidence that eccentric muscle contractions play a major role in muscle damage, yet the true role of exertion and eccentric muscle contractions in the pathophysiology of CM in birds is largely unknown. Observations and controlled studies are required. Recommendations for reducing the severity or duration of eccentric muscle use may help to minimise muscle damage and would be beneficial for prevention. Additionally, there is a paucity of information regarding the role of myoglobin in the pathophysiology of CM in birds and the occurrence of acute renal failure as a sequela. Further investigations of blood or urinary measures of myoglobin and examination of kidney histopathology of affected birds would be beneficial. Potassium as a diagnostic measure requires further assessment and should be included in future analyses to look for changes over time and amongst capture methods, utilising rapid, in-field analysers where possible. Likewise, further studies on post-capture nitrogen metabolism would build on the currently limited information of influences on plasma UA concentration. To investigate differentiation of muscle damage, tissue markers worthy of investigation include serum cardiac troponin-1 and isoenzymes of CK to differentiate cardiac from skeletal muscle damage (Cummins, Auckland, & Cummins, 1987) and to compare other liver enzymes with AST (Lumeij & Westerhof, 1987) to differentiate liver damage from generalised tissue damage.

It would also be interesting to investigate banding records including re-sighting and recapture data to see how many birds have been seen again following cannon-net captures as an indication of survival, comparing to other methods of capture such as mist-netting. Additionally, evaluating the survival of birds that have been re-captured a number of times could provide some interesting data as to the effects of re-captures on survival. Refinement of treatment protocols is necessary and further research involving midazolam as a treatment or preventative (such as intranasal doses in the field after capture, or during treatment) would be worthwhile avenues of investigation. While many reports have shown that capture myopathy can cause significant morbidity and mortality, there are still few studies that address the longer term Implications, such as effects on breeding success, migration and longevity. Additionally, more studies looking at risk factors for muscle damage are required in order to further our knowledge on prevention strategies in birds. Finally, new or refined methods for mass shorebird capture should be investigated.

4.9 CONCLUSIONS & RECOMMENDATIONS

Capture of a wild animal will never be a risk free endeavour. Our goal must be to continually improve and refine capture, handling and transportation protocols in order to minimise harm. Where certain capture methods prove to cause undue harm, we must find alternative means to capture these animals, especially if they are threatened or declining species. This has importance not only for the welfare of the animals, but also to reduce impacts on breeding success (Legagneux, et al., 2012) and bias for survival and morbidity data (Abbott, et al., 2005; Ruder, et al., 2012). Capture myopathy is a devastating disease that causes great suffering for the animal, with a long course of healing that currently has a relatively poor rate of treatment success. Prevention of CM requires knowledge of the species susceptibility and risk factors for that species. Measures for risk include creatine kinase measures as an indicator of muscle damage and occurrence of clinical signs of the disease.

Successful prevention strategies have been limited by incomplete knowledge of risk factors. From the knowledge gleaned to date, the following preventative measures could be summarised: 1) Entanglement (large mesh) nets should be completely avoided for all shorebirds known to be susceptible to capture myopathy. Greater entanglements in the larger holes of the net and increased extraction times appear to be risk factors in these birds; 2) Cannon-net captures are associated with greater muscle trauma in godwits and it is preferable to catch this species with mist-nets or other methods where possible. 3) Where cannon-netting is the only feasible method, then attention should be brought to ensuring quality, trained personnel are present at the captures, ensuring minimal time beneath the nets and speedy, efficient extraction of the birds; 4) Hot ambient temperatures may not be a risk for muscle damage in shorebirds that are acclimatised to their environments, however there may be an increased risk of electrolyte derangements and tissue damage from heat stress, especially where temperatures deviate from the normal mean; 5) Sex of the bird and body mass (not including fat birds) do not appear to be risk factors for capture myopathy, at least in godwits; 6) Other potential ways to minimise CM occurrence should focus on reducing stress, struggling and handling time; 7) Greater reporting of capture complications, collation of data and regular assessment is needed to gather more information.

Treatment of capture myopathy is difficult, yet midazolam shows some promising traits as part of a treatment protocol. Parenteral fluid therapy, non-steroidal pain relief, physiotherapy, adequate nutrition and ensuring waterproofing of shorebirds prior to release are integral parts of a successful treatment regimen. It must be recognised that the

treatment and rehabilitation process may take weeks and euthanasia may be preferred in common species or severe cases. Severe elevations in biochemical parameters including creatine kinase and uric acid, loss of body weight and low packed cell volumes are useful prognostic indicators that could be utilised for the triage of shorebirds with clinical signs of capture myopathy. Establishing true risk factors and finding successful treatment protocols for this devastating disease presents a challenge for veterinary and wildlife professionals that demands further investigation.

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