New Zealand Mustelids and the Ecomorphometrics of mandibles

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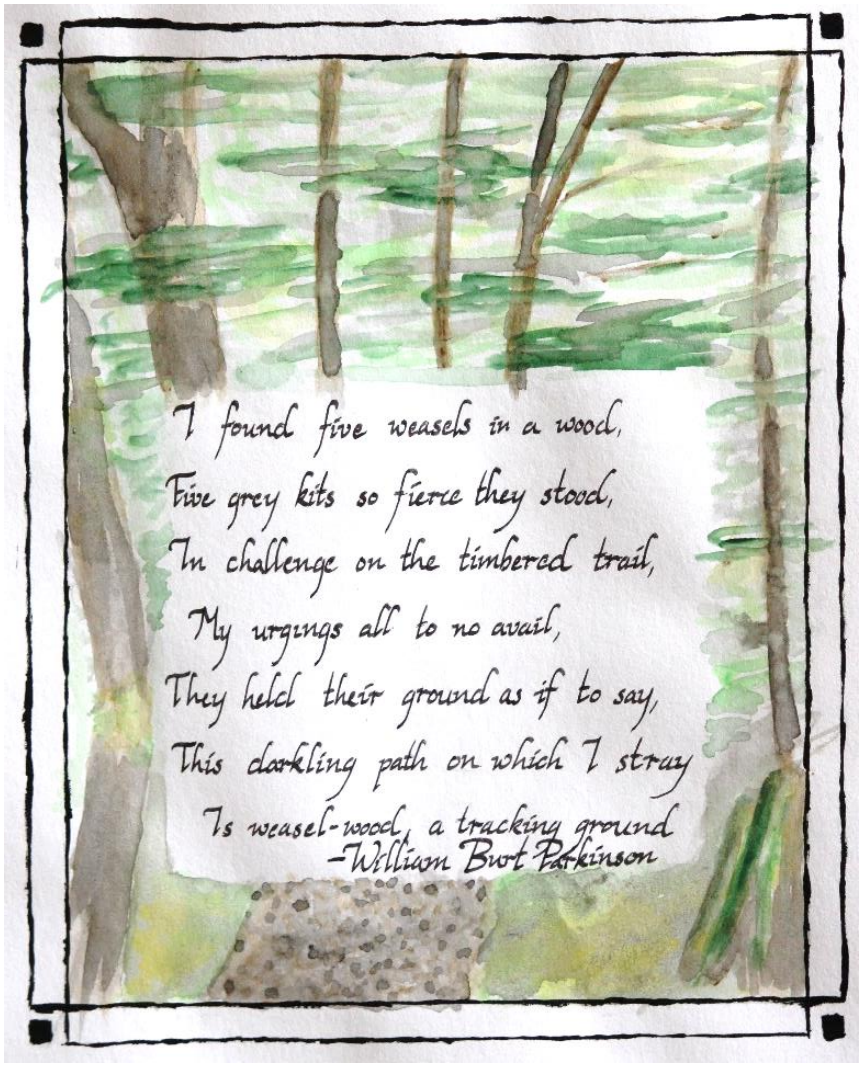
Abstract

Three species of Mustelidae are found in New Zealand: ferrets (*Mustela furo*), stoats (*Mustela erminea*), and weasels (*Mustela nivalis vulgaris*). The introduction and spread of mustelids into a wide range of habitats different from those of their native lands has provided an opportunity to study the impacts that habitat differences might had on mustelid mandible morphology, especially stoats which are more widespread in New Zealand. Geometric morphometrics were used to make comparisons of the morphological variation of mandibles within and between the three New Zealand species. Each mandible had 24 landmarks. There was size sexual dimorphism within each species but no shape sexual dimorphism. However, there was between species allometry and mandible shape differences, which can be related to diet composition and the bite force required to kill prey. The second comparison examined the morphological plasticity of stoat mandibles collected across ten New Zealand habitats and one English location. There was no shape sexual dimorphism and the degree of size sexual dimorphism was different at each location. Male stoats had a high variation in mandible size likely from size plasticity in a response to differential prey availability during growth. Some locations had significantly different mandible shapes from others, these matched differences in biomechanical advantage and likely represents adaptation to the environment. Mandible shape of stoats was correlated with rainfall which has been correlated to mice density. My results also called the into question the correlation between mandible size and skull size, which now requires further study.



Drawing 1. Five Weasels. Poem by William Burt Parkinson. Artist: C. Hill.

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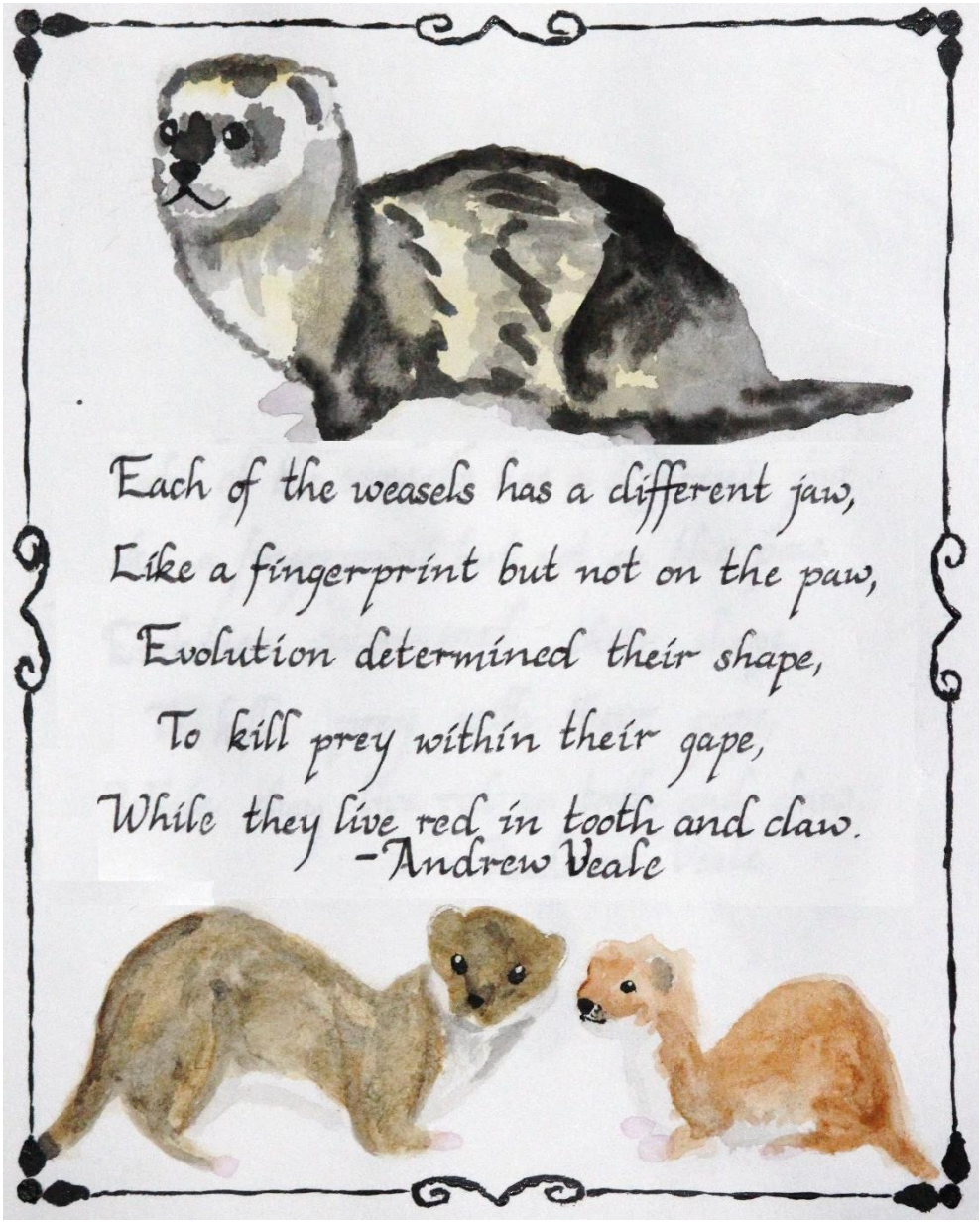
Chapter One

Introduction



Drawing 2. Poem by Andrew Veale written for this thesis. Artist: C. Hill.

Evolution is the result of differential reproductive success of earlier generations in response to their environment (Freeman et al. 2007). Those individuals that had beneficial phenotypes for the environment they were in produced more offspring, therefore increasing the prevalence of their genotype and changing the composition of the gene pool of the current generation (Freeman et al. 2007). Phenotypic plasticity is the variation of a phenotype, from a genotype, over an individual's lifetime and is the response of the individual to the current environment (Freeman et al. 2007). The changes to the phenotype from plasticity are not heritable but the degree of plasticity of a genotype can be heritable, which can be favourable for an increased ability to respond to the environment (Freeman et al. 2007). Islands provide a natural laboratory for studying structural changes in bone because evolutionary processes progress faster on islands (Renaud et al. 2010). These processes typically begin with bone plasticity and then the potential for plasticity that is advantageous is inherited and can become more prevalent (Renaud et al. 2010).

Three species of Mustelidae are found in New Zealand, which is an archipelago: ferrets (*Mustela furo*), stoats (*Mustela erminea*), and weasels (*Mustela nivalis vulgaris*) (King et al. 1982a; King 2005). In the 1880s New Zealand was suffering from a massive invasion of European rabbits (*Oryctolagus cuniculus cuniculus*). The decision was made to introduce their natural predators: ferrets, stoats, and weasels from Britain in the hope they could control rabbit numbers (King et al. 1982a; King 2005). Unfortunately, the introduction of mustelids has had, and is still having severe negative impacts on New Zealand's natural biodiversity (Cuthbert et al. 2000; Dilks et al. 2003; King 2005). The introduction and spread of mustelids into a wide range of habitats different from those of their native lands has provided an opportunity to study the impacts that habitat differences might have had on mustelid morphology, especially stoats which are much more widespread in New Zealand (Baker et al. 1979; King et al. 1982a, b, c, d; Caumul et al. 2005).

The aim of this thesis is to outline how the ecology and habitat of mustelids affects the size and shape of the mandible. The mandible is of interest because its characters directly relate to diet, and potentially also to other environmental factors (Renaud et al. 2010; Suzuki et al. 2011). Mastication is the breakdown of food in the mouth, using the teeth in a grinding and/ or shearing motion prior to swallowing and

digestion (Herring 1993). To break the food down, force is applied by the muscles acting on bone, putting pressure on the teeth which then transfer that pressure to the food by a vertical or transverse motion, or some combination of the two (Herring 1993). The mandible is a lever, the muscles put pressure on one end, which is transferred through the fulcrum (condyle) to the teeth (Herring 1993; Biknevicius et al. 1996; Hildebrand et al. 2001). The diet of the animal is reflected in the mandible. Herbivores and those animals that rely on grinding of plant matter typically have large angular processes and masseter muscles (Herring 1993; Biknevicius et al. 1996; Hildebrand et al. 2001; Christiansen et al. 2007). Carnivores and those that require crushing and shearing forces typically have large coronoid processes and corresponding large temporalis muscles (Herring 1993; Biknevicius et al. 1996; Hildebrand et al. 2001; Christiansen et al. 2007). The shorter and fatter the mandible the more strength it has (Renaud et al. 2015).

I will begin my thesis with a literature review, followed by a description of the methods used during the analyses. The first results chapter compares the morphological variation of mandibles within and between the three New Zealand species. The second examines the morphological plasticity of stoat mandibles collected across the New Zealand habitats. All this information is drawn together in the discussion and conclusions.

1.1 Literature review

1.1.1 Introduction

This section begins with a survey of the methods used to search the available literature, followed by a short overview of the effects of environmental variables on the skeleton, focussing on the mandible. The biology of mustelids in New Zealand, stoats in particular, will be summarised. This will be followed a brief history of morphometric methods and of recent improvements in them. Morphometric techniques and methods used on a range of species, and the basic morphometric analyses already performed on mustelids, will be summarised. This review aims to identify knowledge gaps yet to be filled, which will then form the objectives of the thesis and the methods to be used in the analyses.

1.1.2 Methods

I used the ISI Web of Science database to search the literature, using a series of key-words. For the general introduction on the effects of ecology on the skeleton the key words: "musc* (muscle and musculature), ecolog* (ecology and ecological), effect*, mandib* (mandible and mandibular), carnivor* (carnivore, carnivorous and carnivory), adapt* (adapting, adaption, adaptation, and adaptive), radiation", other key words also used in various combinations were "masticat* (for masticatory and mastication), "mandib* AND biomechanic* (for biomechanical and biomechanics)", and "masseter AND temporalis AND bit* (for biting and bite)". Papers relating to the topic were retained, chosen by reading the abstract. If a paper cited other work for key points, ISI Web of Science was used to search for the cited articles which were then saved. If the paper was highly cited the citation list was also searched for newer relevant information.

Prior to a database search on mustelids the sections on ferrets, stoats, and weasels were read in *The Handbook of New Zealand Mammals* (King 2005) to obtain basic information and the keywords to use in the following database search. The keywords used were: "*Mustela* AND Mustelid* AND New Zealand" and then adding various combinations of the following: "ferret*, weasel*, stoat*, diet*, habitat*, histor* (to search for both historical and history), Brit* (for Britain and British) and dimorphism".

Basic information on morphometrics was found by using the key-words "geometr* AND morphometric*" and "morphometr* AND landmark* AND method*". This was followed by more detailed searches: "morphometr* AND dimorphism*", "morphometr* AND mustel*", "morphometr* AND evolution*", "morphometr* AND mustel* AND allometr*", and "morphometr* AND mandible* AND mustel*". The method for assessing papers and finding new papers used in the mustelids search was repeated here.

1.1.3 Effects of the environment on bone

1.1.3.1 Changes from plasticity

An organism is a series of interconnected parts or traits, some of which are highly integrated and these are called modules, which co-vary to varying degrees with

other modules (Martin-Serra et al. 2015). The connections and integrations come from the interactions both direct and indirect of developmental and signalling pathways and processes, and these processes and pathways can be affected in similar or different ways by the same external stimuli over the individuals lifetime (Klingenberg 2010).

Bone is a dynamic tissue and can change, particularly during the growth period of the animal, to allow the animal to respond to environmental stimuli (Cardini et al. 2008; Anderson et al. 2014). Epigenetics describes the effects of the environment on the genome, altering gene expression in the individual (Cardini et al. 2008). Because the skull and mandible are related to feeding, an important life function subject to strong natural selection, they can illustrate and how bone and muscle interact (Cornette et al. 2013). The mandible is typically more variable than the skull, because the skull is related to more functions (Cardini et al. 2008). These responses to the local environment help the animal survive in the current conditions, and because bone is a living adapting tissue this process can occur even in adulthood (Anderson et al. 2014; Scott et al. 2014). Of course this process does not only occur in the mandible but across the skeleton (Martin-Serra et al. 2015).

Bone plasticity is not just a long term effect but can introduce perceptible changes rather quickly. It has been demonstrated in lab conditions by Anderson et al. (2014) who raised mice on either hard foods or soft foods, and this difference in food consistency caused changes in mandible shape and also the muscle efficiency over the individual's life time. Generational effects of these dietary conditions and therefore effects on the genome, were not studied (Anderson et al. 2014).

Part of the alteration of the genome can include changes in developmental and signalling process which results in the plasticity of the shape enhancing the variation in shape (Klingenberg 2010). For example, different degrees of muscle tension on the mandible bone increases variation in mandible shape correlated with a diet of softer or harder foods (Klingenberg 2010; Anderson et al. 2014).

1.1.3.2 Genetic changes

All bones, and bone and muscle modules, are integrated to varying degrees; within bone modules, such as in the skull, are more integrated than between bone modules,

such as the different bones in the leg (Martin-Serra et al. 2015). Natural selection from environmental stressors favouring some variants more than others can affect the degree of integration between modules, and can affect some modules more than others, and this can also affect bone shape and the degree of shape covariation across modules (Martin-Serra et al. 2015). If there are variations in the available plasticity of the genome within a population, natural selection will result in a change in the relative proportions of traits in the next generation, thereby changing the mean mandible shape over time (Klingenberg 2010; Anderson et al. 2014).

Meloro et al. (2011a) found that within Carnivora, mandible shape is in part determined by ecological variables, such as diet of the species. Within Carnivora there are species-specific differences in mandible shape and the degree to which each muscle is involved in feeding, and this depends on many factors including the degree of carnivory (Meloro et al. 2011a). The two main masticatory muscles pull on the mandible in different directions; Figure 1.1 shows these muscles and their relative attachment sites on a mustelid mandible (Herring 1993; Biknevicius et al. 1996; Hildebrand et al. 2001). Genetic traits determining mandible shape vary between individuals; natural selection between them, in response to environmental conditions, determines the long term differences between species in mandible shape (Wojcik et al. 2003).

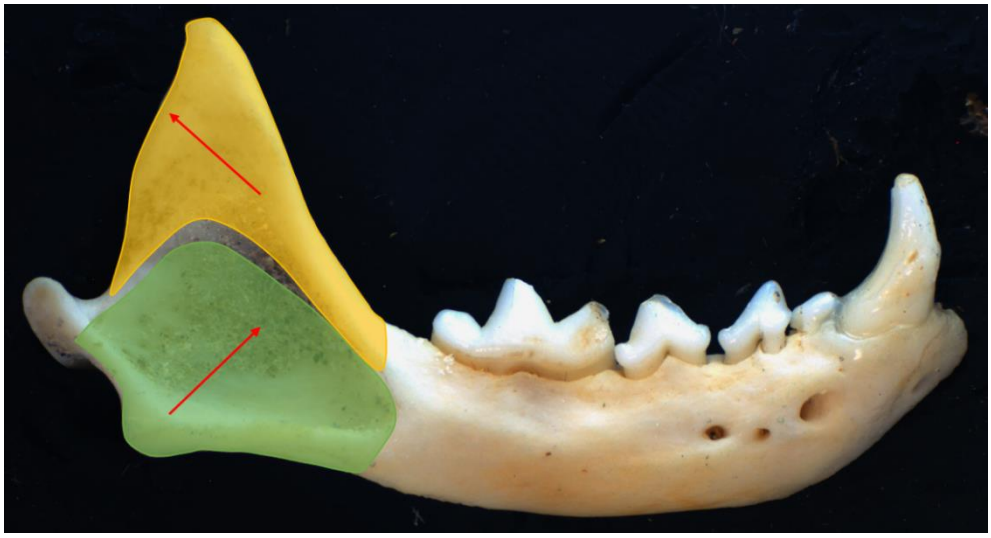


Figure 1.1. The two main masticatory muscle attachment sites (temporalis: orange, masseter: green) on the mustelid mandible. The red arrows indicate the direction the muscles pull. The attachment sites were estimated based on images and descriptions from Rogers (1986), Hildebrand et al. (2001), and Wroe et al. (2013).

Within the order Carnivora is the super-family Musteloidea, the weasels and their relatives (Koepfli et al. 2008; Catalano et al. 2015). Musteloidea is a large and diverse super family which has undergone adaptive radiation across most of the globe since it emerged 32.4-30.9 million years ago (Sato et al. 2012). It has been separated into families and sub families with niches ranging from semi-arboreal, to fossorial, to semi-aquatic (Schutz et al. 2007; Koepfli et al. 2008; Sato et al. 2012; Catalano et al. 2015; Dumont et al. 2016). The family Mustelidae is diverse and widespread and includes: otters, weasels, grisons, martens, and badgers, which span a wide range of diets and lifestyles (Koepfli et al. 2008). Each lifestyle has different environmental conditions resulting in appropriate adaptations of the bones. One example: cranial shape differs in two closely related sympatric species, *Mustela putorius* and *M. eversmanii* (Abramov et al. 2003). The differences have been correlated with adaption to the ecological niche of each species, in particular the available diet, rather than their phylogeny (Abramov et al. 2003).

Mandible shape varies between species within a genus, e.g., in marmots (*Marmota* spp.) (Caumul et al. 2005), mustelids (*Mustela* spp.) (Catalano et al. 2015), and phyllostomid bats (Nogueira et al. 2009). The differences between species of phyllostomid bat mandibles were related to diet and the associated bite force (Nogueira et al. 2009). Mandible shape also varies with diet within a species, e.g., in the Punare rat (*Thrichomys apereoides*) (Monteiro et al. 2003), mice (Renaud et al. 2010) and the common shrew (*Sorex araneus*) (Wojcik et al. 2003). Mandible shape may adapt from the shape seen in the ancestral population within an isolated introduced species that ranges across a diverse environment or habitat with variable diets (Baker et al. 1979).

1.1.3.3 Biomechanical advantage

The biomechanical advantage of the mandible is a measure of mandible geometry and the efficiency of the muscles that create bite force (Anderson et al. 2014; Renaud et al. 2015). The mandible has two sections useful for determining biomechanical advantage. The ramus consists of the muscle attachment points where the forces from the muscles interact with bone and where the lever fulcrum occurs at the condyle (Anderson et al. 2014; Renaud et al. 2015). The second module is the mandible body which holds the teeth and where the downwards biting force is applied. In studies of mice, the inlever is defined as the distance from the

mandibular condyle to the site of muscle attachment on the edge of the mandible, and the outlever as the distance from the condyle to the bite point on the teeth (Renaud et al. 2015). The ratio of inlevers to outlevers is calculated from a simple measurement (Anderson et al. 2014; Renaud et al. 2015). Mustelid inlevers and outlevers are shown in Figure 1.2.

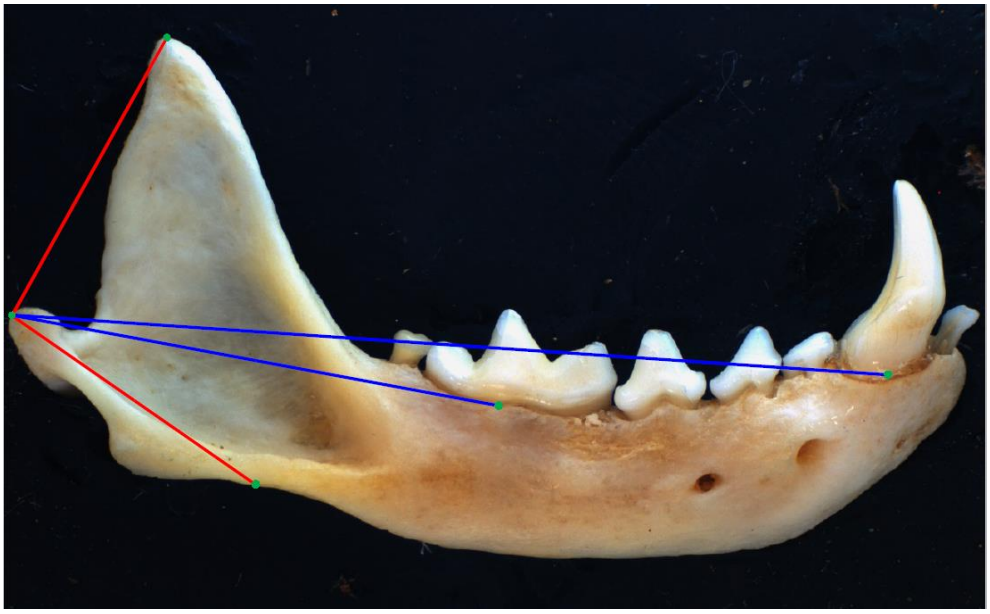


Figure 1.2. Inlever length (red lines, based on muscle insertions) and outlever length (blue lines, based on bite points) (Image is based on Figure 1 from Renaud et al. (2015)).

Christiansen (2008) studied bite force and cranial and mandible shape in felids, comparing extant felids with the extinct sabre-tooth species. Males and females were pooled in each species and each mandible had 17 landmarks. After a Procrustes superimposition, they generated thin plate spline relative warps (TPSrw) (which assign numerical values to shape changes) and then a PCA which is a visual representation of the TPSrw values in descending order of importance (Christiansen 2008). To estimate total bite force, Thomason's dry skull estimates of muscle cross-sectional area were used to identify inlevers and outlevers, and then the bite force quotients were determined before being placed through regression analyses (Christiansen 2008). However, this is a more complicated bite force method than the measure of bite force efficiency used on mice by Anderson et al. (2014) and Renaud et al. (2015), which measured biomechanical advantage by a simpler method of comparing ratios.

1.1.4 *Mustela* within New Zealand

Ferrets, stoats, and weasels were introduced to control rabbit numbers, without success (King et al. 1982a; King 2005). The National Parks Stoat Survey of 1972-1976 (NPSS), collected 1599 specimens from which basic morphological variation with habitat was detected (King et al. 1982c). Since then, new techniques for examining morphology with much greater precision have been developed, and it is these techniques that will be discussed later in this review (Adams et al. 2004; Catalano et al. 2015). This section will give an overview on what is currently known about mustelids in New Zealand.

1.1.4.1 Habitats, diets, and prey availability

The habitats and diets of New Zealand mustelids overlap, although stoats are spread over many more habitats than ferrets or weasels (Cuthbert et al. 2000; King 2005). Ferrets are found predominantly in grasslands, and their prey varies with location (King 2005). Ferrets resident in pastures and mixed habitats primarily prey upon lagomorphs (~60 % of the diet), as they do in Britain; secondary prey are typically small mammals, invertebrates and frogs or fish (Norbury et al. 1996; Jones 2002; King 2005). Ferrets will also prey on mice (*Mus musculus*) and one population of ferrets on the Otago coast prey upon sooty shearwaters (*Puffinus griseus*) when they are abundant and available (Norbury et al. 1996; Jones 2002).

Weasels are the least abundant mustelid in New Zealand, but are found in exotic forests, native forests, and the edges of roads and grasslands (King et al. 1982a; King et al. 1996b). They prefer mice and invertebrates, but also target small birds, eggs, lizards, and occasionally young rats (Murphy et al. 1998).

Stoats have spread across New Zealand, and because they are good swimmers, they have also reached many inshore islands (King 2005). They are found in most forest types (podocarp/broadleaf/hardwood, beech forest, mixed podocarp and beech forests) as well as alpine and grassland areas (King et al. 1982a, c; Murphy et al. 1992; Cuthbert et al. 2000; Smith et al. 2010). Stoats are opportunists, and can change their foraging habits with variable prey densities, and can target flightless birds or birds' eggs in burrows and tree cavities. Table 1 shows the percentage prey makeup of stoat diet across three habitats offering different prey type availability

(King et al. 1982b; Murphy et al. 1992; Smith et al. 2008; Murphy et al. 2016). While mammals typically make up a large portion of stoat diets, they are not a requirement as a population of stoats on mammal-free Secretary Island survives on invertebrates and birds (Murphy et al. 2016).

Table 1. The mean frequency of occurrence (%) of stoat prey across three New Zealand habitats (King et al. 1982b; Murphy et al. 1998; Purdey et al. 2004; Smith et al. 2008).

Prey type	Podocarp/hardwood	Beech	Alpine
Rats	74	1	0
Birds	3	5-39	17
Mice	3	31-87	4
Lagomorphs	12	2	23
Invertebrates	0.9	13	75
Skinks/geckos	0	1	0
Other	6	28	12

1.1.4.2 Genetics

Mandible shape can be used to distinguish between four marten species (*Martes zibellina, M. martes, M. foina, and M. melampus*) (Gasilin et al. 2013). Ferrets, stoats, and weasels are not as closely related as the *Martes* spp., so there should be even clearer differences in mandible morphology of the three species (Sato et al. 2012). Of the three, ferrets and weasels are more closely related to each other than either is to stoats (Sato et al. 2012).

Veale et al. (2015) studied the distribution of five distinct mitochondrial DNA (mtDNA) haplotypes within New Zealand stoats. Haplotypes 1-4 are found in the South Island, but only haplotypes 1 and 5 are found in the North Island. Unfortunately no samples from the central North Island were available, but it is unlikely that they have any different haplotypes (Veale et al. 2015). Haplotype 1 is the most common in New Zealand, and aside from the Wellington area, which has a small proportion of haplotype 5, is the only one in the North Island (Veale et al. 2015).

In the Auckland area, fine scale microsatellite loci detected moderate stoat population structuring (Veale et al. 2014). This result suggested that forest patches

could be treated as different units provided there is low habitat connectivity and any incursions into areas with control are going to be from neighbouring habitats, if at all (Veale et al. 2014). Therefore, extrapolations of likely haplotypes for areas that have not yet been sequenced can be made based on what is in the nearest areas (A. Veale, personal communication, November 17, 2016). Secretary and Resolution islands are likely to have haplotypes 1 and 2, which are present in the nearest areas of Fiordland National Park.

Haplotype 1 is the only haplotype currently found in Britain (Veale et al. 2015). The current hypothesis is that the British stoat population lost its genetic diversity during a bottleneck after the myxomatosis virus quickly and severely reduced numbers of its main prey, European rabbits in the 1950s (Sumption et al. 1985; McDonald et al. 2008; Veale et al. 2015). Because large numbers of British stoats were brought to New Zealand stoats long before this bottleneck, the New Zealand population preserves the genetic diversity of the British ancestral stock (Veale et al. 2015).

The question arises of whether stoat mandible shape correlates more strongly with the ancestral genetic lineage than with local adaptation of the colonising stocks influenced by diet and other environmental variables. However, A. Veale was of the opinion that any morphological differences detected would be most likely due to the environment, with little interference from genetics and founder effects (A. Veale, personal communication, November 17, 2016).

1.1.4.3 The effects of beech masting events in Nothofagus spp. forests

New Zealand, particularly Fiordland National Park, has large areas of beech (Nothofagaceae) forests (King 1983; Dilks et al. 2003). These forests typically have a beech overstorey and a sparse understorey with scattered mountain toatoa (*Phyllocladus alpinus*) and *Griselinia littoralis* and a mossy floor (King 1983; Dilks et al. 2003).

These forest systems, both pure stands and mixed stands with podocarp trees, have a cycling masting event, usually once every three to five years (King 1983; Dilks et al. 2003). The simultaneous flowering and seeding by the trees during these masting events cause pulsing of resources into the forest ecosystem (King 1983; Dilks et al.

2003). These pulses increase the nutritional levels of both native birds and invasive pests, and results in irruptions of invertebrates and mice (King 1983). Stoat diets vary with this cycle, increasing the proportion of mice in the diet in post-seedfall year, and hence large stoat litters can be raised with high juvenile survival rates (King 1983). While the proportion of birds in the diet does not appear to change (birds eaten per head), more birds are being eaten due to the higher number of stoats (King 1983).

Powell et al. (1997) tested the hypothesis that male size, and therefore sexual dimorphism, within and between stoat populations depends on the variation in food resources during the juvenile growth period. They tested this idea on populations of stoats in beech forests observed through two short term food pulses (Powell et al. 1997). Bone length is a finite growing process and therefore accurately represents the condition of the animal during its growth period, although bone shape can change subtly over time (Powell et al. 1997; Scott et al. 2014). The hypothesis predicted that female stoats would be less affected by mice irruptions because their size, at least in European species, seemed to be limited by reproductive requirements (Powell et al. 1997). However, in New Zealand beech forests, both sexes increased in size if their growth period coincided with a mice population irruption, but this size increase was not heritable (Powell et al. 1997). Therefore, in the present thesis, it was expected that all individuals born during a seed year would be larger than those which were not. And as diet has been correlated with mandible shape in other species (Renaud et al. 2010), an increased proportion of mice in the diet during the growth period of those years was expected to have a short-term effect on mandible shape.

Therefore, the questions to be answered are: Is there variation in mandible shape within and between samples of New Zealand stoats? Do genetics or diet play a more influential part in mandible shape, particularly in stoats from different habitats with differing diets?

1.1.5 Morphometrics and its methods

Historically, simple morphological descriptions were used to compare biological organisms, but new methods using quantifiable traits that could be put into

statistical models became more favoured over time (Adams et al. 2004). Soon, pattern changes in morphology detectable from quantifiable traits became possible and interesting, and this was the basis for modern morphometrics (Adams et al. 2004). These quantifiable traits are measurable characters that relate to the shape of the structure being examined (Adams et al. 2004; Klingenberg 2010).

Morphology in the past has been defined by the measuring the length between two points on a structure, correlating that with the length between two other points on the same or another structure, and deducing how the measurements interact and change between groups (Adams et al. 2004). Since the 1990s the use of geometric morphometrics to answer questions in biology has increased, and new and improved techniques are being developed all the time (Adams et al. 2004). The term 'shape' in modern geometric morphometrics includes all the geometric characteristics of the object including spatial relationships of features, excluding size, position and orientation, which can all be controlled for (Klingenberg 2010; McNulty et al. 2015).

The traditional use of geometric morphometrics for taxonomic studies has waned since the rise of affordable genetic taxonomy, although for fossil species where genetics is not possible morphometric taxonomy is still common (Aiello et al. 2007). The recent use of geometric morphometrics is often used in conjunction with ecological and morphology-functional studies (Aiello et al. 2007).

There is some debate about the use of morphometrics in functional studies, because any shape is the product of many interactions: genetics, environmental stressors, and the plasticity of a structure over the organism's lifetime (Klingenberg 2010; McNulty et al. 2015). Identifying which aspect of shape is affected by which factor is not easy, because datasets often lack the necessary details of external stimuli such as climate and community composition to allow for these comparisons, and some, but not all, current statistical tools are appropriate for questions of functional morphology (McNulty et al. 2015).

1.1.5.1 Statistical methods

1.1.5.1.1 General methods

Geometric morphometrics methods that are relevant here analyse shape variation in morphological structures and its covariation with other variables that interact and affect the individual, or over time, the species as a whole. Landmarks are placed on carefully standardised photographs at biologically significant points, and given X,Y, coordinates from an overlain checkerboard (often in computer pixel format) (Adams et al. 2004; Olsen et al. 2015). These shapes are usually put through a superimposition: for example, Procrustes or Booksteins (Adams et al. 2004). Zelditch et al. (2012a) in their text-book "*Geometric Morphometrics for Biologists: A Primer*" discussed the most common methods of analysing the data, and their pros and cons, in detail. One of the most common and informative methods was principal components analysis (PCA), which analyses the spread of each variable. Then from the PCA a shape deformation comparing groups is generated. For further details, see Zelditch et al. (2012a); here I will not rehash what has already been well described only give some examples.

Klingenberg (2013b) discussed different methods of visualising shape changes. Each method, including wireframe superimposition and Procrustes scatter, has its draw-backs and benefits. To clearly see a shape change in mandibular bones, the method I consider is best is the wireframe superimposition based on landmark deformation comparing one group to another (Klingenberg 2013b), such as between sexes or between habitats. This method allows scaling, so size differences can be ignored and shapes can be clearly distinguished (Klingenberg 2013b). The average shape of each group is calculated and then superimposed onto the other by altering the scaling so size differences do not interfere with visualising shape differences (Siegel et al. 1982; Klingenberg 2013b).

Size is important, and mustelid species and sexes are different sizes, but could be controlled for when analysing the shape of mandibles (King et al. 1982c; Catalano et al. 2015). The scaling can be altered when the size difference is known and the main focus is on shape (Klingenberg 2013b), and vice versa. Hence, size and shape can be studied separately. This technique is therefore the right one to answer both of the main questions in this thesis: inter-specific differences in mandible shape,

size, and sexual dimorphism of weasels, stoats and ferrets, and intra-specific shape, size, and sexual dimorphism differences in the stoat mandible across locations.

Caumul et al. (2005) studied morphological variation in the skulls, molars, and mandibles of five species of marmot in relation to environmental factors and genetics. They placed 13 landmarks on the mandibles and each sample had 3-14 individuals. The results for each sample were averaged using Procrustes superimposition before comparing with PCA (Caumul et al. 2005). Caumul et al. (2005) estimated that diet caused 35 % of the variation in the mandible shape, compared with only seven percent related to mtDNA (Caumul et al. 2005). Caumul et al. (2005) found that using only five or less individuals per group meant variance was often inaccurate, and individual variation confounded some results, especially if a landmark was missing because of bone damage. Their conclusion that diet was the biggest identifiable reason for the observed variation mandible shape, even between species, this supports A. Veale's prediction that genetic lineage will have less effect on stoat mandible shape than diet (Caumul et al. 2005).

Catalano et al. (2015) performed a phylogenetic study using morphometrics on the super family Musteloidea, using multiple bones with >360 landmarks total, but with only three individuals of each species (Catalano et al. 2015). Caumul et al. (2005) suggested that the best interspecies analysis use at least five individuals per species, minimising individual variation, which can have a large effect on the results even if a large number of bones is analysed.

Mice have spread over many islands worldwide, providing models to study insular evolution (Renaud et al. 2010). Differences between islands in habitat and diet have consequences for mice living on those islands, expressed as adaptive variations mostly related to food. Therefore, morphological characters directly related to food processing are the logical characters to study, such as the mandible (Renaud et al. 2010). Renaud et al. (2010) analysed average mandible shape in herbivorous and omnivorous wild mice (N=132) and used an elliptic Fourier transformation to reproduce outlines for superimposition and radial Fourier transformations for PCA. The Fourier transformations appeared to only show overall shape changes unlike wire frame analysis which show feature specific deformations (Renaud et al. 2010; Klingenberg 2013b). The results were also analysed using MANOVA and

multivariate regression, confirming that mandible shape significantly differed with diet (Renaud et al. 2010).

Renaud et al. (2010) excluded any tooth measurements as some teeth were loose or missing which also happened here as the majority of my specimens from the NPSS and have been in storage since 1977 (King et al. 1982a). One issue Renaud et al. (2010) had was varied sample size with location (from six to upwards of 30) and this could have affected the results, individual variation will affect the smaller sample sizes more (Caumul et al. 2005; Renaud et al. 2010).

Between studies there is a large variation in opinion on what is considered an appropriate sample size to accurately estimate mean shapes of a group so that individual variation does not skew the mean (Caumul et al. 2005; Christiansen 2008; Renaud et al. 2010; Catalano et al. 2015). Cardini et al. (2015) tackled this problem by examining both size and shape of horse premolars, beginning with a large sample (N=50) and removing individuals until the means and variances started to veer away from the original mean (Cardini et al. 2015). While mean centroid size (structure size) was less affected by sample size, shape was highly affected by small sample size, and the results indicated that 15-20 specimens were needed per sample for accurate means and variances (Cardini et al. 2015). This indicates that the results of previous research may not be as accurate as authors like Caumul et al. (2005) and Catalano et al. (2015) would like. Therefore, in this thesis, I used only samples with at least ten specimens, preferably 20 or more.

1.1.5.1.2 Testing for allometry

There are two schools of thought on how to deal with allometry. The first is the Huxley-Jolicoeur school which considers that size and shape co-vary as a single morphological unit that cannot be separated and only quantified (Klingenberg 2016). The second is the Gould-Mosimann school, which considers that size and shape are correlated but the effects of size can be quantified, accounted for, and removed (Klingenberg 2016). The variation in shape that is left can then be accounted for by other factors. Klingenberg (2016) explains that the choice of which school is followed is up to the decision of the researcher. Here I followed Gould-Mosimann.

Regression is a useful tool for determining allometry in shape. Recently Klingenberg (2016) reviewed allometry and its place in geometric morphometrics. There are three types of allometry: ontogenetic allometry (the changes in shape over time with the growth of the individual), static allometry (the differences in shape with size within a species of a single age group, or across the sexes or distinct populations), and evolutionary allometry (the differences in shape with size across differing species, that are usually closely related). These types of allometry can interact and can be hard to separate out if the study design does not account for them, for example only studying adults removes the effects of ontogeny (Klingenberg 2016). For the section comparing species the evolutionary allometry is appropriate, static allometry is appropriate for all investigations comparing the sexes and also for the section comparing populations of stoats and the investigation into the effects of beech masting.

Isometry can act as the null hypothesis for allometry, because isometric features scale up linearly with size (Klingenberg 2016). One of the current acceptable methods to quantify allometry in geometric morphometrics is to use multivariate regression on the Procrustes superimposed shapes against the standard size variable, usually centroid size. The residuals of the regression can then be used in further analyses to determine the variation in shape between groups with the allometric component removed (Klingenberg 2016).

There are two options for the regression, pooled within-group regression and non-pooled (Klingenberg 2016). Pooled within-group regression should be used when comparisons between sexes, populations, or species are being made, and is equivalent to a multivariate analysis of covariance. While pooled within-group regression assumes that all groups have a similar size range and similar regression coefficients, it can still act as a logical and sufficient test for allometry even with this criterion relaxed. Klingenberg (2016) ended the discussion by saying that more work is required to fine tune the regression methods, and that either of the schools of thought can be used as both are valid as a base for allometry tests, it all depends on the individual preferences of the researcher.

1.1.5.1.3 Modularity

A new trend in evolutionary-developmental biology is to use geometric morphometrics to investigate modularity within structures (Klingenberg 2010). Modularity is the assessment of integration of and covariation of parts (Klingenberg 2010). The integration of these modules is not always visible but can be inferred from modularity assessments (Klingenberg 2010).

There are four different classes of modularity: developmental, genetic, functional, and evolutionary (Klingenberg 2010). Developmental modules come from the interactions between signalling in localised areas during growth. Genetic modules come from loci and loci linkage which can in turn affect the integration and covariation of developmental modules (Klingenberg 2010). Functional modules, as the name suggests, provide specific functions. For example, the mandible muscles specified by genetic modules produce forces on the bone that contribute to the function of eating.

Evolutionary modules are those formed over time by selection based changes in frequency distribution of genetic traits that form a complex, which in turn affects the other three types of modules (Klingenberg 2010). Evolutionary integration has been defined as how the process of evolutionary changes in multiple parts are coordinated, and how that integration varies across a set of related species (Klingenberg 2013a). The integration within populations and species can act as a basis for comparison to be used in evolutionary studies (Klingenberg 2013a). The majority of modularity studies investigate how shape can be affected by the different forms of modularity and how modularity and phenotypic plasticity interact (Klingenberg 2010).

For example, the mandible is constructed by two developmental modules, the ramus and the mandible body. These two modules develop along two separate pathways starting in the embryo (Anderson et al. 2014). Variation in muscle tension on the mandible bone changes the bone's structure and shape, which can then become visible if the difference in shape is pronounced enough (Anderson et al. 2014).

The functional modules are change depending on the level of function to be studied (Klingenberg 2009; Renaud et al. 2010; Klingenberg 2013a; Anderson et al. 2014).

The commonly defined functional modules are the coronoid, angular process, condyle, the molar region, and the incisor region (Anderson et al. 2014). The first two relate to the temporalis and masseter muscle attachment, the condyle is the fulcrum lever point, and the last two are the chewing teeth and the piercing or scraping teeth (Anderson et al. 2014; Renaud et al. 2015). Because different levels of modularity can apply to the same structure they need to be studied under different contexts, and the study must be designed accordingly (Klingenberg 2013a). Lab techniques and tests in artificial environments can help exaggerate the action of functional modules, and so identify the levels of integration (Klingenberg 2010). Anderson et al. (2014) proved this in their modularity analysis of laboratory mice feed on extreme hard foods and extreme soft foods.

To determine if there are modules within a structure such as the mandible, first the hypothesised modules need to be identified and assigned landmarks and then run through a modularity hypothesis test (Klingenberg 2013a). This will determine if the covariation within the hypothesised modules is greater than between the hypothesised modules, which is required for the definition of recognised modules. Partial least squares analysis (PLS) looks at the strength and patterns of integration by decomposing matrices of covariance between shapes. Both the covariance and the related shape deformations provide information about the modularity (Klingenberg 2013a).

The sample size in a modularity analysis must be larger than the number of principal components that account for the majority, say 95 %, of the variance from the total shape (Klingenberg 2013a). As Klingenberg (2016) pointed out, allometry can affect shape, and therefore allometry may be integrated into the modules and may have an effect on the covariation (Klingenberg 2013a). Like whole shape multivariate regression, the modules can be run through the multivariate regression and allometry accounted for (Klingenberg 2013a).

Klingenberg (2013a) described the techniques used to analyse evolutionary modularity but standard evolutionary modularity tests are currently difficult to run for a within-taxa evolutionary analysis, and that more work is required to help establish patterns of integration within taxa (Klingenberg 2013a). In addition, Cardini et al. (2008) showed that mandibles are more susceptible to shape changes

from environmental influence on phenotypic plasticity than other structures such as some areas of the skull. Mandibles are less accurate for evolutionary modularity studies as their shapes are likely to be different from the evolutionary template due to epigenetics and plasticity (Cardini et al. 2008). Therefore, evolutionary modularity tests would not be appropriate in this thesis.

1.1.5.2 Morphometrics software

The many available software and packages for study of morphometrics all have something different to offer. R has a large number of packages, but each is written by a different person or group, and each package requires the compatible version of R for it to work (Zelditch et al. 2012b).

The TPS series of software by (Rohlf 2015), is a series of individual programs that run different analysis ranging from data file set up (tpsUtil) to landmark placement (tpsDig2) to more complicated analyses like partial least-squares analysis (tpsPLS) and regression (tpsRegr) (Rohlf 2015). The digitisation software (tpsDig2) seems to be the most commonly used, and is cross compatible with most of the other morphometrics software, including R and packages like the IMP series, as well as MorphoJ (Sheets 2001; Klingenberg 2011; Zelditch et al. 2012b; Rohlf 2015). Like the tps series, the IMP series is a compilations of different programs, it is easier to use than the tps series and has a full set of manuals, but Regress8, the regression program, follows the Huxley-Jolicoeur school of allometry, whereas I prefer the Gould-Mosimann school (Sheets 2001).

MorphoJ is a free and comprehensive morphometrics program written for biologists by Klingenberg (2011) and was the best program to use in this research. It has manuals freely available online and in pdf versions, which also come with explanations and reasoning behind each test (Klingenberg 2014). Another benefit of the MorphoJ software is it has most of the common analyses (for example PCA, discriminant function analysis, CVA) and a few others which include incorporating genetics and shape into phylogenetic trees (Klingenberg 2011).

MorphoJ software also has some limitations. It has only Procrustes superimpositions, whereas other programs have at least two options, typically Procrustes and Booksteins superimpositions. It is also missing the Anderson's chi-

squared test for the significant eigenvalues in the PCA (Sheets 2001; Klingenberg 2011). However, Klingenberg has said that the significance test is not always useful, and the data patterns can provide more information for biological specimens and their differences. If required, significance testing can be used in conjunction with the data patterns (C. P. Klingenberg, personal communication, September 8, 2016).

Another advantage of the MorphoJ software is that the outputs are visually more helpful compared with those from the tps and IMP series, as they are more customisable and the data and images can be exported in useful formats if other programs are required, for example, exporting centroid size data into STATISTICA version 12 for the centroid size analyses (Klingenberg 2011; StatSoft Inc. 2014). The Regression analysis from MorphoJ is also more useful, not only does it follow the Gould-Mosimann school, but there are options for regressing shape onto other variables such as age for ontogenetic studies (Klingenberg 2011). As morphometrics increases in popularity there are more and more programs to do any set of analyses that a researcher can wish for, the issue is just narrowing down the useful analysis (Aiello et al. 2007; McNulty et al. 2015).

1.1.6 Mustelids in morphometrics

Catalano et al. (2015) investigated the phylogenetic relationships of 22 species of Musteloidea using morphometric analyses of several bones, including: cranium, mandible, axis, cervical vertebra six, scapula, humerus, ulna, pelvis, and femur. The mandible results are most directly related to this thesis, based on 23 landmark points. I used these as a base for the landmarks in this thesis, except that the teeth landmarks were omitted (Catalano et al. 2015).

The American mink (*Mustela vison*) was taken to Europe, Asia, and Russia to supplement the fur trade. Some mink were released to the wild and some escaped from fur farms, to establish feral populations which compete with the native mustelids (Kruska et al. 2003). Using basic measurement morphometrics Kruska et al. (2003) have shown that, since their domestication and subsquent escape the skull and mandible of feral mink now differ to their wild relatives. This case can act as a model to show how adaptation to a new environment can result in population differences in bone shape over a short space of time, in evolutionary terms.

Domestication of the American mink started in 1866, and European releases in the 1920s. Ferrets have been domesticated since Roman times, so the domestication process could also have affected their New Zealand descendants, perhaps to cause the similar changes (Kruska et al. 2003).

While investigating allometry, Suzuki et al. (2011) compared the skulls and mandibles of two weasel species, *Mustela itatsi* and *M. sibirica*. I intended to use a similar technique to quantify allometric differences in mandible shape between the three *Mustela* species in New Zealand. Suzuki et al. (2011) had over 30 male and female specimens for the larger *M. sibirica* but no females were available for *M. itatsi*, so only males (N=39) were analysed for this species. I did not use their landmarks and general shape analyses methods because they used 45 distance measurements which do not allow for whole shape examination. There was allometric sexual dimorphism in *M. sibirica* mandibles (Suzuki et al. 2011). The two species also showed allometric functional differentiation, most pronounced at sites of muscle attachments used for eating, reflecting diet differences (Suzuki et al. 2011). The three mustelid species in this study, with their different sizes and diets, were likely to show a similar trend.

Sexual dimorphism is common among mustelids, and body size is the most common form of it, at least so far in the literature, usually expressed in simple measurement morphometrics. ANOVAs comparing groups is the most common method for determining whether there is dimorphism (Loy et al. 2004). Loy et al. (2004) studied the sexual dimorphism of two *Martes* spp., both size and shape, using geometric morphometrics. They found that size was a component of the shape sexual dimorphism but did not account for all of it, and neither did it account for the inter species differences in shape. They interpreted these results to be correlated with the different diets typical of the two species, which produce different stressors on the feeding related structures (Loy et al. 2004).

Abramov et al. (2003) used simple measurement-based morphology to study sexual dimorphism in skull size of European mink (*Mustela lutreola*), but did not mention shape. They did carry out a discriminant analysis, which classified and then reclassified individuals based on the measurements, and correctly grouped each individual by gender (Abramov et al. 2003). They also mentioned that the skulls of

male *Mustela putorius* and *M. eversmanii* are usually 16 % larger than females (Abramov et al. 2003). These are the two species most closely related to *M. furo*, the ferrets found in New Zealand, so it was likely that the degree of sexual dimorphism in them would be similar (Sato et al. 2012). Discriminant function analysis is also available for shape variables, and is an easy technique to analyse whether two groups are different.

Elsasser et al. (2008) used discriminant analyses to separate *M. erminea* from *M. frenata*, and Gasilin et al. (2013) used it to distinguish between four species of the genus *Martes.* However, Elsasser et al. (2008) and Gasilin et al. (2013) used basic measurement morphology. Gasilin et al. (2013) also lacked enough specimens for some of the groups with sample numbers as low as six, although the technique worked even with the small sample size.

Canonical Variates Analysis (CVA) is a method similar to discriminant function but for more than two groups. It was used to identify the largest differences between seven species of British mustelids (Lee et al. 2004). This was effective in separating out the species and identifying which characteristics are different and which are similar amongst the species. Stoats and weasels were grouped with only a small separation, but ferrets were quite different (Lee et al. 2004). These were mostly linear skull measurements and not whole shape measurements, but it does indicate that ferrets should have the most distinct mandible if the trends follow the skull (Lee et al. 2004).

King et al. (1982c) analysed nine different body measurements for the stoats collected during the NPSS, including: total length, mandible length, and condylobasal length of the skull. These measurements were used to investigate growth patterns and sexual dimorphism, and variation body and skull size with location (King et al. 1982c). On average male stoats were 36 % heavier than females, and mandibles were on average 13.7 % longer, but there is currently no information on sexual dimorphism in mandible shape of New Zealand stoats. Because the degree of sexual dimorphism in size changes with the feature being examined, there could be shape differences and maybe allometry between the mandibles of the two sexes (King et al. 1982c), correlated with the differences in their diets (King et al. 1982b).

New Zealand male stoats from beech forests were three percent longer in condylobasal length and four percent larger in body length compared to males from podocarp and mixed forests, a trend also seen in other measurements (King et al. 1982c). Females showed a similar but less extreme difference (King et al. 1982c). The likely explanation is that food pulses generated by the beech mast cycle increased growth, as skull length is finite and determined by food availability in the birth year (Powell et al. 1997). Many of the stoats collected for NPSS were caught in the post-seedfall summer of 1976-77, but the potential influence of this effect was not allowed for in the reported analyses.

King (1991b) tested a hypothesis from Erlinge (1987), predicting that body size in stoats is correlated with the average size of mammalian prey. If correct, this hypothesis predicts that New Zealand stoats should be larger than their ancestral stock in Britain, as the average mammalian prey is larger in New Zealand. The results supported the hypothesis, but it was acknowledged that invertebrates were excluded, because they are rarely eaten by British stoats but frequently by New Zealand stoats (King 1991b). There was not enough known about the foraging strategies of New Zealand stoats, and as yet we still do not know much, because the focus in New Zealand is typically on how to kill these animals (King 1991b; King et al. 2007b). Male British stoats sampled since the population recovery from myxomatosis did have a higher component of lagomorphs in their diet compared to New Zealand stoats, whereas the larger possums in the diet of New Zealand stoats are likely to be carrion and do not require high force applied by the mandible for a killing blow (King et al. 1982b; McDonald et al. 2000). While mandible length changes with habitat, and is likely related to diet or other environmental factors such as temperature or altitude, shape has not previously investigated. Newer morphometric techniques now make this possible.

1.2 Objectives and hypotheses

It is well known that ferrets are bigger in body and skull size than stoats, which are bigger than weasels (King 2005), but as yet there has been no interspecific comparison of the New Zealand mustelid mandible shapes, so there is no way to predict whether any changes observed will be related to genetics or diet or the potential extent of phenotypic plasticity (Meloro et al. 2011a). There is also no

current information about sexual dimorphism in the mandible shape of these species, or on modularity and bite force efficiency. Therefore, the material available invited answers to several questions.

1. Was there sexual dimorphism in shape as well as in size? The null hypothesis was that there was no sexual dimorphism in shape.

2. Did the degree of sexual dimorphism differ between species? The null hypothesis was that there were no differences in the degree of sexual dimorphism.

3. Were any detectable differences in mandible shape, across the species, isometric or allometric with size? The null hypothesis of this study was that ferret mandibles would be a scaled up version of the mandible of weasels and stoats, hence all the shapes should be isometric.

4. If allometric variation in shape was found, did size account for all or just a component of it?

5. How did shape differ between the species? Was there any modularity in the mandibles of each species, and was it detectable across the species?

6. Did the bite force efficiency of the mandibles differ between the species? The null hypothesis was that there was no difference in bite force efficiency.

Further knowledge gaps to be filled include the potential differences in mandible shape of stoats collected from different habitats around New Zealand. The spread of stoats into a wide range of habitats different from their native ones has provided an opportunity to study the effects of habitat differences on stoat mandible morphology (Baker et al. 1979; King et al. 1982a, b, c, d; Caumul et al. 2005). Stoat ecology has been studied extensively in New Zealand, looking at a range of factors that include: diet, genetics, reproduction, pest control, and pulsating resources (King et al. 1982a, b, c, d, e; Murphy et al. 1992; King et al. 1996a; Powell et al. 1997; Cuthbert et al. 2000; King 2002; Veale et al. 2015; Murphy et al. 2016). Shape may change with sexual dimorphism, environmental conditions, or diet,

which led to several questions in intra-specific variation similar to the inter-species comparisons. Based on results of the Powell et al. (1997) study on the effect of beech seed fall and stoat body and skull size, both male and female mandible size should fluctuate across habitats with varying food density, and sexual dimorphism should remain fairly similar across habitats because of this. The differences in mandible characters between stoats collected in England and in the New Zealand should also follow the same trends previously reported for skull and body size (King et al. 1982c; Powell et al. 1997; Piontek et al. 2015). New Zealand stoats should be larger and have a lower sexual dimorphism (King et al. 1982c; King 1991b; Powell et al. 1997).

1. Was there sexual dimorphism in mandible shape as well as in size *within* different locations from New Zealand? The null hypothesis was that there was no sexual dimorphism in shape controlling for location.

2. Did the degree of sexual dimorphism differ *between* locations? The null hypothesis was that there were no differences in the degree of sexual dimorphism.

3. Were any detectable differences in mandible shape isometric or allometric with size? The null hypothesis of this study was that there would be no within location-between sex effects of size on shape, and that there would be no effect of size on shape between locations, hence all the shapes should be isometric.

4. If allometric variation in shape was found, did size account for all or just a component of it?

5. Was mandible morphology affected by the increased food available to young born during a beech (*Nothofagus* spp.) seed masting year? Overall body size, diet, and expected lifespan are known to change with a beech seed year, but changes in mandible shape have not yet been examined (King et al. 1982b, c; King 1983, 2002). The null hypothesis was that there would be no effect of beech seed masting on mandible shape.

6. How did shape differ *between* locations after the sexes were pooled for each location? The null hypothesis was that there was no difference in shape across locations. Was there any modularity detectable across the locations?

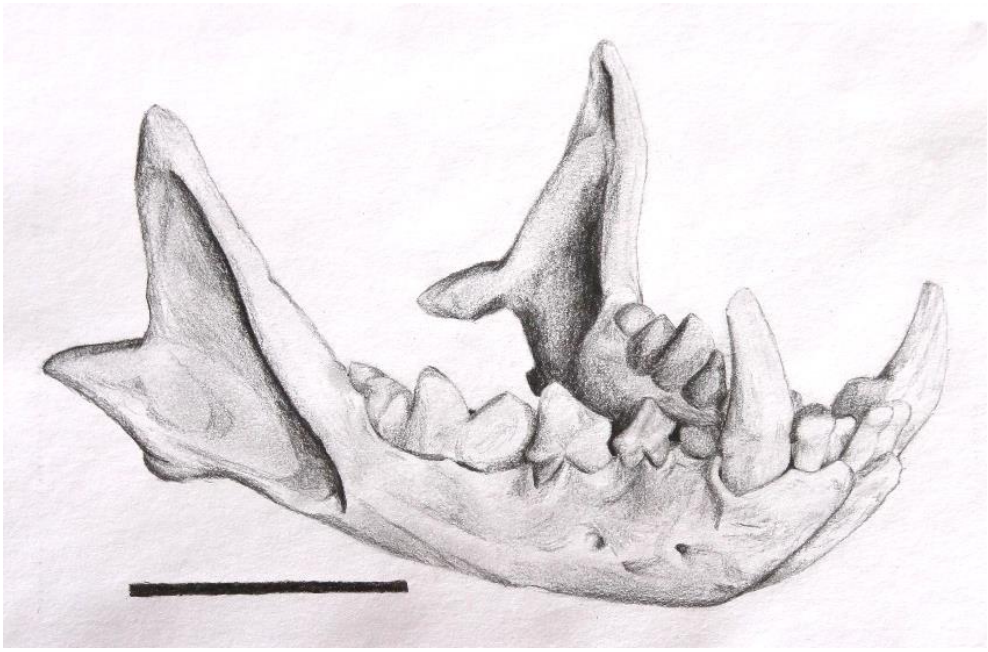
7. Did the bite force efficiency of the mandibles differ between the locations? The null hypothesis was that there was no difference in bite force efficiency.

8. Was there any covariance between mandible shape and environmental or dietary factors? The null hypothesis was that shape was independent of other factors.

New morphometric techniques provide the opportunity to study these differences and give information on micro-evolutionary processes and adaption (Baker et al. 1979; Renaud et al. 2010).

Chapter Two

Methods



Drawing 3. Full mandible of a male stoat from Packington Park, Warwickshire, England. Black bar is 10 mm. Artist: C. Hill.

2.1 Samples

2.1.1 Sample sourcing

Between 1972 and 1980, 1599 stoats, 40 weasels, and 56 ferrets were collected from sample areas of ten National Parks plus four other areas of New Zealand (King et al. 1982a) during the DSIR National Parks Stoat Survey (NPSS). Existing data on the skulls and mandibles have since been logged into a database with the relevant information including: sex, age, year collected, body size, and place of collection. The mustelids were caught using a variety of methods and by various groups, and the sample space represented over two million ha of New Zealand's 26.8 million ha. Field methods and lab protocols were described by King et al. (1982a).

Other specimens include 15 male weasels from Pureora collected 1982-87, and 37 male and 32 female ferrets from Waotu caught in 2004 and 2005, both in Central North Island (King et al. 1996c; King et al. 2007a). As the small female weasels are relatively harder to catch, specimens from more than one location were pooled (Craigieburn Forest Park, Nelson Lakes National Park, Mahina Bay, Mount Cook National Park, and Paraparaumu) (King 2005), and some female weasels caught as recently as 2016 were added to this study (Paraparaumu).

Some stoats from the Coromandel peninsula were collected specifically for the purposes of this study. Stoats from Resolution Island and Secretary Island previously trapped and stored (P. McMurtrie, personal communication, April 4, 2016), Pureora Forest Park (King et al. 1996a), and Grebe Valley (Purdey et al. 2004) were also available. To represent the ancestral stoat population, 24 stoats, I used 12 of each sex trapped in Packington Park, Warwickshire, England from the mid-recovery phase (1977-1989) after the myxomatosis epidemic (Sumption et al. 1985; McDonald et al. 2008).

2.1.1.1 Data limitations

The material available limited the types of questions that could be asked. Among the NPSS stoats, some local samples were large enough to subdivide by gender or season, and others were not. Inadequate material ruled out some locations completely, e.g. Tongariro NP and Mount Bruce. Some locations e.g., Mount Egmont National Park had good skull samples, but all the mandibles were missing

as they had been sent off to a lab in US for age determination. The samples were returned to New Zealand by the lab but, as it was not foreseen they would be needed again at the time, they were destroyed in order to avoid the costs of bringing them back through New Zealand customs.

One potential question to be investigated initially was how age, sex, and location affected the growth and shape of the mandible. Unfortunately, with so many variables to consider, there was simply not enough young stoats of each age category for any sort of analysis. This was particularly unfortunate as there is no existing analysis of mandible growth and shape, although Powell et al. (1997) and King et al. (1982c) both examined how body length and condylobasal length changed with growth.

Another limitation to my data was the need to find new samples. The NPSS programme did not cover all of New Zealand or all the known genetic variation (King et al. 1982a; Veale et al. 2015). In an effort to add missing details into the analyses I sought to collect more stoats from community groups. Consequently, some additional stoats were collected up to and over 40 years after the NPSS specimens. The recent specimens were often difficult to collect, because extensive pest control at some locations has reduced the number of individuals left. DOC200 spring traps, a common stoat trapping method, often crush the skull and mandible of captured stoats, which rendered them unusable for this study.

2.1.1.2 Ethics

Ethics approval was not required as all specimens were trapped by third parties in the normal course of their work, and the carcasses were saved and sent in for research. No trapping was performed by the researcher during the course of this study.

2.1.2 Sample preparation

Cleaned and stored mandibles required no preparation. Fresh weasel and stoat skulls and mandibles were cleaned by cooking them in a sodium perborate solution (100 mg/ml) in a Contherm Digital Series Five oven at 60°C, as recommended by McDonald et al. (1999). The required cooking time varied depending on the

specimen. The female weasel mandibles were very small so required only 24 hr of incubation time, compared to the 48-70 hr for most of the stoats. Stoat mandibles that were well-fleshed but still partially frozen when they went in the oven took the longest time. Stoat mandibles from Resolution and Secretary Islands that were decomposed to the point where only bone and some scraps of flesh were left took the shortest time, 2-24 hr.

Sexes were identified using descriptions from *The Handbook of New Zealand Mammals* (King 2005). All specimens were kept organised in their location groups and by sex within location groups. After the specimens were dried they were put into individual sealable Glad plastic bags and labelled with their sex and a unique identification code (site###).

2.1.3 Objectives and sample sizes

It was important to include only full-grown adult animals in this study. Weasels were considered independent and sexually mature at three to four months old, so all wild weasels caught and used in this study were considered adults (King 2005). Although they may not be fully grown until six months old, but unfortunately this was not discovered until after all analyses had been completed (C. M. King, personal communication, January 27, 2017). The ages of wild-caught ferrets can be determined using tooth cementum layers, so only ferrets from 0.5 years old were used in this study (King 2005). Adult stoats can be distinguished into age classes using a variety of skull characteristics, most relevant to this study were the lines in the canine teeth (Powell et al. 1997).

Objective one compared intra-specific variation within *Mustela* spp., minimising local variation wherever possible by taking one location to represent each species: Westland National Park in 1972-1976 for stoats, Waotu in 2004-2005 for ferrets, and Pureora Forest Park in 1983-1987 and 2016 for weasels. These locations produced 18 adult males each of ferrets and stoats, and 15 male weasels. The female analysis used 18 stoats (Westland National Park), and 17 ferrets (Waotu). Because only seven female weasels were available, they had to come from different locations: Craigieburn Forest Park, Mount Cook National Park, Mahina Bay (Wellington), Nelson Lakes National Park, and Paraparaumu. Female weasels are lighter than

males and do not roam as far, so are less likely to come across a trap and activate it (King 2005).

For Objective two, samples were chosen using the NPSS and P&K Microsoft Excel databases by "source, age group, sex, seed year birth" (National Park Stoat Survey database (King et al. 1982a), and the Powell and King database used for studies on the effect of beech seed masting events on stoat ecology (King 1983; Powell et al. 1997)). Both Excel databases can be found on the appendices disk at the end of this thesis. Locations with 10-12 available specimens per sex in the adult age group were chosen for further analysis, and seed years and non-seed years were treated as separate groups within each site. For the locations that were not part of NPSS, the stoats were grouped by location and sex, and 10-12 of each sex per location were used.

Samples identified as suitable on the databases were then manually inspected, and any specimens with incomplete mandible bones were discarded. Specimens with missing teeth were acceptable, as the teeth sockets were still visible. Some of the mandibles were missing or too damaged for use, which, as mentioned earlier, severely limited the numbers available for analyses.

2.2 Photography method

A stereo microscope (model: Leica MZ12) and its attached camera (model: Zeiss AxioCam HRc) were used to take two dimensional photos of each specimen. Each image was automatically transferred to AxioVision version 4.8.2 software, which ran on a desktop computer with a Windows XP operating system. The camera and microscope were set up to avoid parallax and potential effects of camera orientation (Mullin et al. 2002).

Before specimens were photographed a microscope scale ruler was placed in the field of view at the correct magnification for the specimen (Ferrets: 0.1512x, stoats: 0.3024x, and weasels: 0.378x) and the scale calibrated for the site. To calibrate: the first specimen is placed on the stage and the objective and zoom are fixed, the specimen is then removed and a glass mm ruler is placed on the stage. A photo is taken, and the "scalings" tab is used to set the scale by placing the cursor across the

first 20 mm of the ruler. This scale is saved and then applied to mandible photos using the scale bar option in the photograph capture screen.

This procedure was repeated for each location, and all specimens from each location were photographed in one session. The right mandible was used where possible; if the left mandible had to be photographed, the image was later flipped, see section 2.3 Landmarks. The first photo from each location had a scale embedded into the photo, using the scale bar option. Each specimen was placed on the stage on a black plasticine block roughly five millimetres high and positioned horizontally, so the outside face of the mandible was facing the camera. The plasticine allowed each specimen to be placed at the same angle as the previous one.

Around the plasticine base and specimen was a polystyrene disposable cup with the end removed, which helped to diffuse the light of the small spotlights used to highlight the curves and create shadows on the specimen in the photo. The shadows and highlights aided in accurate landmark placement, and the lights were moved to the best area needed for each specimen; the focus, exposure, and white balance were also adjusted where needed to maximise the photo clarity of each specimen. The lights were designed and made by B. O'Brien for the microscope. Light intensity was controlled by a BioENG 4 channel Luxeon controller (B. O'Brien, personal communication, 03 September 2015). Figure 2.1 shows the microscope stage and light setup including the cut polystyrene cup used as a light diffuser. The photos from each location were saved in .tif format into separate folders to avoid confusion later on in the analysis.

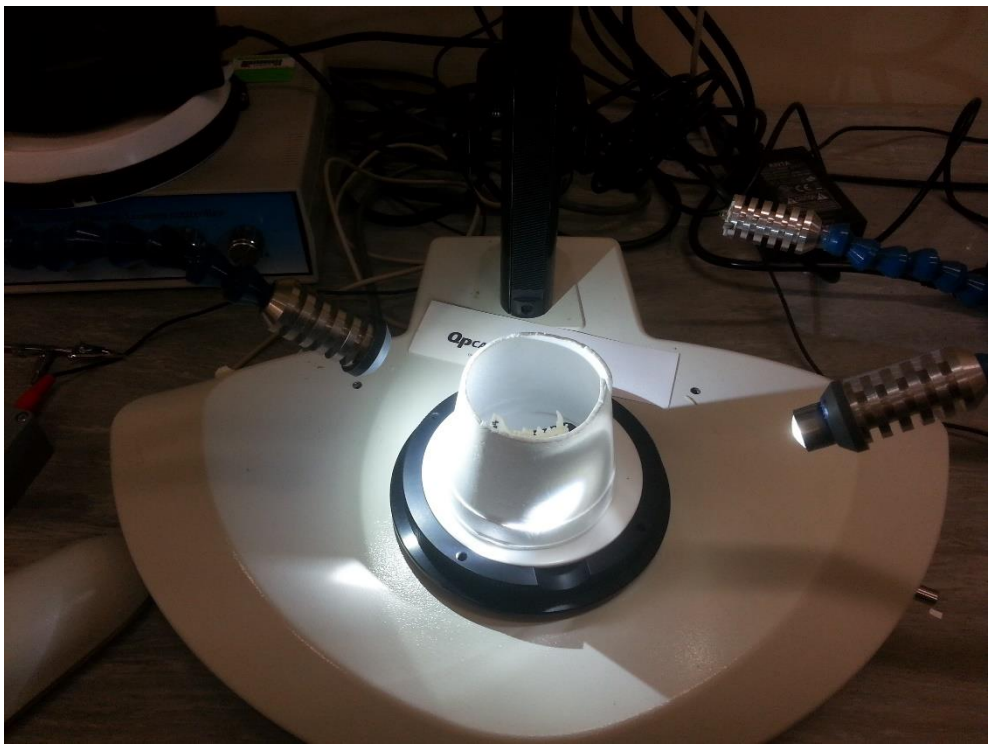


Figure 2.1. The microscope stage and light set up for photographing specimens.

2.3 Landmarking method

Landmarking is an advanced method of analysing size and shape of images in photographs (Zelditch et al. 2012a). To digitally landmark the photos for the analyses, tpsUtil (version 1.65) was used to create a .TPS file that links the photos of all specimens from a location together. The tps software series can be found in Rohlf (2015). Each species or location had a different .TPS file labelled with the species or location abbreviation. tpsDig2 (version 2.22) opened the .TPS file. If a photo of a left mandible required flipping, this was done first and then the file and the photo were saved. Once this was done for each photo, the whole file was saved and exited before reopening the file to landmark. Flipping left mandibles to face the right would not affect overall results (A. West, personal communication, 10 December 2015), but ensured consistent presentation. The scale for the photos was set using the measure option on the tool bar, by measuring the number of pixels on the embedded scale bar on the first photo.

The landmarking process was extended. Twenty-two landmarks and four curves (Figure 2.2) were placed on each mandible photograph, in the same way for all objectives, based on landmarks illustrated by Zelditch et al. (2012a); Catalano et al.

(2015). The .TPS file with the curves was then re-opened in tpsDig2 and the curves were appended to the file. Notepad software was used to open the .TPS file, and for each specimen the ID section was completed with the classifier string appropriate for the specimen (Table 2).

However, after feedback from other morphometric scientists and an article on mouse morphometrics by Pallares et al. (2016), I determined the dimensionality was too large, so the landmarks were reduced to 24 without curves (Figure 2.2). For confirmation, two sample groups (the stoat sites) were landmarked and then re-landmarked as practice. After all the specimens from a location had been landmarked, each set was checked and landmarks were adjusted if required, as sometimes a second look was needed to ensure they were in the correct position. Physical descriptions of the landmarks can be found in the Appendices disk.

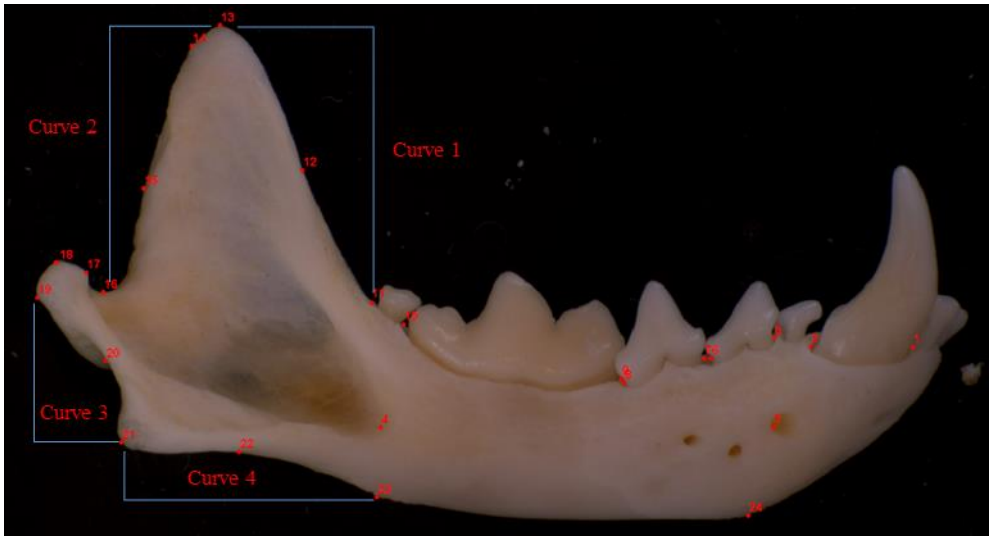


Figure 2.2. Twenty-four digitised landmarks, and the original proposed curves on a right mandible from a stoat (CP014MN). The full curves are not shown, only the area they covered is indicated here. For an explanation of the specimen classifier in brackets see Table 1.

Table 2. Classifier strings used in each objective. Sections included: species, source: the location where the specimen came from, ID: the unique three number code for the specimen, sex, year: the year the specimen was collected, and seed year: whether the specimen was born in a beech seeding year.

Objective one	Objective two
Species: ## (FE, ST, or WE) ID: ### Sex: M or F Year of capture: #	Source:## ID:### Sex: M or F Seed year: Y or N
FE008F4	CP014MN

2.4 Statistical analyses

2.4.1 Objective 1: Interspecific morphological differences between New Zealand mustelid mandibles

For this objective, specimens of the three species of mustelids were sourced from locations shown in Figure 2.3, the total sample numbers can be seen in Table 3.

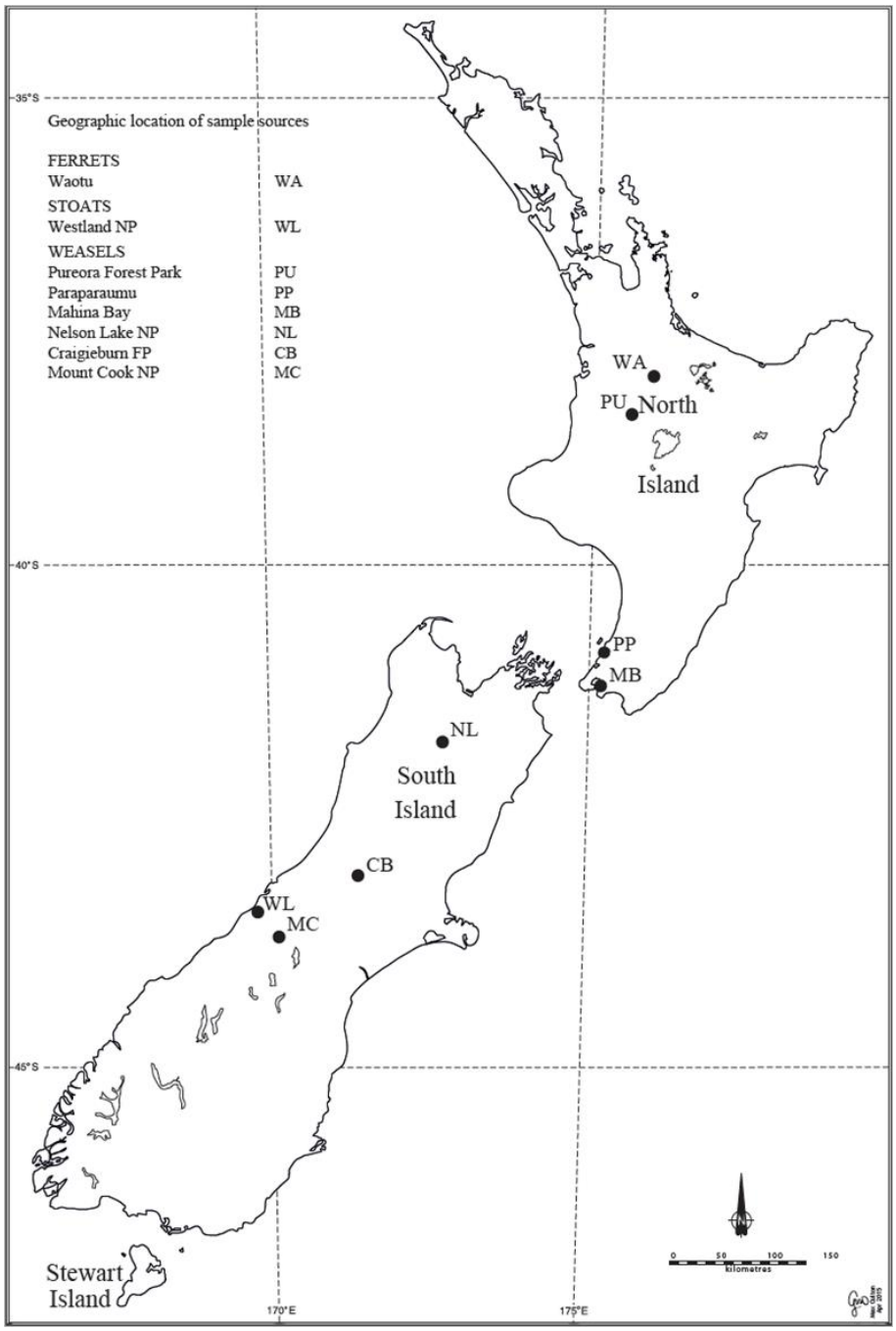


Figure 2.3. New Zealand geographical map showing the source locations of the ferrets, stoats, and weasels (Original image by M. Oulton, modified by C. Hill).

Table 3. Total sample numbers of ferrets, stoats, and weasels used in this objective.

Species	Males	Females	Total
Ferrets	18	17	35
Stoats	18	18	36
Weasels	15	7	22
			93

2.4.1.1 Software preparation

The aim of this objective was to investigate potential differences in mandible size and shape between the sexes within the three species of mustelid (ferrets, stoats, weasels), and differences between species. The .text shape files described by the landmarks, and required for some of the software (IMP based software, Sheets (2001)), were generated by exporting the data sets (separately for males and females of each species) from MorphoJ (version 1.06d) (Klingenberg 2011), including the raw coordinates and the centroid sizes. The resulting file was then copied into Microsoft Word, where the labels and log centroid sizes were edited out and saved as a .text file. The files were then combined to form species-combined files as well as sex-combined files, which allowed for the within-species and the between-species analyses. The group file was created following the guide in the PCAgen6 manual. The methods for completing the statistics for all the IMP based software were followed directly from the manuals for each program (Sheets 2001).

2.4.1.2 Size analysis

Centroid size data were exported to Excel from MorphoJ and loaded into STATISTICA version 12 (StatSoft Inc. 2014). Variations in size within-species by sex and within-sex between species were tested with ANOVA and Newman-Keuls post-hoc tests (Loy et al. 2004), descriptive statistics (mean and standard deviations) and Brown-Forsythe tests for homogenous variances. Categorised probability plots to check for normality were also generated. The average sexual dimorphism was calculated from the average male and female centroid size of each species.

2.4.1.3 Regression analysis

Before any shape analyses were performed, MorphoJ was used to perform a Procrustes superimposition, to remove any biases introduced by orientation, and

scaling. This was done by reducing the sums of squares to the lowest possible level between the shapes (Zelditch et al. 2012a). To test for allometry a pooled within-group multivariate regression analysis was performed in MorphoJ. At the same time permutation tests against the null hypothesis were performed (10,000 permutations), and a regression wireframe deformation was generated.

Statistics were calculated for the total, predicted, and residual sums of squares and the percentage of shape explained by the regression, and a p-value. This was done first during the within-species analysis comparing the sexes, and then later it was used to compare the species without separating the sexes. If the regression and therefore evidence of allometry was significant, then the residuals of regression were obtained and all analyses were performed on the residual data. Using the residuals removed the consequences of allometry (variation in shape correlated with size), therefore leaving only variation in shape to be accounted for by other factors.

Any images that required editing, such as colour changes or shape filling was done using Inkscape™ 0.91, a freely available image editing software. Only regressions which proved significant were presented in the results, all other non-significant regressions were placed onto the appendices disk at the end of this thesis.

2.4.1.4 Principal components analysis

Based upon the methods of Siahsarvie et al. (2012) and Bower et al. (2015), the data for each species (ferrets, stoats, and weasels) were put through a PCA analysis to investigate the difference between males and females and to identify any outliers within the species data set. The PCA analyses was conducted between sexes within species, and between species with sexes combined. PCA analyses were done twice, once before regression and, if the regression was significant, again after regression. Each PCA also had the 90 % confidence ellipses added to the graph.

The group-coded Procrustes superimpositions were obtained using PCAgen8. The group consensus shapes were the ones obtained during the discriminant analysis. The group shapes were edited in Inkscape to produce solid shapes. These were then overlaid onto any PCA graphs that showed a clear division between groups. Only Principal components (PCs)/ axes that showed a division between the groups being analysed were presented (unless there was no division, in which case PC one and

two were presented), and only for PCs that accounted for more than five percent of the variance, or occurred before the inflection point, which ever came first.

2.4.1.5 Eigenvalue analysis

Eigenvalues, obtained during PCA analyses, give information of the concentration of variation across dimensions (Zelditch et al. 2012a; Klingenberg 2013a). Based on the explanations by Zelditch et al. (2012a) it was expected that the graph will be the reverse of an exponential graph where the first few dimensions' account for the majority of the variation and then the amount of variation per dimension tapers off, the point where the taper begins is known as the inflection point (Zelditch et al. 2012a; Klingenberg 2013a). The dimensions provide information about the shape changes, or the aspects or subsets of the shape that change (Zelditch et al. 2012a). The eigenvalues were saved and graphs showing the percentage of variance explained for each eigenvalue/ PC were generated in Excel. The cumulative percentage was also calculated.

Following Zelditch et al. (2012a), only eigenvalues that explained five percent or more of the variance have been presented. There are other options for significant eigenvalues, including (1) presenting all PC's that account for the first 90 % of variance, (2) only the eigenvalues that occur before the inflection point on the eigenvalues scree graph, and (3) Kaiser's modified rule: only eigenvalues with a value 0.7 or higher are counted (Izenman 2008; Zelditch et al. 2012a).

The eigenvalues were tested for distinct variances using Anderson's Chi-squared test of eigenvalues in PCAgen8 (values over 5.99 were considered significant). Zelditch et al. (2012a), in "*Geometric Morphometrics for Biologists: A primer*", mentions only the five percent method, the inflection point, and Anderson's Chi-squared test, so these were the methods that were used. Tables were generated that show the eigenvalue, the percentage of variance each eigenvalue explained, the cumulative percentage, and an indication of the inflection point if there was one. PCs with distinct eigenvalues were used to create wireframe deformation graphs within MorphoJ. Wireframes for PCs which showed variation between the groups were also presented.

2.4.1.6 Discriminant function analysis

Discriminant function analysis was performed in MorphoJ, within and between species. The discriminant function analysis is a canonical variate analyses (CVA) for only two groups. It gives the Procrustes superimpositions of the means of each group, which also serves as the deformation graph moving from one group mean to the next. The analysis included the Procrustes distance between means, the Mahalanobis distance between means, the Hotelling's T^2 test, the parametric p-value, and the p-values after a 10,000 permutation test.

The Mahalanobis distance between means takes the within-group variation into account during the calculation, unlike the Procrustes distances between means, which takes only the group mean itself into account. Mahalanobis distances in MorphoJ also base their significance of bootstrapping and not degrees of freedom, and can therefore be more accurate when sample sizes are small (Brombin et al. 2009).

Classification/ misclassification tables were also created, presented as tables containing all the discriminant function analysis information, except in the case of the between species analyses. For them, only the deformation images and classification/ misclassification information were presented, and all other statistics were obtained as part of the CVA. The cross-validation tables are the preferred form of information for investigating the separation of the groups, and so the percentage of misclassified individuals was calculated from these and presented (Klingenberg 2011). To begin with the entire classification/ misclassification tables were going to be presented, but they proved to be large and difficult to read, and so they were condensed following the methods of Pallares et al. (2016).

2.4.1.7 Canonical variates analysis

A canonical variate analysis (CVA) calculated in MorphoJ, can be performed only on three groups or more, so no within-species analysis could be conducted using this method (Siahsarvie et al. 2012; Zelditch et al. 2012a; Renaud et al. 2015). Canonical variates analysis simplifies the differences between already defined groups. Estimating the distances between characteristics that vary between groups but do not vary within groups can help with describing differences in shape (Zelditch et al. 2012a). All CVs that accounted for up to 100 % of the variance were

presented with their associated wireframes, and a table was compiled showing the generated Procrustes and Mahalanobis distances and p-values from permutation tests.

2.4.1.8 Modularity analysis

Modularity is the assessment of integration of and covariation of parts (Klingenberg 2010). The modularity analysis I used required the landmarks to be split into developmental and functional modules. Functional modules are grouped to form developmental modules (Figure 2.4A and B), based on those used by Anderson et al. (2014) and Renaud et al. (2015). The two developmental modules were the ramus and the mandible body (Figure 2.4A). The five functional modules were: the condyle, angular process, the coronoid process, the molar section of the mandible body, and the incisor/ canine region of the mandible body (Figure 2.4B).

The modularity testing was performed first for each species individually and then on all the species combined, both on the raw Procrustes superimposed landmarks and the landmarks corrected for allometry (using the multivariate regression methods outlined earlier). This was done first for the two developmental modules and then the five functional modules, following the methods outlined by Klingenberg (2011) for analysing contiguous partitions only with a full enumeration of partitions. The number of permutations depends on how many contiguous partitions are possible, which depends on the number of total landmarks and the number of landmarks per module.

Wherever the modularity hypothesis for the developmental modules was supported, a partial least squares (PLS) analysis of two blocks within one configuration was conducted with the same partitioning. The PLS results gave an RV coefficient and a P-value after 10,000 permutations. The scatterplot of corresponding PLS scores (saving those that accounted for more than five percent of the variance) as well as the associated deformation graphs were saved. Also generated was a table of values from the PLS which included: the axes singular values, the percentage of the total squared covariance for each PLS axes, the correlation scores between the block for each axes and the p-value calculated for each axis generated during the permutation test (Klingenberg 2011). Percentage scree graphs were created for the percentage of total covariation, similar to the eigenvalue scree plot, and tables which showed

the data for all axes that accounted for five percent or more of the total squared covariance.

The significant modules were then separated, and each module was then run through: ANOVAs on centroid sizes, Procrustes superimpositions, regression analyses, discriminant function analyses, PCAs, and if applicable CVAs. The appropriate tables and graphs were created following the same methods used for the whole shape analysis.

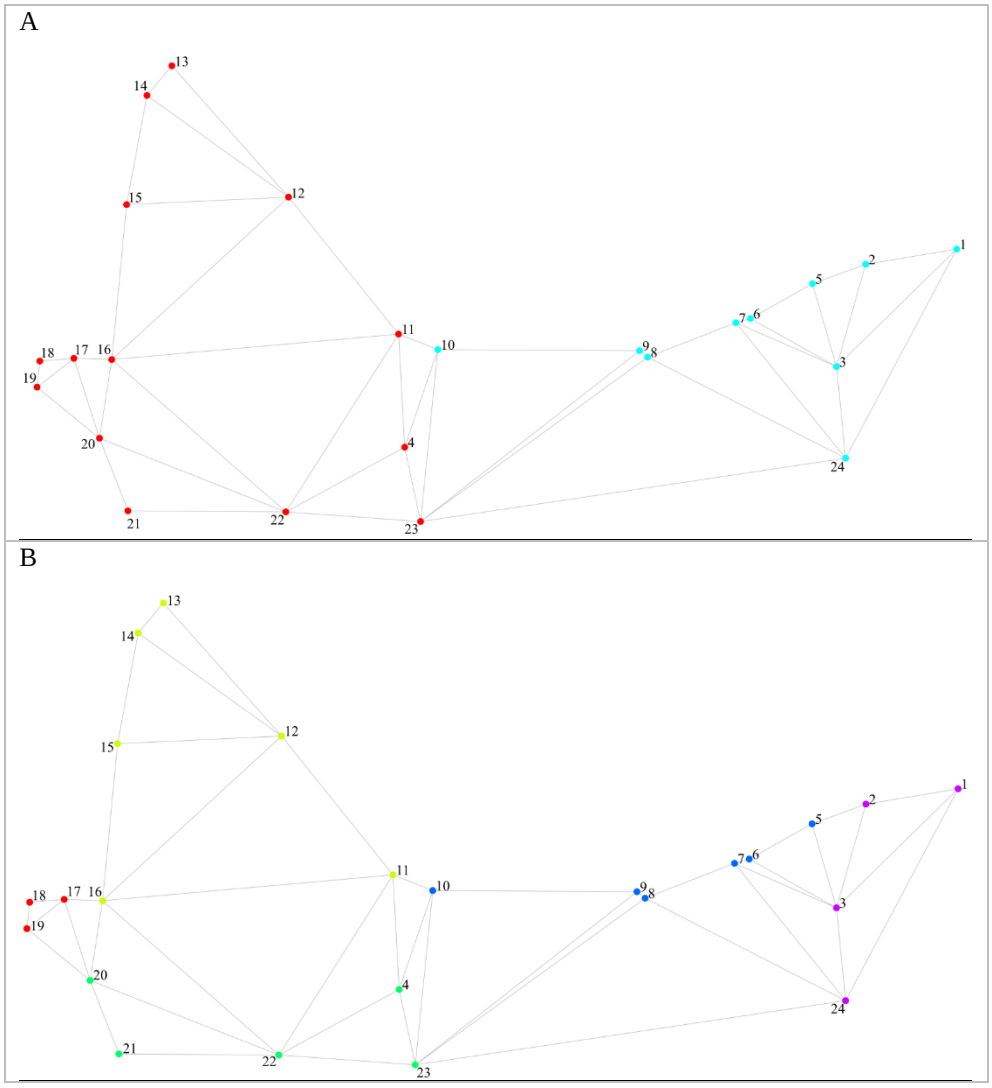


Figure 2.4. Wireframe layouts used in the modularity analyses. A) The developmental modules are the ramus (red) and mandible body (light blue). B) The functional modules are the condyle (red), angular process (green), coronoid process (yellow), molar section of the mandible body (blue), and incisor/ canine region of the mandible (purple). The pale grey lines indicate the connectivity used in the adjacency graph in MorphoJ.

Table 4. Landmarks organised into developmental modules and functional modules.

Developmental modules	
Ramus	4, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23
Mandible body	1, 2, 3, 5, 6, 7, 8, 9, 10, 24
Functional modules	
Condyle	17, 18, 19
Angular process	4, 20, 21, 22, 23
Coronoid process	11, 12, 13, 14, 15, 16
Molar section of the mandible body	5, 6, 7, 8, 9, 10
Incisor/ canine section of the mandible body	1, 2, 3, 24

2.4.1.9 Biomechanical advantage

The biomechanical advantage of the mandible is a measure of mandible geometry and muscle efficiency used for bite force (Anderson et al. 2014; Renaud et al. 2015). In studies of mice, relationship between inlevers (the line of action of the muscle to mandible-skull attachment point) to outlevers (line of action of the bite point to the mandible-skull attachment point) is a simple ratio measurement (Anderson et al. 2014; Renaud et al. 2015). The inlever is the distance from the condyle to the site of muscle attachment, the outlever is the distance from the condyle to the bite point (Renaud et al. 2015).

Biomechanical advantage was examined by the methods used by Renaud et al. (2015) to study the change in house mouse (*Mus musculus domesticus*) mandibles after an anthropogenic disturbance on Guillou Island, a sub-Antarctic island. For the present study the outlevers were defined differently, because the feeding behaviours of *Mustela* spp. are different from those of mice (Renaud et al. 2015). *Mustela* spp. have two bite points on the mandible: the lower canine (C) for the killing blow and the lower carnassial (M1) for the shearing of meat. Therefore, the two outlevers were measured as the distance from the condyle to each of these two points (Figure 1.2). Renaud et al. (2015) placed the outlevers on the crowns of the teeth, but many of the mustelid teeth were missing on my specimens, so the outlevers were shifted to the mandible. The canine outlever was placed in the centre of the canine socket, and the carnassial outlever was placed on the socket for the middle one of the three roots (Figure 1.2).

The inlevers were based on the two main jaw muscles used by *Mustela* spp.: from the condyle to the tip of the coronoid process where the lower edge of the temporalis muscle (T) attaches, and from the condyle to the middle of the ridge to which the masseter muscle (M) attaches to the ramus and the angular process at the edge of the jaw (Figure 1.2). The measurements were made in millimetres from the scale imbedded in the file, using the tpsDig2 software and the measurement function, and entered into an Excel file.

Four biomechanical advantage measurements were made on each mandible (inlever/outlever): temporalis muscle/canine (T/C), temporalis muscle/carnassial (T/M1), masseter muscle/canine (M/C), masseter muscle/carnassial (M/M1) (Renaud et al. 2015). The differences between each group were tested using Kruskal-Wallis analysis of variance for each ratio measurement using STATISTICA, comparing sexes within species and within sexes across species.

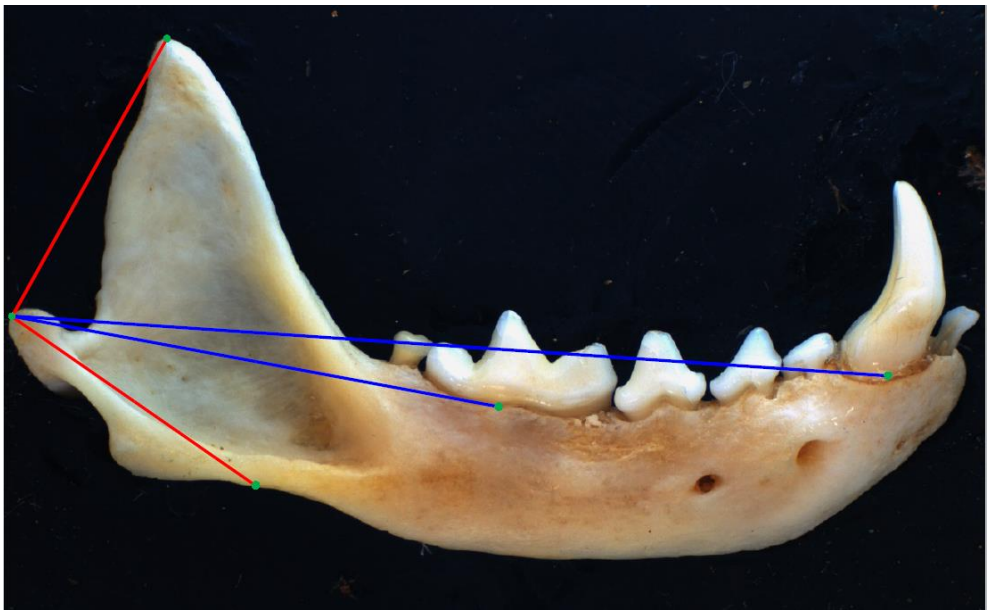


Figure 2.5. Inlever length (red lines, based on muscle insertions) and outlever length (blue lines, based on bite points) (Image is based on Figure 1 from Renaud et al. (2015)).

2.4.2 Objective 2a: Variation in size and shape of mandibles from New Zealand stoats over a range of habitats

This objective investigated the differences in mandible shape of stoats across a range of sources, between sexes within locations and then between locations with the sexes grouped. There were ten New Zealand locations in total (Figure 2.6),

although EV and HV were subdivided between specimens born in beech seed fall years (EVY and HVY) and those that were not (EVN and HVN). The location of Packington Park, Warwickshire, where the English group (EN) representing the ancestral stoat population was collected, is not shown. The sample numbers can be found in Table 5.

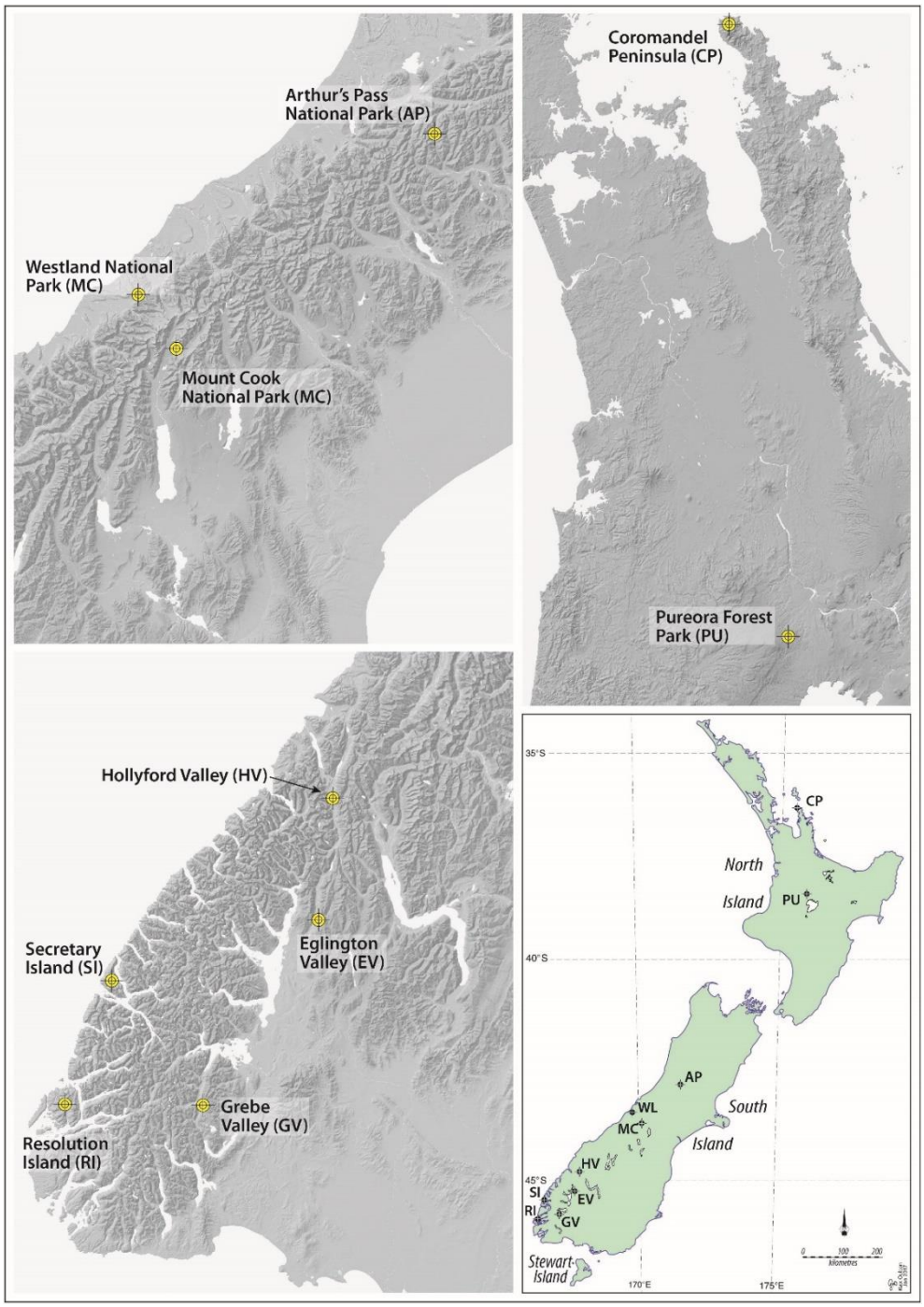


Figure 2.6. New Zealand map showing the source locations of the stoats used for Objective two (Image by M. Oulton, locations supplied by C. Hill).

Table 5. Total sample numbers of male and female stoats from each location.

Location	Males	Females	Total
CP	12	10	22
PU	10	10	20
AP	12	12	24
WL	18	18	36
MC	12	12	24
HVN	12	12	24
HVY	10	3	13
EVN	12	13	25
EVY	12	11	23
GV	12	12	24
SI	8	10	18
RI	8	13	21
EN	12	12	24
			298

2.4.2.1 Software preparation

The preparation of files for software were implemented following the same methods used for the across species comparisons.

2.4.2.2 Size analysis

The size analyses were conducted in the same way as the between species analyses, first comparing the sexes within locations and then comparing each sex across locations. The average sexual dimorphism was calculated from the difference between the average male and female centroid size of each site. All statistics were the same as those for comparing sexes within sites.

The ANOVA showed at least two locations were significantly different from each other, but the post-hoc tests did not find any difference in their standard deviations and means. This was probably because the sample sizes were small compared with the number of locations, so the confidence intervals were wide. Therefore, instead of doing one ANOVA for each sex and then using the post-hoc to determine the local differences, separate ANOVAs tested each pair-wise comparison, and then the F-value and p-values were reported as one table per sex.

2.4.2.3 Regression analysis

The regression analyses followed the same procedures as for the between species tests, by first checking for allometry between sexes within each site. Any significant allometry was corrected for, and the results of the tests with and without allometry were presented for each procedure. Only regressions which proved significant were presented in the results section, and all other non-significant regressions were placed onto the appendices disk at the end of this thesis. The final regression investigated allometry across all New Zealand stoats and the one English group, the locations were used as the "pools".

2.4.2.4 Principal components analysis

The PCAs followed the same methods as for Objective one. Comparisons were conducted between sexes within sites, and between locations with sexes combined, twice each if applicable, once before regression and once after regression.

No 90 % confidence ellipses were added to the PCA comparing locations, as the graphs were too difficult to read. Instead, ellipses showing the likely position of the mean for each were overlain onto the graph using the option in MorphoJ. Resolution and Secretary Islands were compared against each other without the other locations, as they are known to be similar in habitat and absence of rats and possums, but only Resolution has mice.

2.4.2.5 Eigenvalue analysis

These methods were identical to those described above for comparing the species.

2.4.2.6 Discriminant function analysis

Discriminant function analysis was performed in MorphoJ, on the data for within locations (comparing sexes) and between them (sexes pooled). Wireframes were presented for the locations that showed significant differences from each other after the classification. Misclassification tables were presented in the same way as the previous analyses comparing the species.

2.4.2.7 Canonical variates analysis

CVAs were conducted comparing all locations. Otherwise the methods followed those outlined for the between-species analyses.

2.4.2.8 Modularity analysis

No modularity analysis was necessary to compare the sexes as no within-location differences were detected within the Westland stoats in the interspecies comparisons, but for the location comparisons all stoats were pooled for modularity tests using the same modules and connectivity as for the interspecies tests.

2.4.2.9 Biomechanical advantage

The biomechanical advantage tests first compared the sexes. Lastly the sexes were grouped by location and the effect of location on biomechanical advantage was examined by the same methods described above.

2.4.2.10 Partial least-squares analysis

MorphoJ software was used compare external factors and their degree of covariation with mandible shape across the sites, using a 2-block partial least-squares (PLS) analysis. This was done twice, first defining the external factors as environmental variables, and then as diet (Monteiro et al. 2003; Loy et al. 2004).

Overall there were nine variables in the environmental block. Six environmental variables were: range of altitude (two variables), annual range of monthly average temperature (two variables), mean annual rainfall, and orthogonal contrasts to separate different vegetation types (Table 6) (King et al. 1982a; Monteiro et al. 2003). Because the vegetation type is a categorical variable with five levels, it was factored into 'dummy' variables called linear orthogonal contrasts, where each type assumes a value (for example: 2, 1, 0, −1, or -2) and the groups were separated (Table 6). Orthogonal contrasts can be combined with continuous variables in linear models (Monteiro et al. 2003).

Latitude was also used in the environmental variables matrix, as a way of identifying geographical gradients in the morphological–environmental association (Monteiro et al. 2003; Yom-Tov et al. 2010). Longitude varied too little between sites to be useful. Haplotypes identified by Veale et al. (2015) were also added into the block, both the major haplotype and the minor haplotype were used, in separate columns. The most common haplotype was classified as the major and the least common as the minor haplotype (Veale et al. 2015). Variable values can be found in Table 7. Most of the environmental data came from King et al. (1982a), although

some was not specific to the study areas. For locations CP, PU, GV, SI, RI and EN, the data had to come from elsewhere. For RI, published studies were missing some information. Weather data for PU and SI came from weather stations in the study area (Weather station Pureora 2234 and Secretary Island 9533 (NIWA 2016)); for CP, from the nearest airport's weather station; for GV, from a nearby weather station outside the valley in farmland (Borland Burn); for EN, from a nearby county, the only area with available historic weather data (King et al. 1982a; King 1983; King et al. 1996a; King et al. 1996c; Purdey et al. 2004; Clayton et al. 2011; Canty and Associates LLC 2016; Met Office 2016; Meteorological Service of New Zealand Ltd 2008-2016 2016; Murphy et al. 2016; NIWA 2016).

The PLS axes that accounted for more than five percent of the covariance were presented. The singular values, covariance, PLS coefficients, correlation values, the RV value and their associated permutation p-values were reported.

Table 6. Location habitat types, indicating the major components of the habitat over-story, and the orthogonal contrast dummy code used for the PLS analysis.

Habitat name	Major components of the habitat over-story	Dummy code	Reference
Beech	A beech (*Nothofagus* spp.) dominated forest, understorey is often scarce	2	King et al. (1982a)
Grass	Open tussock grass land with sparse shrubs and trees	1	King et al. (1982a)
Alpine	A mixture of beech, tussock and alpine (above the tree line) zones	0	King et al. (1982a)
Mixed	A mixture of podocarp/broad leaf forest with some beech trees which become more common with increasing altitude. There is typically a rich understorey and species include many fruiting species; in the north of the North Island forests may have Kauri (*Agathis australis*) in the overstorey. Note: this category is a gradient with locally variable proportions of each species	-1	Nicholls (1976); King et al. (1982a); McMurtrie et al. (2011); Clayton et al. (2011)
Agricultural mixed land	A patchwork of agricultural and forested land that may or may not include native plants	-2	King et al. (1996a)

Table 7. Environmental factor values for the partial least-squares analysis of shape and environment (King et al. 1982a; King 1983; King et al. 1996a; King et al. 1996c; Purdey et al. 2004; Clayton et al. 2011; Canty and Associates LLC 2016; Met Office 2016; Meteorological Service of New Zealand Ltd 2008-2016 2016; Murphy et al. 2016; NIWA 2016).

Location	Latitude	Altitude range (m)		Average temperature range (°C)		Annual Mean rainfall (mm)	Genetics-haplotypes		Habitat code
		Low	High	Monthly low	Monthly high		Major	Minor	
CP	-36.5466	0	859	8.50	18.00	1579	1	1	-1
PU	-38.4389	550	700	6.00	15.30	1759	1	1	-2
AP	-42.8643	380	910	3.90	15.50	5074	1	3	0
WL	-43.4601	120	150	6.70	14.90	5130	1	3	-1
MC	-43.5947	690	910	0.80	14.10	4071	1	3	1
HV	-44.6813	90	1100	1.00	10.00	4250	1	2	-1
EV	-45.0932	270	1800	0.00	8.00	2300	2	1	2
GV	-45.6733	244	945	3.60	14.40	334	1	2	2
SI	-45.2388	0	1196	9.10	15.00	4004	1	2	-1
RI	-45.6757	0	1069	10.00	10.00	4000	1	2	-1
EN	52.4627	90	100	5.30	13.15	52	1	1	-2

Diet data from King et al. (1982b), King (1983), King et al. (1996a), McDonald et al. (2000), Gillies (2016), and Murphy et al. (2016) were entered into a diet matrix. The main components of the diet were defined as the variables, and the relative frequencies of occurrence per habitat as the values, modelled on the table from King et al. (1982b). The methods used for calculating frequency of occurrence were the same as used by King et al. (1982b) Table 3, although some sources did not separate the diets into the same number of categories, so after some trial and error, certain categories were grouped to obtain the general trends. For example, King et al. (1982b) separated weta into further classifications: tree, ground, small cave, and large cave weta, but here all insects were grouped as one variable.

The percentage frequencies were separated into classes, similar to the orthogonal variables from the habitat codes (Table 8), based on the same percentage groupings used by Nogueira et al. (2009) in their study of phyllostomid bat bite force and PLS method of diet. The diet data matrix (Table 9) was used in a second 2-block PLS to compare diet and its degree of linear association with mandible shape. The significance of the PLS calculations were tested using the permutations test available in MorphoJ. For some locations such as CP and EN there were no previous diet data. The CP diet data were assumed to be similar to diets from stoats in similar Kauri (*Agathis australis*) podocarp forests in Northland, New Zealand forests (Gillies 2016). The English stoats came from an area near a game park and agricultural area of England, and so their diets were assumed to be similar to those of stoats from similar areas from across England (McDonald et al. 2000).

Table 8. Codes for the diet data used in the partial least squares analysis of diet and mandible morphology, codes used from Nogueira et al. (2009).

Percentage frequency in diet	Dummy code
0%	0
0.1-10.9 %	1
11-25.9 %	2
26-50.9 %	3
51-75.9 %	4
76-90 %	5
90.1 -100	6

Table 9. Diet composition of New Zealand and English stoats in a diet matrix used in a partial least-squares analysis. Diet data obtained from (King et al. 1982b; King 1983; King et al. 1996a; McDonald et al. 2000; Purdey et al. 2004; Gillies 2016; Murphy et al. 2016).

Location	Large mammal	Rat	Bird	Mouse	Insects	Lizards
CP	1	3	2	2	3	3
PU	3	2	3	2	4	1
AP	3	1	3	2	3	1
WL	3	2	3	2	4	0
MC	4	1	3	1	3	3
HV	2	1	3	2	3	0
EV	2	1	4	3	4	1
GV	2	1	4	1	4	0
SI	0	0	3	0	6	0
RI	0	0	2	2	6	0
EN	4	1	2	2	1	0

2.4.3 Objective 2b: Short term plasticity of stoat mandibles in response to resource pulsing

2.4.3.1 Software preparation

The preparation of files for software were implemented following the same methods used for the across species comparisons.

2.4.3.2 Size analysis

The HV and EV data were also examined for differences between males born in seed years and in non-seed years, and between female seed year and non-seed year births. Two-way ANOVAs also investigated the interaction between sex and seed year on centroid size, on HV and EV stoats. Size has generally been less affected by small sample size than has shape (Pallares et al. 2016) but as the sample of adult HV males born during a seed year contains only three individuals the results must be regarded with some caution.

2.4.3.3 Regression analysis

The need to test for the effects of seed birth year (seed or non-seed) on mandible shape required pooled-within group regressions for each valley. The groups were separated by sex and by birth year, which were the same groups in the two-way ANOVAs performed on centroid sizes. Because estimates of shape are greatly

affected by small sample size, all results including Hollyford males born during a seed year (N=3) must be treated with extreme caution (Cardini et al. 2015; Pallares et al. 2016).

2.4.3.4 Principal components analysis

The HV and EV comparisons of sex and birth year examined all four groups, within each location, on a single PCA, and patterns across sexes, birth year or both together were identified.

2.4.3.5 Eigenvalue analysis

These methods were identical to those described above for comparing the species.

2.4.3.6 Discriminant function analysis

The HV and EV comparisons of sex and birth year examined all four groups.

2.4.3.7 Canonical variates analysis

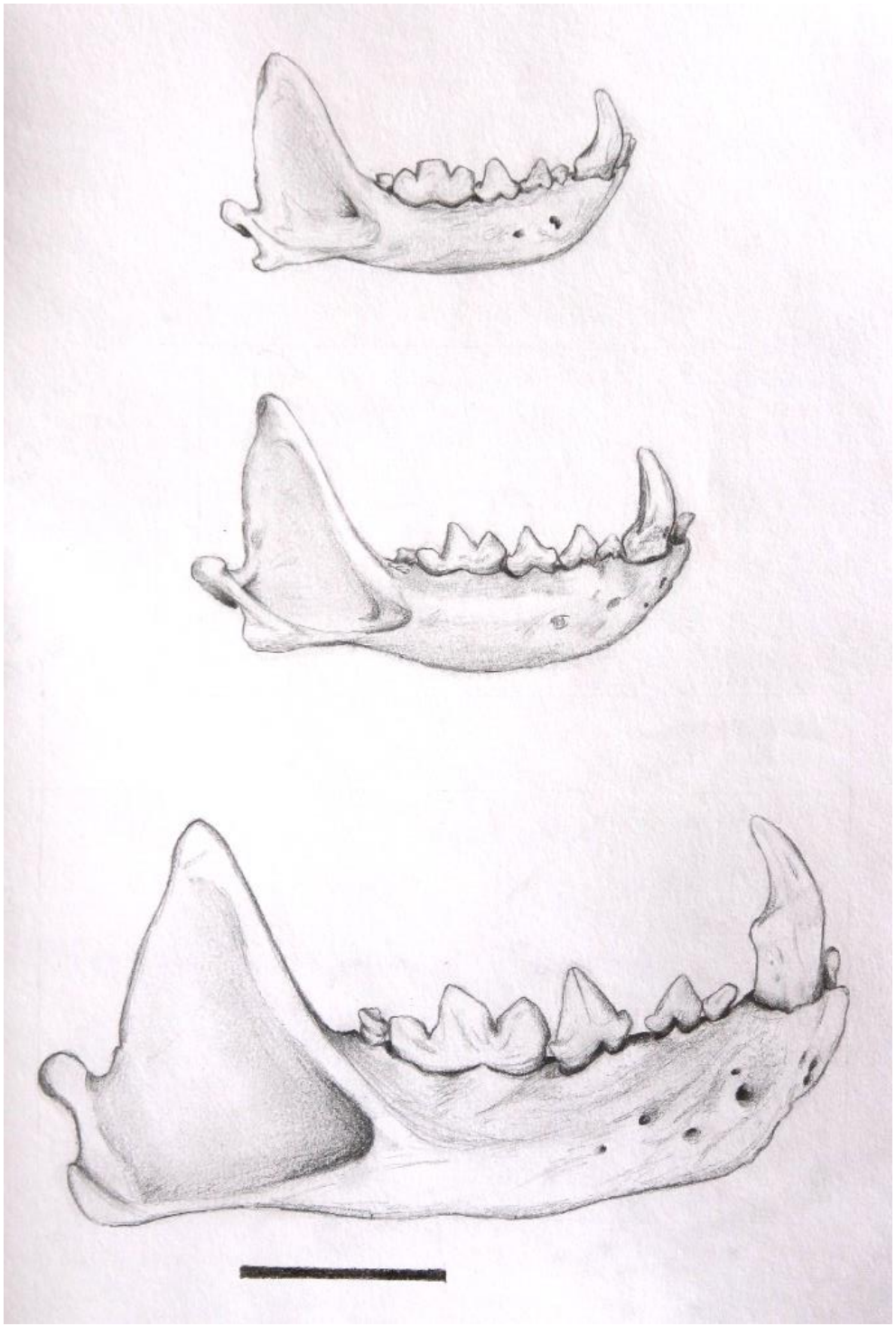
The first set of CVAs were conducted for HV and EV, comparing their sex and birth year groups.

2.4.3.8 Biomechanical advantage

Stoats born the Hollyford valley and then the Eglinton Valleys during seed years and non-seed years.

Chapter Three

Interspecific morphological differences of New Zealand mustelid mandibles



Drawing 4. *Mustela* spp. hemi-mandibles. Top: weasel (*M. nivalis*), middle: stoat (*M. erminea*), bottom: ferret (*M. furo*). Black bar is 10 mm. Artist: C. Hill.

3.1 Introduction

While phylogeny is interesting the mustelid phylogeny has been studied both using morphological features like Catalano et al. (2015) and also genetics (Koepfli et al. 2008; Sato et al. 2012) so this will not be studied any further. Particularly as bone does have phenotypic plasticity and therefore cannot be a true representation of phylogeny (Caumul et al. 2005; Klingenberg et al. 2010; Adams et al. 2011). This chapter compares the mandibles of the three species of New Zealand mustelids (*Mustela furo, M. erminea,* and *M. nivalis*). It is well known that ferrets are bigger than stoats, which are bigger than weasels, but there have been no interspecific comparisons of mandible shape and size (King 2005). Therefore, the material available invited answers to several questions.

1. Was there sexual dimorphism in mandible shape as well as in size? The null hypothesis was that there was no sexual dimorphism in shape.

2. Did the degree of sexual dimorphism differ between species? The null hypothesis was that there were no differences in the degree of sexual dimorphism.

3. Were any detectable differences in mandible shape, across the species isometric or allometric with size? The null hypothesis of this study was that ferret mandibles would be a scaled up version of the mandible of weasels and stoats, hence all the shapes should be isometric.

4. If allometric variation in shape was found, did size account for all or just a component of it?

5. How did shape differ between the species? Was there any modularity in the mandibles of each species, and was it detectable across the species?

6. Did the bite force efficiency of the mandibles differ between the species? The null hypothesis was that there was no difference in bite force efficiency.

Detailed methods are described in Chapter Two. Intra-species analyses compared sexes with ages pooled, all individuals who were considered of adult age were used; for ferrets and stoats this was based on cementum layers, weasels are independent and sexually mature at 3-4 months old, so all weasels caught and used in this study were considered adults (King 2005). Although they may not be fully grown until six months old, but unfortunately this was not discovered until after all analyses had been completed (C. M. King, personal communication, January 27, 2017). For interspecies analyses, the sexes were pooled. All non-significant results were placed into Appendix one on the appendices disk at the end of this thesis.

3.2 Within species analyses

3.2.1 Ferrets (*Mustela furo*)

3.2.1.1 Size

Male ferrets were significantly larger (15.38 %) than females in ANOVA tests ($F(1)=133.9239$, $p<0.0001$) (Table 10). The normality plots showed that the ANOVA was a valid test (Figure 3.1).

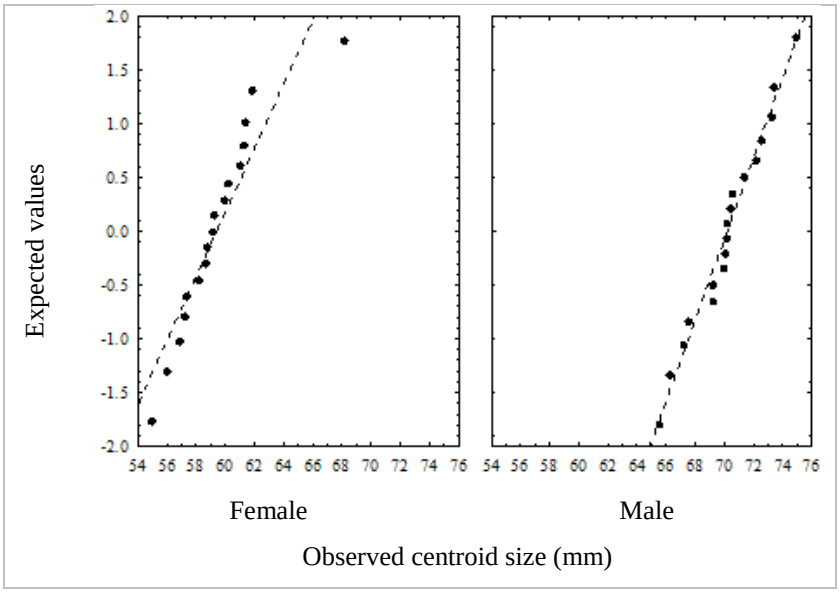


Figure 3.1. Normal probability plots generated as part of the centroid size ANOVAs comparing male and female ferrets.

Table 10. Centroid size means and standard deviations split by sex and within species ANOVAs, significant values (p<0.05) are highlighted in bold.

	Males		Females									
	Mean	Std. Dev.	Mean	Std. Dev.	Effects SS	Effects df	Effects MS	Error SS	Error df	Error MS	F	p-value
Ferrets	70.2178	2.5106	59.4163	3.0021	1020.0476	1	1020.0476	251.3486	33	7.6166	133.9239	**<0.0001**
Stoats	45.2217	1.1890	40.8782	0.9655	169.7943	1	169.7943	39.8820	34	1.1730	144.7521	**<0.0001**
Weasels	34.8236	0.8296	28.1828	0.4564	210.4819	1	210.4819	10.8842	20	0.5442	386.7643	**<0.0001**

3.2.1.2 Shape

3.2.1.2.1 Regression and allometry

The pooled-within group regression was used as a test for allometry between male and female ferrets, by determining whether shape depended on centroid size. It did not produce significant results.

3.2.1.2.2 Principal components analysis

Principal component one (PC1) accounted for 39.99 % of the variance in shape (Figure 3.2A) and was significant according to the chi-squared tests ($\chi 2$=8.9614). PC1 was the only PC above the inflection point on the eigenvalue analyses (Figure 3.2B). The inflection point is the point on the graph where the taper begins, this usually indicated where the PC's are no longer different from each other. However, there was no clear division between males and females along PC1, although females showed a greater spread along PC1 and males were grouped in the middle. There was more variation within the sexes than between the sexes.

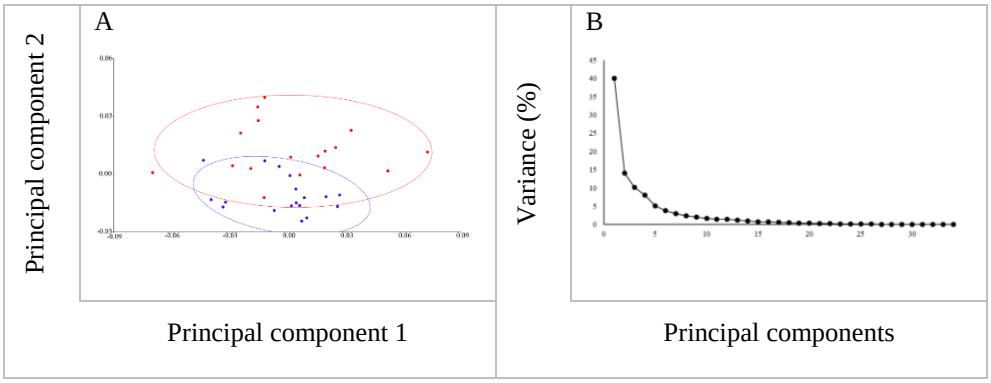


Figure 3.2. A) PCA of female and male ferret mandibles. Circles indicate the 90 % confidence ellipses. Males: blue, females: red. B) Eigenvalue and percentage of variance scree plot from the ferret PCA.

There was a clear division along PC2, but the axis values show that this was a very small difference. Females were found in the lower PC2 values, and males towards the top. PC2 accounted for 14.03 % of the variance, but it was not significant ($\chi 2$=0.8696). In the percentage of variance PC scree plot (Figure 3.2B), PC2 was barely separated from the remaining PCs. Traditionally, only those eigenvalues that explain greater than five percent of the variance are reported, as they were most likely to have biological significance, these would be the first five eigenvalues (Table 11) but the inflection point and the chi-squared analyses show that they were not significant here.

A deformation wireframe plot along PC1 (Figure 3.3A), found that the mandible shape was influenced by an extension of the angular process, the length of the arch for the masseter muscle attachment was extended, and the body of the mandible shortened. The PC2 deformation wireframe (Figure 3.3B) was generated because of the split of sexes on this axis, and indicated that the mandible body was lengthened and the coronoid process (muscle attachment) shortened along this axis, the longer mandible body indicated females may be less efficient at transferring power to the canine.

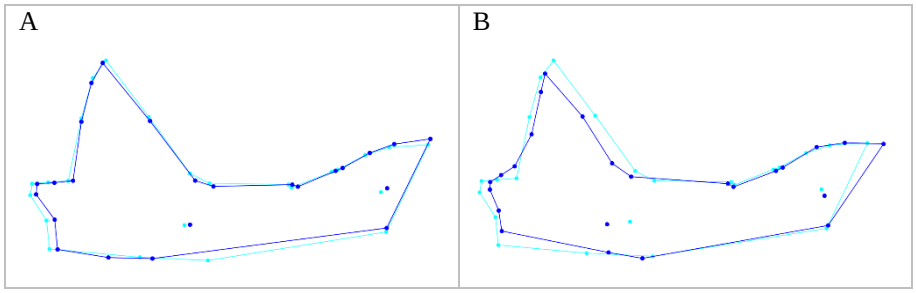


Figure 3.3. Procrustes deformation wireframe plot of ferret mandibles based on the deformation implied by A) PC1; B) PC2. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

Table 11. Eigenvalues relating to the within species PCAs, eigenvalues that account for five percent or more of the variance are reported, significant eigenvalues ($\chi 2<5.99$) are highlighted in bold, and eigenvalues before the inflection point on the scree graphs are italicised.

		EV1	EV2	EV3	EV4	EV5	EV6	EV7
Ferrets	Eigenvalue	***0.0008***	0.0003	0.0002	0.0002	0.0001		
	Variance (%)	***39.9930***	14.0280	10.1810	8.0220	5.0990		
	Cumulative %	***39.9930***	54.0210	64.2010	72.2230	77.3220		
Stoats	Eigenvalue	*0.0004*	*0.0002*	*0.0002*	*0.0002*	*0.0001*	*0.0001*	*0.0001*
	Variance (%)	*21.6140*	*14.0290*	*12.0010*	*10.2210*	*9.0620*	*6.7750*	*4.1670*
	Cumulative %	*21.6140*	*35.6430*	*47.6440*	*57.8640*	*66.9270*	*73.7010*	*77.8690*
Weasels	Eigenvalue	*0.0006*	*0.0005*	0.0004	0.0002	0.0002		
	Variance (%)	*20.9990*	*19.6740*	13.7000	11.4420	6.1780		
	Cumulative %	*20.9990*	*40.6720*	54.3730	65.8150	80.5770		

3.2.1.2.3 Discriminant function analysis

A Procrustes- based superimposition is the graphical output after the mandible shape of each individual is overlaid on all other from the group. In this case they show the difference in shape between male and female ferret mandibles. Figure 3.4A shows all individuals used in the ferret analyses. Figure 3.4B shows that the mean shapes of male and female ferrets, from the discriminant function analysis, (not including centroid size) found no statistical differences between males and females. The Procrustes shape deformations (Figure 3.4B) found very little change in the overall shape, only a small change in the shape of the coronoid process in both sexes, and a slightly shorter mandible body in males. This was the same trend seen in the PC2 wireframe just less exaggerated.

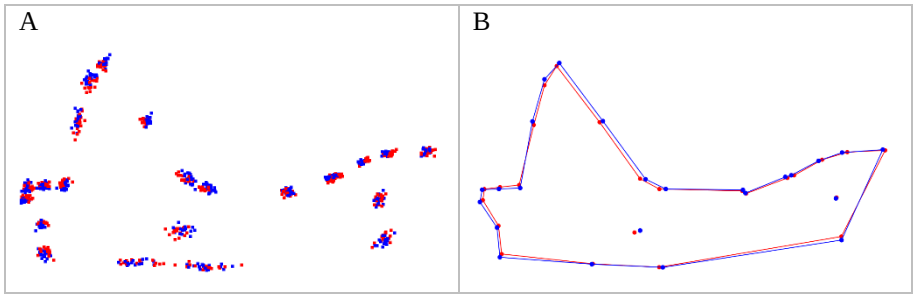


Figure 3.4. A) Procrustes superimpositions of all ferret mandibles. B) Procrustes superimpositions of the mean male and female mandible shapes. Males: blue, females: red.

3.2.1.2.4 Modularity analyses

The hypothesis of developmental modularity was not supported (p-value=1.0000) and neither was the functional modularity hypothesis (p-value=0.7755), therefore no further testing was conducted.

3.2.1.2.5 Biomechanical advantage

The biomechanical advanatge of the mandible is a measure of mandible geometry and muscle efficiency used for bite force. The explanation on how this is done can be found in the Methods, Section 2.4.1.8. Kruskal-Wallis ANOVAs testing biomechanical advantage (Table 12) found no significant differences between male and female ferrets, except for the temporalis/canine (T/C) ($H(1,N=35)=9.027$, $p=0.0027$), males had a greater efficiency, which was expected based on the results from the PCA and discriminant function analyses.

Table 12. Within species Kruskal-Wallis ANOVAs of biomechanical advantage of male and female mandibles, significant values (p<0.05) are highlighted in bold (T: temporalis, M: masseter, C: canine, M1: carnassial).

	Ferrets				Stoats				Weasels			
	H-value (1, N=35)	p-value	Average male	Average female	H-value (1, N=36)	p-value	Average male	Average female	H-value (1, N=22)	p-value	Average male	Average female
T/C	9.0207	**0.0027**	0.3924	0.3745	3.4845	0.0619	0.3731	0.3597	0.1006	0.7511	0.3755	0.4560
T/M1	0.1569	0.6921	0.7121	0.7078	2.0270	0.1545	0.6686	0.6503	0.3590	0.5491	0.7065	0.6947
M/C	0.7364	0.3908	0.3516	0.3479	1.0250	0.3113	0.3432	0.3364	0.1006	0.7511	0.3243	0.3972
M/M1	0.3148	0.5747	0.6384	0.6579	0.2563	0.6127	0.6150	0.6085	1.5217	0.2174	0.6103	0.5957

3.2.1.3 Ferret section summary

There was no significant allometry affecting the shape of male and female ferret mandibles, and the largest change in the mandibles of ferrets, as indicated by the PCA, did not group male and females differently. These results indicated that there was no statistically or biologically significant difference in mandible function between the sexes.

3.2.2 Stoats (*Mustela erminea*)

3.2.2.1 Size

Like the ferrets, male stoat mandibles were significantly larger (9.60 %) than females in ANOVA tests ($F(1)=144.7521$, $p<0.0001$) (Table 10). The normality plots showed that the ANOVA was a valid test (Figure 3.5).

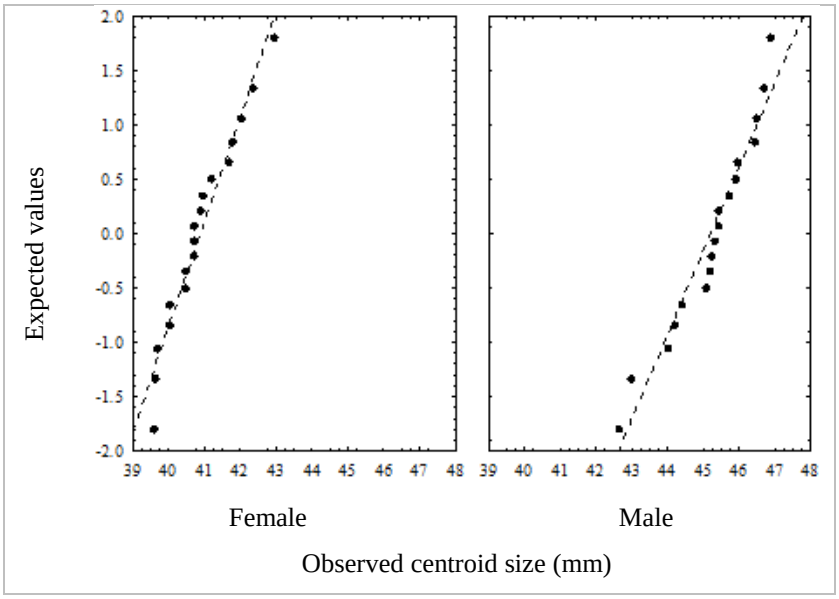


Figure 3.5. Normal probability plots generated as part of the centroid size ANOVAs comparing male and female stoats.

3.2.2.2 Shape

3.2.2.2.1 Regression and allometry

There was no significant effect of size on stoat mandible shape, and therefore the raw data without allometric correction were used for the rest of the analyses.

3.2.2.2.2 Principal components analysis

PC1 accounted for 21.61 % of the variance in shape ($\chi 2=1.6209$) (Figure 3.6A), but there was no clear division between males and females along this axis. There was

also little to no division along PC2, which accounted for 14.03 % of the variance, although the females did have a greater spread of points. The eigenvalue analysis indicated the first five eigenvalues may have biological significance (Table 11), but chi-squared statistical tests did not confirm that prediction for this analyses. None of the eigenvalues or the PCs were significant. The percentage of variance PC scree plot (Figure 3.6B) found a separation between PC1 and PC2, but not enough to be significant, and the inflection point is located after PC7. As none of the eigenvalues or the PCs were significant, no deformation plot was generated.

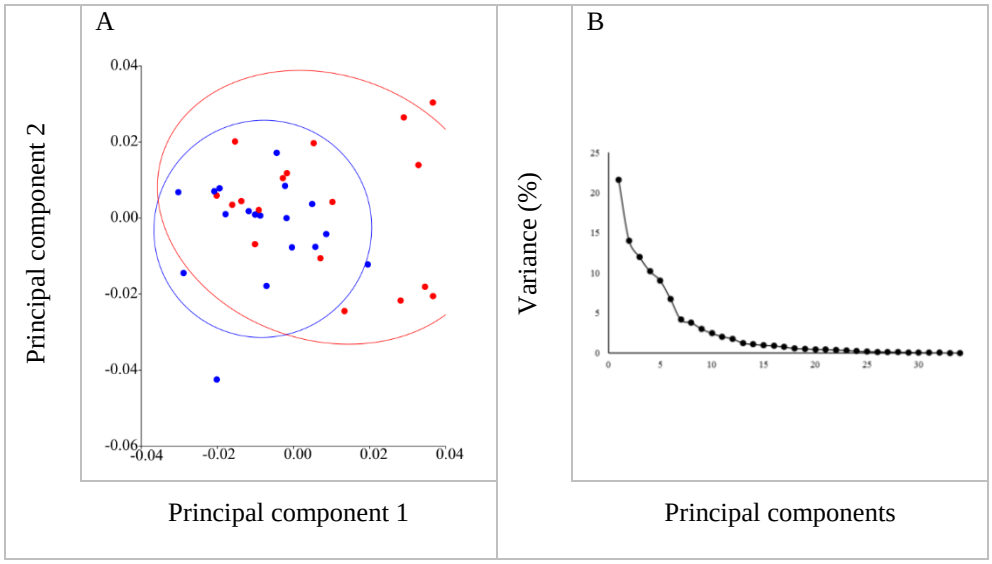


Figure 3.6. A) PCA of female and male stoat mandibles. Circles indicate the 90 % confidence ellipses. Males: blue, females: red. B) Eigenvalue and percentage of variance scree plot from the stoat PCA.

3.2.2.2.3 Discriminant function analysis

Procrustes- based superimpositions show the difference in shape between male and female stoat mandibles. Figure 3.7A shows all individuals used in the stoat analyses. Figure 3.7B shows the mean Procrustes superimposed shape of male and female stoats, from the discriminant functional analysis which indicated that there was no statistical difference between males and females only a small change in the angle between the coronoid process and the condyle.

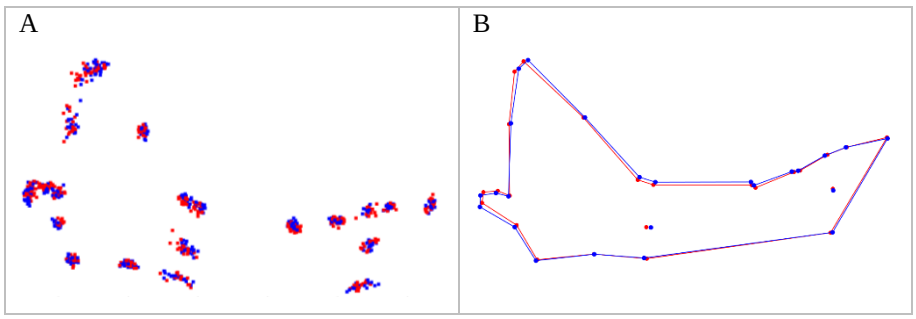


Figure 3.7. A) Procrustes superimpositions of all stoat mandibles. B) Procrustes superimpositions of the mean male and female mandible shapes, generated by the discriminant function analysis. Males: blue, females: red.

3.2.2.2.4 Modularity analyses

The hypothesis of developmental modularity was not supported (p-value=0.2131) and neither was the functional modularity hypothesis (p-value=0.4567), therefore no further testing was conducted.

3.2.2.2.5 Biomechanical advantage

The results of the biomechanical advantage Kruskal-Wallis ANOVAs showed that there was no significant difference between male and female stoats (Table 12).

3.2.2.3 Stoats section summary

There were no statistically significant differences between the mandible shapes of male and female stoats, detectable by the above previous analyses. Therefore, it is unlikely that there is any biologically significant difference in mandible function between these male and female stoats.

3.2.3 Weasels (Mustela nivalis)

3.2.3.1 Size

Like ferrets and stoats, male weasel mandibles were significantly larger (19.07 %) than females in ANOVA tests ($F(1)=386.7643$, $p<0.0001$) (Table 10). This was the largest difference between sexes in any of the three mustelid species examined here. The normality plots showed that the ANOVA was a valid test (Figure 3.8).

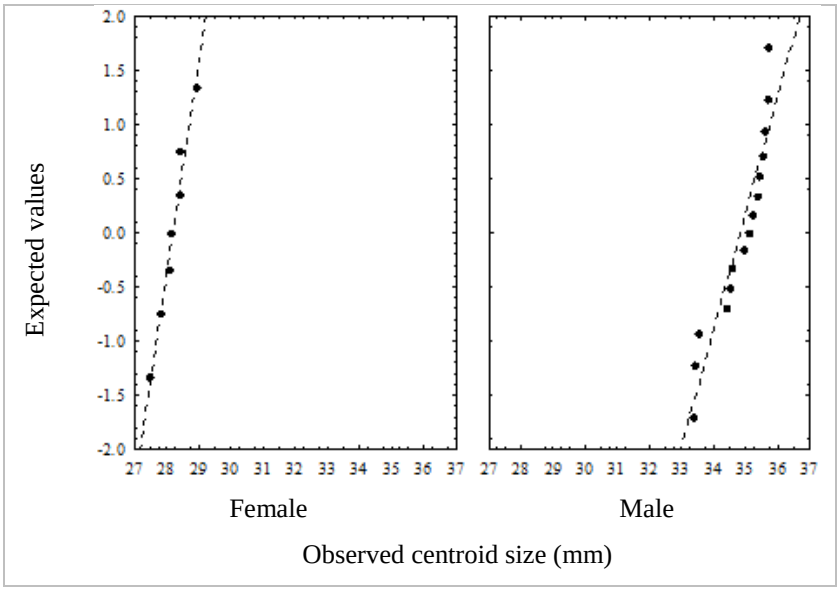


Figure 3.8. Normal probability plots generated as part of the centroid size ANOVAs comparing male and female weasels.

3.2.3.2 Shape

3.2.3.2.1 Regression and allometry

There was no significant effect of size on shape and therefore, as for the ferrets and stoats, the rest of the analyses used the raw data without allometric correction.

3.2.3.2.2 Principal components analysis

PC1 accounted for 21 % of the variance in shape ($\chi 2$=0.0222) (Figure 3.9A) but there was no clear division along this axis. Females had a greater spread of points found at both the high and low values. PC2 accounted for 19.67 % of the variance but there was no division along this PC. The weasels' eigenvalue analysis indicated the first six eigenvalues may have biological significance (Table 11 and Figure 3.9B). However, the chi-squared statistical tests, indicated that none of the PC's were significant. None of the PCs were separated from the remaining PCs, the inflection point was also less defined than the other two species, although PC3 could be considered the inflection point.

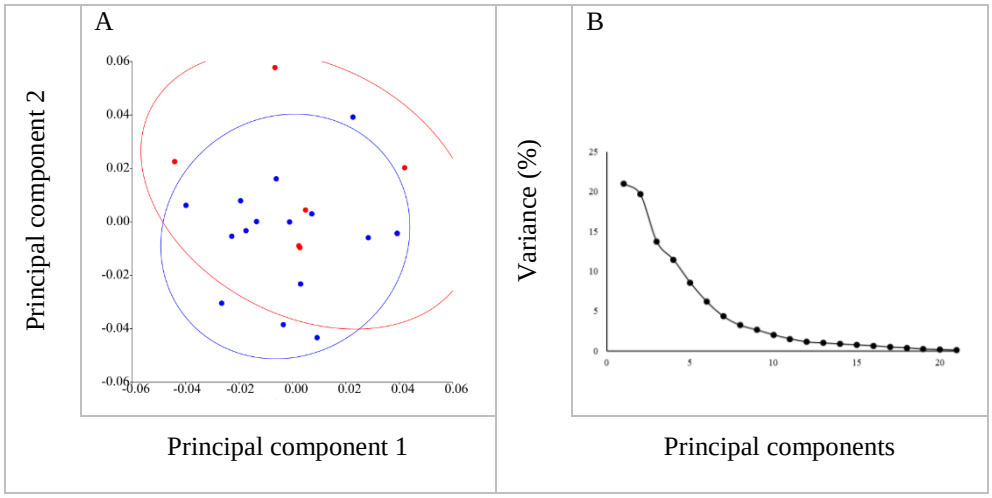


Figure 3.9. A) PCA of female and male weasel mandibles. Circles indicate the 90 % confidence ellipses. Males: blue, females: red. B) Eigenvalue and percentage of variance scree plot from the weasel PCA.

3.2.3.2.3 Discriminant function analysis

Procrustes- based superimpositions show the difference in shape between male and female weasel mandibles. Figure 3.10A shows all individuals used in the weasel analyses. Figure 3.10B shows the mean Procrustes superimposed shape of male and female weasels, obtained from the discriminant function analysis which indicated that there was no statistical difference between males and females. The Procrustes shape deformations (Figure 3.10B) showed very little change in the overall shape, females had a slightly thinner mandible body. Which indicates a possibly weaker bite force.

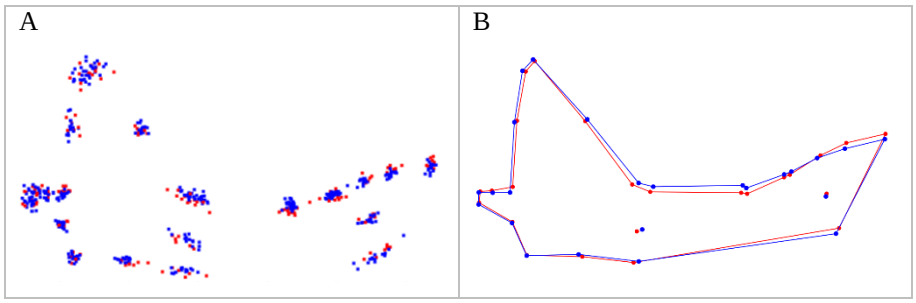


Figure 3.10. A) Procrustes superimpositions of all weasel mandibles. B) Procrustes superimpositions of the mean male and female mandible shapes, obtained from the discriminant function analysis. Males: blue, females: red.

3.2.3.2.4 Modularity analyses

The hypothesis of developmental modularity was not supported (p-value=0.2549) and neither was the functional modularity hypothesis (p-value=0.3814), therefore no further testing was conducted.

3.2.3.2.5 Biomechanical advantage

The results of the biomechanical advantage Kruskal-Wallis ANOVAs showed there was no significant difference between male and female weasels (Table 12).

3.2.3.3 Weasels section summary

The differences between male and female weasel mandibles were not statistically significant. The largest variation in mandibles of weasels, as indicated by the PCA, did not definitively group male and females differently. The discriminant function analyses also failed to detect differences between their mean shapes, suggesting no biologically significant shape difference between the sexes.

3.2.4 Section summary

None of the analyses conducted here found any evidence of consistent statistical or biologically significant differences in shape between male and female mandibles of any of the three species. Sexual dimorphism was apparent only in the size of the mandibles, to a degree which differed within each species. Weasels showed the greatest sexual dimorphism, and stoats the smallest. Hence, the sexes were pooled for the following interspecies analyses.

3.3 Between species analyses

This section shows the mandible comparisons of ferrets, stoats, and weasel after the sexes have been combined.

3.3.1 Size

As expected, the ANOVA results showed that the size of the mandibles of male ferrets, stoats, and weasels were all statistically significant from each other $F(2)=11173.9384$, $p<0.0001$. In the Newman-Keuls post-hoc tests, all p-values were 0.0001 (Table 13). The ANOVA comparing the females of the three species agreed ($F(2)=5751.3558$, $p<0.0001$), also supported by the Newman-Keuls post-hoc test, where all p-values were 0.0001. The differences between ferrets and weasels were similar in both males and females, but the difference between male ferrets and stoats was larger than between the females, and the opposite was true between the stoats and weasels.

Table 13. Centroid size between species ANOVAs, and Newman-Keuls post-hoc tests, significant values (p<0.05) are highlighted in bold. The magnitude of the difference in size between the species was calculated, the difference is presented as the percentage amount that one species was larger than the other.

									Newman-Keuls post-hoc test p-value		
	Effects SS	Effects df	Effects MS	Error SS	Error df	Error MS	F	p-value	Ferrets versus stoats	Ferrets versus weasels	Stoats versus weasels
Males	11173.9384	2	5586.9692	140.8196	48	2.9337	1904.3833	**<0.0001**	**0.0001**	**0.0001**	**0.0001**
Females	5751.3558	2	2875.6779	161.2952	39	4.1358	695.3179	**<0.0001**	**0.0001**	**0.0001**	**0.0001**
						Percentage size difference the species		Males	35.5980	50.4063	22.9936
								Females	31.2004	63.5672	31.0567

3.3.2 Shape

3.3.2.1 Regression analysis

The pooled-within group regression not only tested for allometry in the three *Mustela* spp., but also served as a multivariate analysis of covariance. The regression calculated the change of shape against log centroid size (Figure 3.11); the associated statistics can be found in Table 14. The results indicated there was allometry in the jaw architecture of the three *Mustela* spp., 5.94 % of the shape of the mandibles can be accounted for by size, and the permutation test p-value was below the 0.05 threshold, all further analyses were done on both the raw coordinates and the regression residuals.

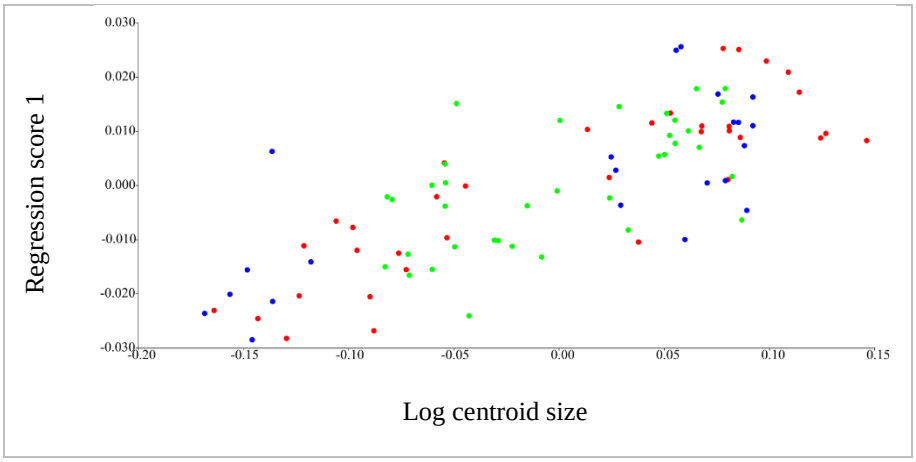


Figure 3.11. Pooled-within group regression analysis of shape on log centroid size, as a test for allometry in mandibles of ferrets, stoats, and weasels. The associated statistics for this regression can be found in Table 14. Ferrets: red, stoats: green, weasels: blue.

Table 14. Between species pooled-within group regression analysis. The predicted (%) is the percentage of shape accounted for by size. The permutation test against the null hypothesis of independence is presented, significant values (p<0.05) are highlighted in bold.

Total SS	Predicted SS	Residual SS	d.f.	Predicted (%)	Permutation test p-value (10000 runs)
0.1798	0.0107	0.1691	2, 90	5.9400	**<0.0001**

3.3.2.2 Principal components analysis

Before allometric correction PC1, (χ2=45.6630), accounted for 51.11 % of the variance, and PC2 (χ2=13.2574) accounted for 11.77 % of the variance (Figure 3.12A). The first two eigenvalues in the PCA before allometric correction, and the first three in the PCA after allometric correction, were likely to be biologically

significant (Table 15). Chi-squared statistical tests showed that the first two eigenvalues for the PCA without correction were significant. Only the first two eigenvalues were above the inflection point for the PCA without correction (Figure 3.13A), which meant that the two methods for evaluating significant PCs agree that the first two were significant. The inflection point is the point on the graph where the taper begins, this usually indicated where the PC's are no longer different from each other.

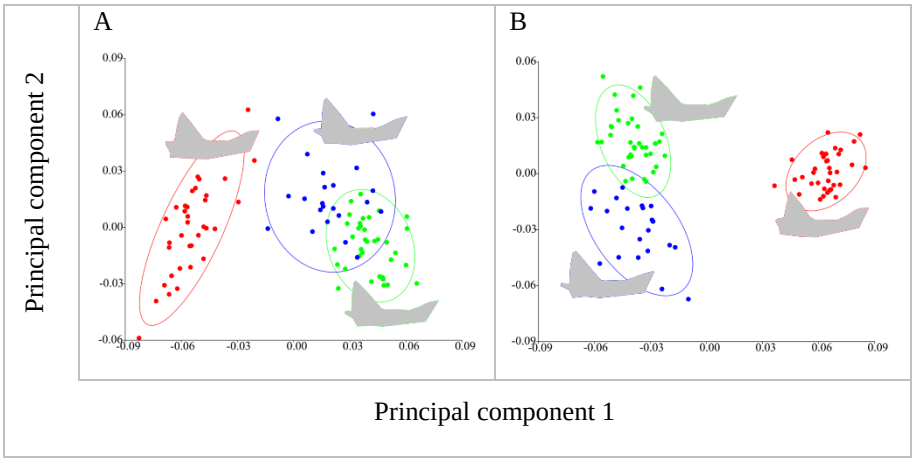


Figure 3.12. PCA of ferret, stoat, and weasel mandibles. A) without allometric correction; B) with allometric correction. Ferrets: red, stoats: green, weasels: blue. The Procrustes mean shape of each species was overlain on the graph bordered in the colour of the species' symbols.

Table 15. Eigenvalues relating to the between species PCAs, eigenvalues that account for five percent or more of the variance are reported, significant eigenvalues ($\chi 2<5.99$) are highlighted in bold, and eigenvalues that occur before the inflection point are italicised.

		EV1	EV2	EV3
Before allometric correction	Eigenvalue	***0.0020***	***0.0005***	0.0002
	Variance (%)	***51.1100***	***11.7680***	5.4650
	Cumulative %	***51.1100***	***62.8780***	68.343
After allometric correction	Eigenvalue	*0.0026*	*0.0005*	*0.0004*
	Variance (%)	*55.4080*	*10.9000*	*8.5630*
	Cumulative %	*55.4080*	*66.3090*	*74.8720*

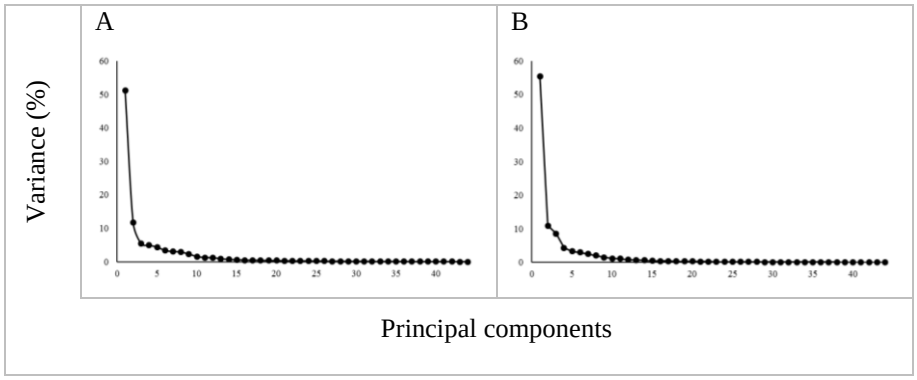


Figure 3.13. Eigenvalue and percentage of variance scree plot from the between species PCA A) without allometric correction; B) with allometric correction.

There was a clear division between the species along PC1, although weasels and stoats did overlap. Along PC2 there was no true division between the species, because although there was as much separation of stoats and weasels along this axis as there was along PC1, there was more within species variation than between species variation along this axis.

Figure 3.14A shows the wireframe deformations for PC1 and PC2. From the negative values to the positive values (from ferrets to stoats and weasels) the angular process moved forward under the coronoid process, and the coronoid process angled further backwards. The angle between the body of the mandible and the coronoid process and the arch of the masseter muscle attachment to the bone also increased in depth but reduced in length. This corresponds with a reduction in the ability of the mandible to transmit the force from the muscle to the teeth. Along PC2 the largest changes were the reduction in depth of the mandible body while the coronoid tilted forwards and was reduced in width. The reduction in width corresponds with a reduction in mandible strength but the shorter tilted forward coronoid process indicates a greater bite force efficiency.

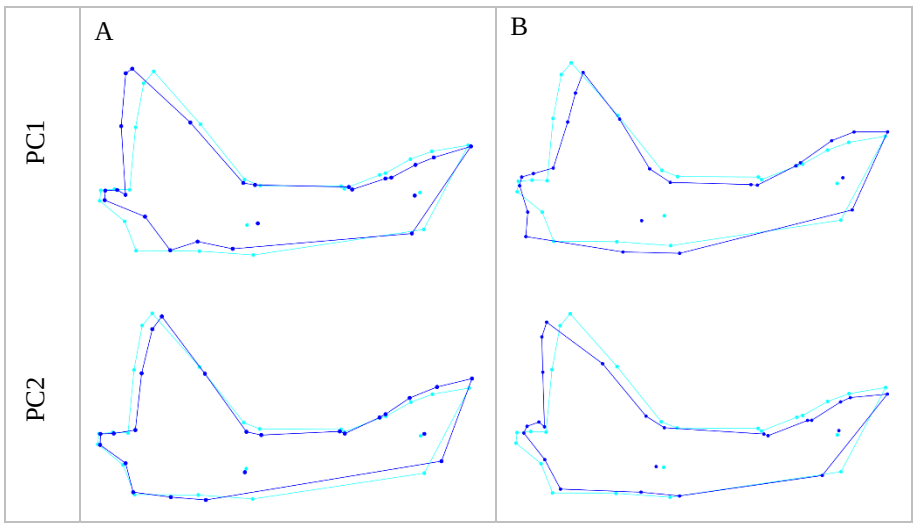


Figure 3.14. Procrustes wireframe deformation plot of *Mustela* spp. mandibles based on the deformation implied by the PCA A) without allometric correction; B) with allometric correction. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

The PCA with allometric correction showed that the first three eigenvalues in the PCA were significant based on the five percent variance and the inflection point (Table 15, Figure 3.12B, and Figure 3.13B). The chi-squared test could not be performed on the allometric corrected data as PCAgen8 does not support the data type that MorphoJ uses as output after the correction. PC1 accounted for 55.41 % of the variance, PC2 10.90 %, and PC3 8.56 % (Table 15).

Ferrets were clearly separated from the other two species on PC1, but weasels and stoats were not. PC2 in the analysis after allometric correction showed some separation of the three species, including a subdivision between stoats and weasels. PC3 showed no division of the species and therefore was not presented at all. Ferrets had a tighter grouping than the others, weasels had the most spread of the three species. Although all the species grouped tighter within themselves after the allometric correction.

Figure 3.14B shows the deformations for PC1 and PC2. Along PC1 the deformations were similar in the stoat and ferret wireframes from the discrimination function analysis, performed on the allometric corrected data. From the negative values to the positive values (stoats and weasels to ferrets) the angular process moved further behind the coronoid process, and the coronoid process angled further forwards, the coronoid process also decreased in size. The bottom area of the ramus (posterior vertical part of the mandible) increased in overall size, this included the

condyle and the angular process. The angle between the body of the mandible and the coronoid process and the depth of the masseter muscle attachment arch also decreased. This was the same trend seen the wireframe deformation from PC1 before allometric correction. In the wireframe for PC2 showed the coronoid tilted back, the condyle tilted up and the mandible body thinned (Figure 3.14B). Therefore, the implications on mandible strength for both PC wireframes after allometric correction are the same as those from before allometric correction.

3.3.2.3 Discriminant function analysis

Procrustes- based superimpositions show the difference in shape between all ferret, stoat, and weasel mandibles, controlling for the differences in size. Figure 3.15A plots all individuals used in the interspecies analyses. Figure 3.15B shows the mean Procrustes-superimposed shape of ferrets, stoats, and weasels, from the discriminant function analysis prior to allometric correction. The data before and after allometric correction confirmed that there was a statistical difference in mandible shape between all three species, after correcting for centroid size.

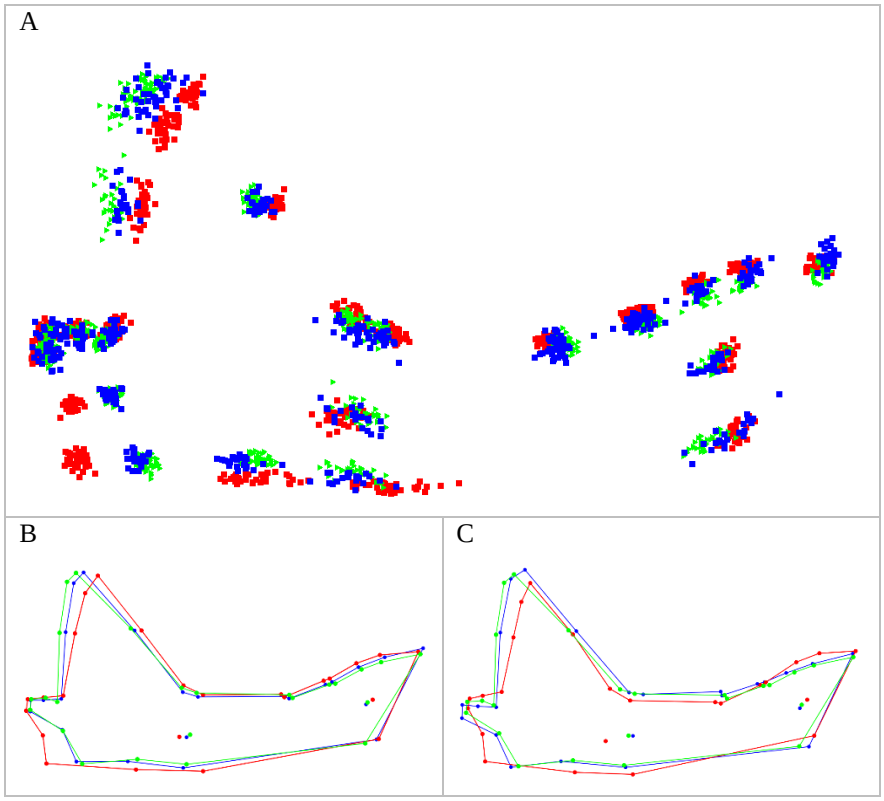


Figure 3.15. A) Procrustes superimpositions of individual ferret, stoat, and weasel mandibles. B) Procrustes superimpositions of the mean ferret, stoat, and weasel mandible shapes, obtained from the discriminant function analysis, before allometric correction. C) Procrustes superimpositions of the mean ferret, stoat, and weasel mandible shapes, obtained from the discriminant function analysis, after allometric correction. Ferrets: red, stoats: green, weasels: blue.

Before the regression analysis the classification and misclassification tables almost fully separated all the species (stoats and weasels did not completely separate); after allometric correction, the classification/ misclassification tables separated the species with 100 % accuracy (Table 16).

Table 16. Discriminant function analyses, classification/ misclassification analysis presented as the percentage of misclassified individuals calculated from the cross validation table. The top (pale green) triangle was for the data before allometric correction, the bottom (white) triangle was for the data after allometric correction.

	Ferrets	Stoats	Weasels
Ferrets		0 %	0 %
Stoats	0 %		10.3448 %
Weasels	0 %	0 %	

The Procrustes shapes (Figure 3.15) show the differences between the group means for both sets of data. Prior to allometric correction (Figure 3.15B), the mandible body (anterior horizontal section) was relatively similar across the three species. The biggest differences were in the shape of the ramus. The angle between the coronoid process and the body of the mandible was different in all species, and the length of the curve where the edge of the masseter muscle connect to the base of the mandible was longer and inverted in ferrets compared with the other two species.

After the allometric correction altered the shape, the coronoid and angular processes were noticeably different in ferrets, and the angle between the coronoid process and the mandible body also decreased further. The coronoid process decreased and the masseter muscle attachment arch became further inverted, the angular process' shape also decreased in size. While the ferrets showed the largest change in shape, the allometric correction changed the other species more subtly after, mostly in the condyle.

The differences in shape both before and after allometric correction indicate that ferrets have a stronger mandible shape that has a greater efficiency at transmitting force from the muscle area to the teeth. This greater efficiency also means they require less muscle area.

3.3.2.4 Canonical variates analysis

Canonical variates analysis (CVA) maximises the differences between groups to better distinguish between them. Like the previous discriminant function analysis and PCA, this analysis has been done both on the original data and on the data with the allometric correction. Prior to allometric correction the Procrustes and Mahalanobis distances showed ferrets and stoats had the most dissimilar mandible shape, and stoats and weasels the most similar (Table 17).

After the allometric correction, the distance measures indicated that ferrets and weasels were the most dissimilar although the stoats and weasels remained the most similar (Table 17). The results of both analyses were quite similar, although stoats and weasels were more similar in the CVA before allometric correction, as can be seen in the CVA graphs (Figure 3.16A and B) and in Table 17, which also gives the eigenvalues and the variance they account for.

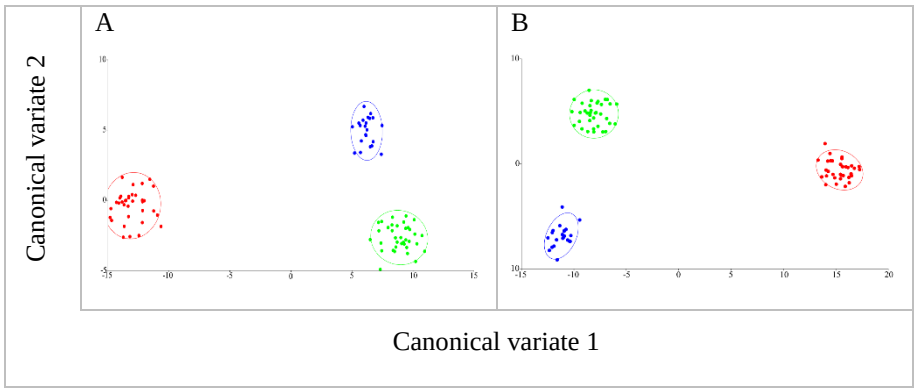


Figure 3.16. CVA plots. A) without allometric correction of *Mustela* spp. mandibles; B) with allometric correction. Ferrets: red, stoats: green, weasels: blue.

Table 17. Statistical results from the CVA. A) CVA eigenvalues; B) Mahalanobis and Procrustes distances between means calculated during the CVA (bottom white triangle) with their associated p-values (top green triangle) after 10000 permutations. Significant values (p<0.05) are highlighted in bold.

A)

		EV1	EV2
Before allometric correction	Eigenvalue	106.892	8.8416
	Variance (%)	92.36	7.64
	Cumulative %	92.36	100
After allometric correction	Eigenvalue	148.501	20.6959
	Variance (%)	87.768	12.232
	Cumulative %	87.768	100

B)

	Mahalanobis distances				Procrustes distances			
Before allometric correction		Ferrets	Stoats	Weasels		Ferrets	Stoats	Weasels
	Ferrets		**<0.0001**	**<0.0001**	Ferrets		**<0.0001**	**<0.0001**
	Stoats	22.0187		**<0.0001**	Stoats	0.0961		**<0.0001**
	Weasels	19.95	8.052		Weasels	0.078	0.0392	
After allometric correction		Ferrets	Stoats	Weasels		Ferrets	Stoats	Weasels
	Ferrets		**<0.0001**	**<0.0001**	Ferrets		**<0.0001**	**<0.0001**
	Stoats	24.039		**<0.0001**	Stoats	0.1047		**<0.0001**
	Weasels	27.2893	12.0308		Weasels	0.1067	0.0501	

The wireframe deformations for the analysis without allometric correction showed the largest difference between the ferrets versus the stoats and weasels together was the angular process, with some differences in the tilt of the coronoid process (Figure 3.17A). There was little difference between the stoats and weasels.

The wireframe deformations for the analysis with allometric correction confirmed these differences in the angular process, and like the previous wireframes on the corrected data (PCA and discriminant function analysis), the coronoid process was shorter in ferrets (Figure 3.17B). These differences reinforce the findings from the PCA and discriminant function analysis; ferrets have a more efficient mandible for transmitting force to the teeth. Between stoats and weasels there was a difference in the angular process, but this was still quite small. The CVA grouped the three species differently based on these differences even though PCA did not, probably because the CVA exaggerates the differences between groups.

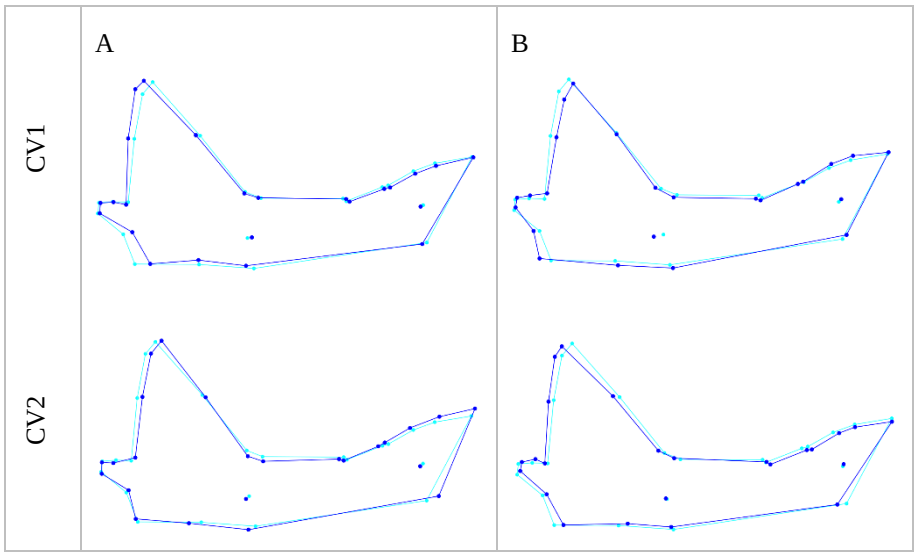


Figure 3.17. CVA wireframe deformation plots of *Mustela* spp. mandibles based on the deformation implied by the analysis. A) without allometric correction; B) with allometric correction. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

3.3.2.5 Modularity analyses

The hypothesis of developmental modularity was not supported, p-value=0.0678, while the p-value of the functional modularity hypothesis testing was significant p-value=0.0475. The distribution graph of the contiguous partitions was skewed with a tail extending toward the lower values, and therefore no further testing was needed (Figure 3.18). After the regression analyses neither hypothesis was considered

significant: developmental modularity p-value=0.1695, functional modularity hypothesis p-value=0.1133.

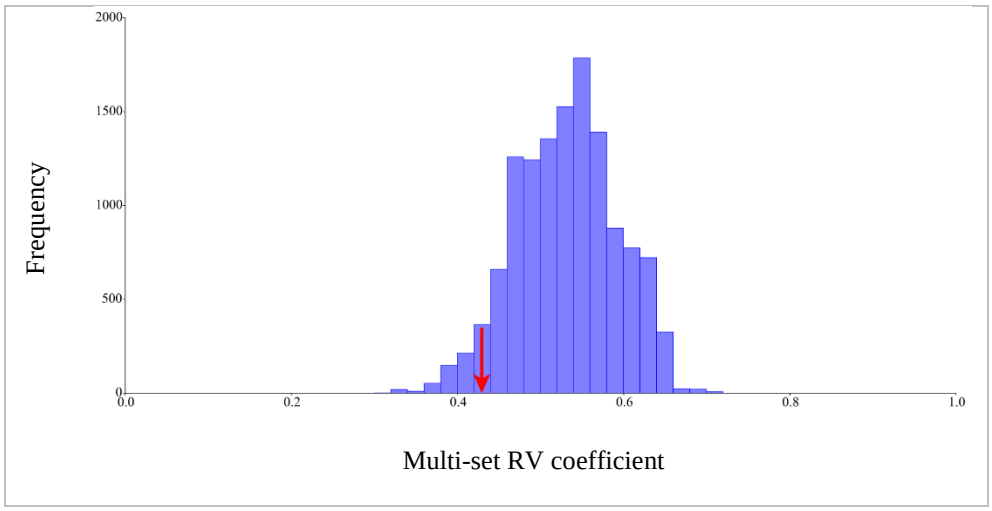


Figure 3.18. Frequency distribution graph from the functional modularity hypothesis test. The red arrow indicates the RV coefficient for the hypothesis, 0.4291.

3.3.2.6 Biomechanical advantage

The biomechanical advantage could only be analysed on the raw coordinates without allometric correction. In this context, a greater biomechanical advantage can be taken to imply greater relative strength. Kruskal-Wallis ANOVAs showed that there was a statistically significant difference between ferrets, stoats, and weasels for a least some of the measurements (Table 18). Ferrets differed from stoats in biomechanical advantage of the T/C, temporalis/ carnassial (T/M1) and masseter/ carnassial (M/M1) areas. The change in shape of the coronoid process, and therefore of the temporalis muscle attachment, was one of the largest differences between these species based on the PCA and CVA deformations, showing that ferrets have a larger biomechanical advantage than stoats when comparing the averages (Table 18). Ferrets had a greater biomechanical advantage than weasels in the masseter/ canine (M/C) and M/M1. In two of the four measurements, T/M1 and M/C, weasels had greater biomechanical advantages than stoats. These results reinforced the findings of the previous analyses.

Table 18. Between species Kruskal-Wallis ANOVAs of biomechanical advantage of the mandible, significant values (p<0.05) are highlighted in bold (T: temporalis, M: masseter, C: canine, M1: carnassial).

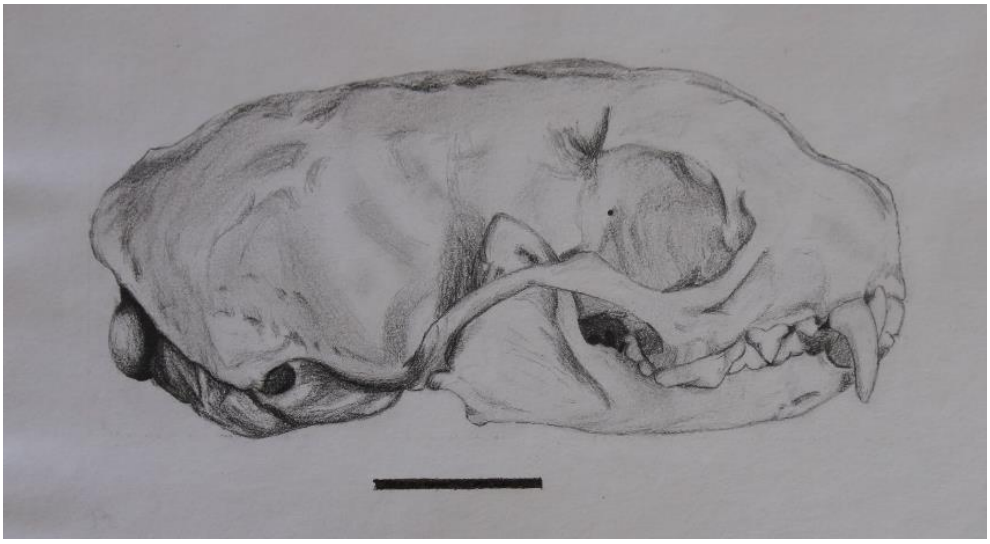
Biomechanical advantage	Average			Ferrets v. Stoats		Ferrets v. Weasels		Stoats v. Weasels	
	Ferrets	Stoats	Weasels	H-value (1, N=71)	p-value	H-value (1, N=57)	p-value	H-value (1, N=58)	p-value
T/C	0.3837	0.3664	0.4011	11.5894	**0.0007**	1.3545	0.2445	2.4664	0.1163
T/M1	0.7100	0.6595	0.7027	30.8583	**<0.0001**	0.2687×10^{-3}	0.9869	8.1366	**0.0043**
M/C	0.3498	0.3398	0.3475	2.3048	0.1290	9.4968	**0.0021**	6.7396	**0.0094**
M/M1	0.6479	0.6118	0.6057	9.3593	**0.0022**	10.1127	**0.0015**	1.4445	0.2294

3.3.3 Section summary

The consensus of statistical analyses, shape deformations and groupings indicate that ferret, stoat, and weasel mandibles were not just statistically different but biologically different as well. There was a small degree of allometry, but not all the shape variation between groups was explained by the regression. Ferrets were the most dissimilar from the other two species and the mandible geometry indicated ferrets had a greater relative muscle strength, particularly of the temporalis muscle measurements which is used for catching prey, the larger angular process in ferrets also indicate a higher requirement for mastication.

Chapter Four

Mandible plasticity and adaptations of New Zealand *Mustela erminea* mandibles across locations



Drawing 5. Skull of a male stoat from Packington Park, Warwickshire, England. Black bar is 10 mm. Artist: C. Hill.

4.1 Introduction

This chapter investigates the extent of variation in size and shape of the mandibles of New Zealand stoats (*Mustela erminea*), between sexes and between locations. Such variation has been found in other species such as mice (Anderson et al. 2014), Punare rat (*Thrichomys apereoides*) (Monteiro et al. 2003), and the common shrew (*Sorex araneus*) (Wojcik et al. 2003).

Stoats were introduced from Britain onto rabbit-infested pastures of New Zealand over the decade 1883-92, and spread rapidly throughout both South and North Islands (King, in press. Liberation and spread of stoats (*Mustela erminea*) and weasels (*M. nivalis*) in New Zealand, 1883-c.1920. NZ J Ecol. Islands are a natural laboratory for studying phenotypic changes (Renaud et al. 2010). The spread of stoats into a wide range of New Zealand habitats different from those to which their British ancestors were adapted has provided an opportunity to study the potential impacts of habitat differences on stoat mandible morphology (Baker et al. 1979; King et al. 1982a, b, c, d; Caumul et al. 2005).

The ecology of stoats in New Zealand has been studied extensively, documenting their diet, genetics, reproduction, and population responses to pulsed resources (King et al. 1982a, b, c, d, e; Murphy et al. 1992; King et al. 1996a; Powell et al. 1997; Cuthbert et al. 2000; King 2002; Veale et al. 2015; Murphy et al. 2016). Therefore, the material available invited answers to several questions.

1. Was there sexual dimorphism in mandible shape as well as in size *within* different locations from New Zealand? The null hypothesis was that there would be no sexual dimorphism in shape controlling for location.

2. Did the degree of size sexual dimorphism differ *between* locations? The null hypothesis was that there would be no differences in the degree of sexual dimorphism between locations controlling for year collected.

3. Were any detectable differences in mandible shape isometric or allometric with size? The null hypothesis of this study was that there would be no within location-between sex effects of size on shape, and that there would

be no effect of size on shape between locations, hence all the shapes should be isometric.

4. If any allometric variation in shape was found, did size account for all of it, or just a component of it?

5. Was mandible morphology affected by the increased food available to young born during a beech (*Nothofagus* spp.) seed masting year? Overall body size, diet, and expected lifespan are known to change with a beech seed year but changes in mandible shape have not yet been examined (King et al. 1982b, c; King 1983, 2002). The null hypothesis was that there would be no effect of beech seed masting on mandible shape.

6. How did shape differ *between* locations after the sexes were pooled for each location? The null hypothesis was that there would be no difference in shape across locations. Was there any modularity detectable across the locations?

7. Did the bite force efficiency of the mandibles differ between the locations? The null hypothesis was that there would be no difference in bite force efficiency.

8. Was there any covariance between mandible shape and environmental or dietary factors? The null hypothesis was that shape was independent of other factors.

Detailed methods are described in Chapter Two. All individuals used were considered of adult age, based on cementum layers if available, or based on mandible length, the average New Zealand adult mandible length for each sex with standard errors was available from (King et al. 1982c). Within-location analyses compared sexes with ages pooled; the effect on beech seed masting was investigated, sexes were kept separates; for between-location analyses, the sexes were pooled. Significant results are described here; all non-significant results were placed in Appendix two on the appendices disk at the end of this thesis.

4.2 Do mandibles of *Mustela erminea* differ between sexes from the same location?

4.2.1 Size and sexual dimorphism

The difference between centroid sizes of male and female stoats from each location were examined using ANOVAs; each location was analysed separately. Male stoats were significantly larger (by 9.36-14.63 %) (Table 19) than females ($p<0.0001$) (Table 20). The normality plots confirmed that the ANOVAs were a valid test (these can be found in Appendix two). Of the New Zealand stoats, Secretary Island (SI) had the smallest size sexual dimorphism and Eglington valley stoats that were born during a seed year (EVY) had the largest. The English (EN) stoats had larger sexual dimorphism in mandible size of any New Zealand stoats.

Table 19. Centroid size means and standard deviations split by sex from different locations around New Zealand and one English location, the average sexual dimorphism is also presented.

Site	Sex	N	Means	Standard deviation	Size sexual dimorphism (% males larger)
CP	Female	10	40.9333	1.1492	10.7175
	Male	12	45.8470	1.8125	
PU	Female	10	40.9824	1.0083	10.9434
	Male	10	46.0184	1.3794	
AP	Female	12	41.6849	1.0080	11.4287
	Male	12	47.0636	1.3442	
WL	Female	18	40.8782	0.9655	9.6049
	Male	18	45.2217	1.1890	
MC	Female	12	41.1702	1.3320	10.8874
	Male	12	46.2002	2.0679	
HVN	Female	12	40.3944	1.1077	11.0518
	Male	12	45.4134	1.9770	
HVY	Female	10	40.8566	1.1441	8.5441
	Male	3	44.6736	0.5320	
EVN	Female	13	41.1780	1.0817	11.6403
	Male	12	46.6027	1.4103	
EVY	Female	11	41.5406	1.3514	13.5248
	Male	12	48.0376	1.2084	
GV	Female	12	41.5385	0.8452	11.6802
	Male	12	47.0319	1.2718	

SI	Female	10	41.9437	1.8495	9.3573
	Male	8	46.2737	1.1817	
RI	Female	13	40.0790	1.2836	9.7428
	Male	8	44.4053	1.9857	
EN	Female	12	40.8914	1.5203	14.6327
	Male	12	47.9005	2.2431	

Table 20. Within location ANOVAs comparing male and female centroid sizes, significant values (p<0.05) are highlighted in bold.

	Effect SS	Effect df	Effect MS	Error SS	Error df	Error MS	F	p-value
CP	131.6938	1	131.6938	48.0239	20	2.4012	54.8451	**<0.0001**
PU	126.8046	1	126.8046	26.2755	18	1.4597	86.8674	**<0.0001**
AP	173.5863	1	173.5863	31.0501	22	1.4114	122.9916	**<0.0001**
WL	210.4819	1	210.4819	10.8842	20	0.5442	386.7643	**<0.0001**
MC	151.8063	1	151.8063	66.5569	22	3.0253	50.1787	**<0.0001**
HVN	151.1428	1	151.1428	56.4935	22	2.5679	58.8589	**<0.0001**
EVN	183.6263	1	183.6263	35.9190	23	1.5617	117.5815	**<0.0001**
GV	181.0661	1	181.0661	25.6513	22	1.1660	155.2923	**<0.0001**
SI	83.3269	1	83.3269	40.5591	16	2.5349	32.8713	**<0.0001**
RI	92.6946	1	92.6946	47.3742	19	2.4934	37.1763	**<0.0001**
EN	294.7688	1	294.7688	80.7702	22	3.6714	80.2885	**<0.0001**

4.2.2 Shape

4.2.2.1 Regression and allometry

The pooled-within group regressions run for each location separately, used as tests for allometry, did not produce significant results in any location, except for Hollyford valley stoats who were not born during a seed year (HVN) (Table 21 and Figure 4.1). In HVN stoats 9.2 % of the shape of mandibles was accounted for by size (p=0.0108). All following analyses comparing males and females from Hollyford valley were conducted on both the raw data and the allometric corrected data.

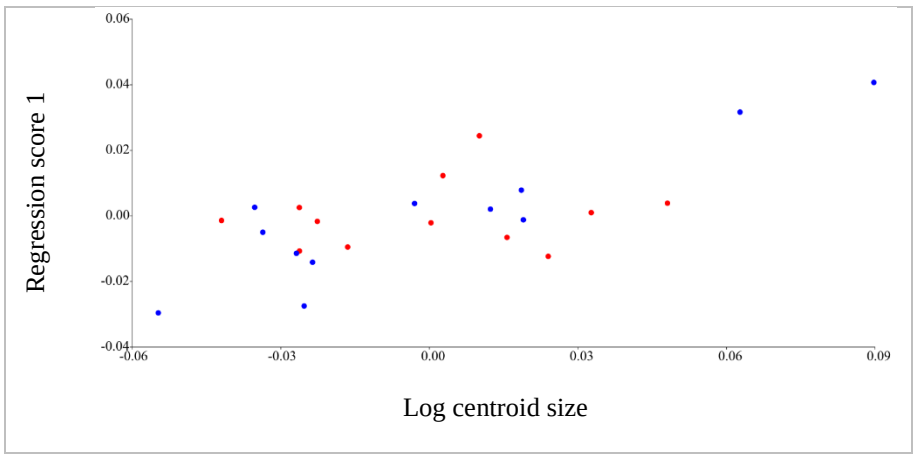


Figure 4.1. Pooled-within group regression analysis of shape on log centroid size, as a test for allometry in non-seed year Hollyford valley stoats. The associated statistics for this regression can be found in Table 21. Males: blue, females: red.

Table 21. Pooled within-group (sex) regression analyses of mandible shape and centroid size as a test for allometry, The permutation test p-value is against the null-hypothesis, significant values (p<0.05) are highlighted in bold.

	Total SS	Predicted SS	Residual SS	d.f.	% predicted	Permutation test p-value (10000 runs)
HVN	0.0336	0.0031	0.0305	1, 22	9.2000	**0.0108**

4.2.2.2 Principal components analysis

The PCAs showed almost no separation in shape between the sexes at most locations, and there was no apparent trend in shape variation between the sexes across sites. At some location males (blue) show more shape variation and at others the females (red) show more variation. Figure 4.2 is a representative of the PCAs, all others can be found in the disk at the end of the thesis.

The majority of the percentage of variance PC scree plots (Figure 4.2) have an inflection point, except Resolution Island (RI) and HVN (prior to allometric correction) which do not. The inflection point is the point on the graph where the taper begins, this usually indicated where the PC's are no longer different from each other. There was no separation of PC1 from the other PCs, other than on Secretary Island (SI) which did show some separation, but males and females were not separated along PC1.

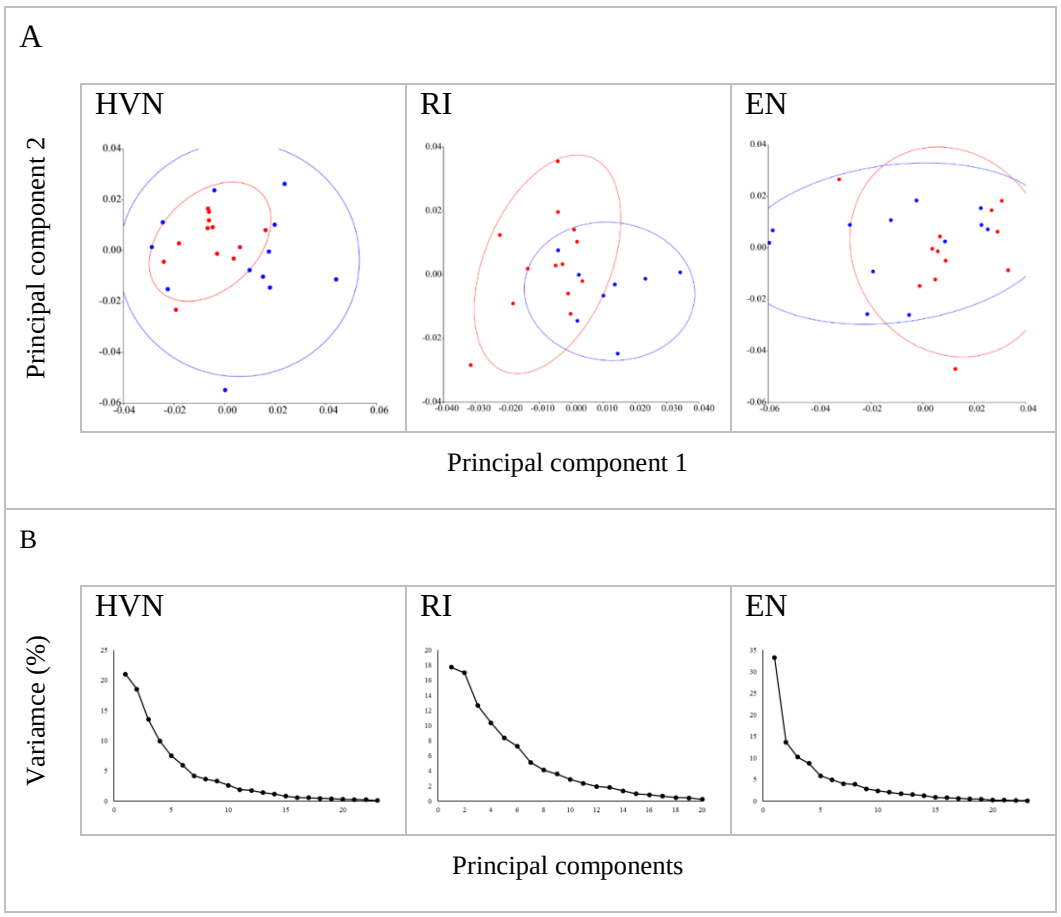


Figure 4.2. A) PCAs of male and female stoat mandibles from 3 of 11 locations, before allometric correction. Circles indicate the 90 % confidence ellipses. Males: blue, females: red. B) Eigenvalue and percentage of variance scree plot from the male and female PCAs before allometric correction from 3 of 11 locations.

Eigenvalue analyses indicated the first five to six eigenvalues may have biological significance for most of the PCAs (Table 22). Typically an eigenvalue that accounts for more than five percent of the variance is likely to have biological significance, if the normal "scree" slope is shown on the graph (Zelditch et al. 2012a). No shape deformations were generated because Chi-squared statistical tests, indicated that there were no significant PCs, this in conjunction with the lack of inflection points and the non-typical scree plots, indicated there was unlikely to be any biologically significant PCs. The scree plots differed between the New Zealand and EN stoats only in that the EN stoats exhibited the typical ideal pattern for the scree slopes even though there was no difference between the sexes along that axis.

Table 22. Eigenvalues relating to the within location PCAs shown here, all other eigenvalue results can be found in Appendix two on the disk, Hollyford valley results from both prior to and after allometric correction were included, eigenvalues that account for five percent or more of the variance were reported, significant eigenvalues ($\chi 2 < 5.99$) are highlighted in bold, and eigenvalues before the inflection point on the scree graphs are italicised.

		EV1	EV2	EV3	EV4	EV5	EV6	EV7
HVN prior to allometric correction	Eigenvalue	0.0003	0.0003	0.0002	0.0002	0.0001	0.0001	
	Variance (%)	21.0100	18.5270	13.5040	9.9320	7.5120	5.9490	
	Cumulative %	21.0100	39.5360	53.0400	62.9730	70.4850	76.4340	
HVN after allometric correction	Eigenvalue	*0.0006*	*0.0003*	*0.0002*	0.0001	0.0001		
	Variance (%)	*35.0390*	*14.8480*	*12.3070*	7.1940	6.4120		
	Cumulative %	*35.0390*	*49.8870*	*62.1950*	69.3890	75.8010		
RI	Eigenvalue	0.0002	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001
	Variance (%)	17.7190	16.9970	12.6720	10.3670	8.3700	7.2400	5.1380
	Cumulative %	17.7190	34.7160	47.3880	57.7550	66.1250	73.3650	78.5030
EN	Eigenvalue	*0.0007*	0.0003	0.0002	0.0002	0.0001		
	Variance (%)	*33.2190*	13.6600	10.2160	8.7250	5.8470		
	Cumulative %	*33.2190*	46.8800	57.0950	65.8200	71.6670		

After allometric correction, the PCA of the HVN stoat data indicated a difference between male and female stoats (Figure 4.3, Table 22). The before-correction PCA showed females tightly grouped within the males, but the after-correction PCA separated the sexes along PC1 (Figure 4.3A).

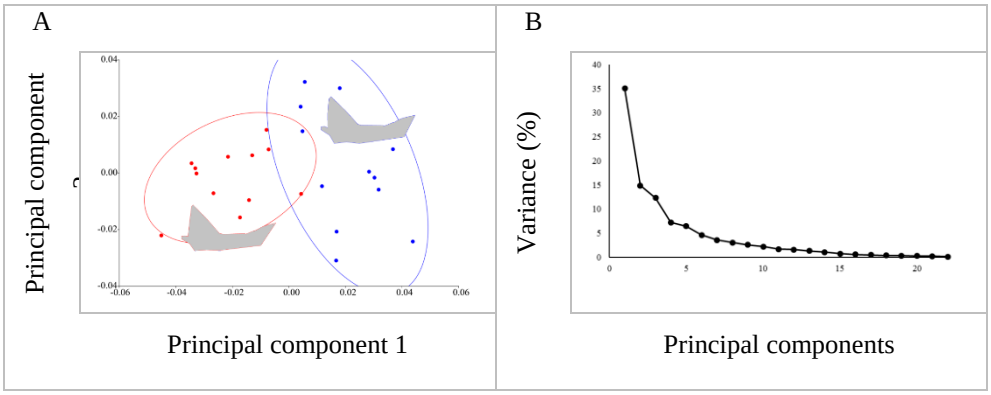


Figure 4.3. A) PCA of the Hollyford valley stoats after allometric correction. Circles indicate the 90 % confidence ellipses. Males: blue, females: red. B) Eigenvalue and percentage of variance scree plot from after the allometric correction.

On the eigenvalues scree plot (Figure 4.3A) PC1 was separated from the other PCs, there was also a clearer inflection point, this indicates that PC1 is likely to have biological significance. PC1 accounted for 35.04 % of the variance in shape (Table 22).

The Procrustes wireframe deformation plot shows the change in the average shape across the selected axis, the starting shape (pale blue) was the average at -0.1 and the end shape (royal blue) was the average shape at 0.1 (Figure 4.4). In this case it is showing the general but exaggerated trend from female mandibles (pale blue) to male mandibles (royal blue) (Figure 4.4). The mandible body of males is deeper, and the front of the mandible thicker. The coronoid process is tilted towards the rear and the condyle tilted up, reducing the angle between the coronoid process and the condyle. The angular process is further forward under the coronoid, and the arch where the masseter muscle joins the base of the mandible is deeper. This means that males would have a greater ability to transmit muscle force to the carnassial but less so to the canine.

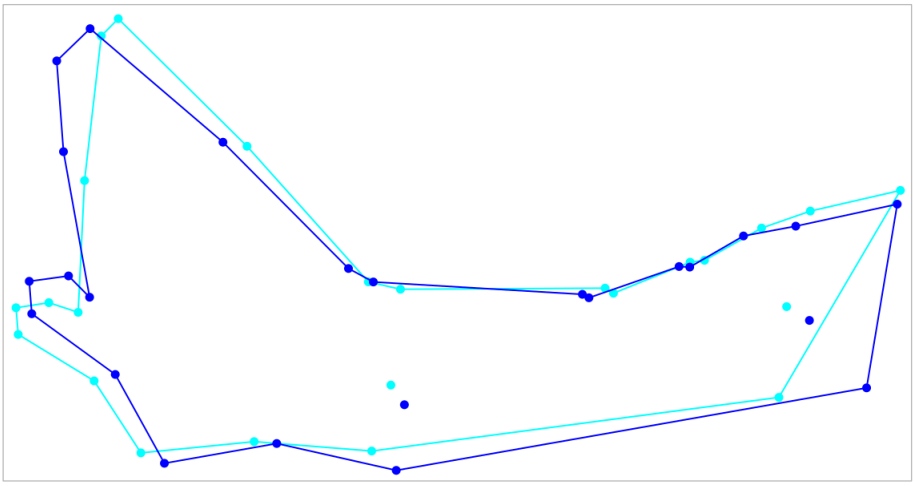


Figure 4.4. Procrustes deformation wireframe plot of Hollyford valley stoat mandibles based on the deformation implied by PC1 from the PCA after allometric correction. Starting shape (negative end of axis) is the closest to the females: pale blue, end shape is closest to the males (positive end of axis): royal blue.

4.2.2.3 Discriminant function analysis

A Procrustes- based superimposition is the graphical output after the mandible shape of each individual is overlaid on all other from the group. In this case they show the difference in shape between male and female stoat mandibles, Figure 4.5 shows four of the 11 different locations as a representative of all locations, all others can be found in Appendix two on the disk. Fiordland stoats born during a seed year (HVY and EVY) were analysed in Section 4.3. The left column shows the Procrustes superimpositions of all individuals from each location, the right hand column shows the mean Procrustes superimposed shape of male and female stoats for that location, obtained from the discriminant function analysis. In all locations there appeared to be greater variation in the ramus section (posterior vertical section) of the bone compared with the mandible body (anterior horizontal section), where the points are more tightly grouped.

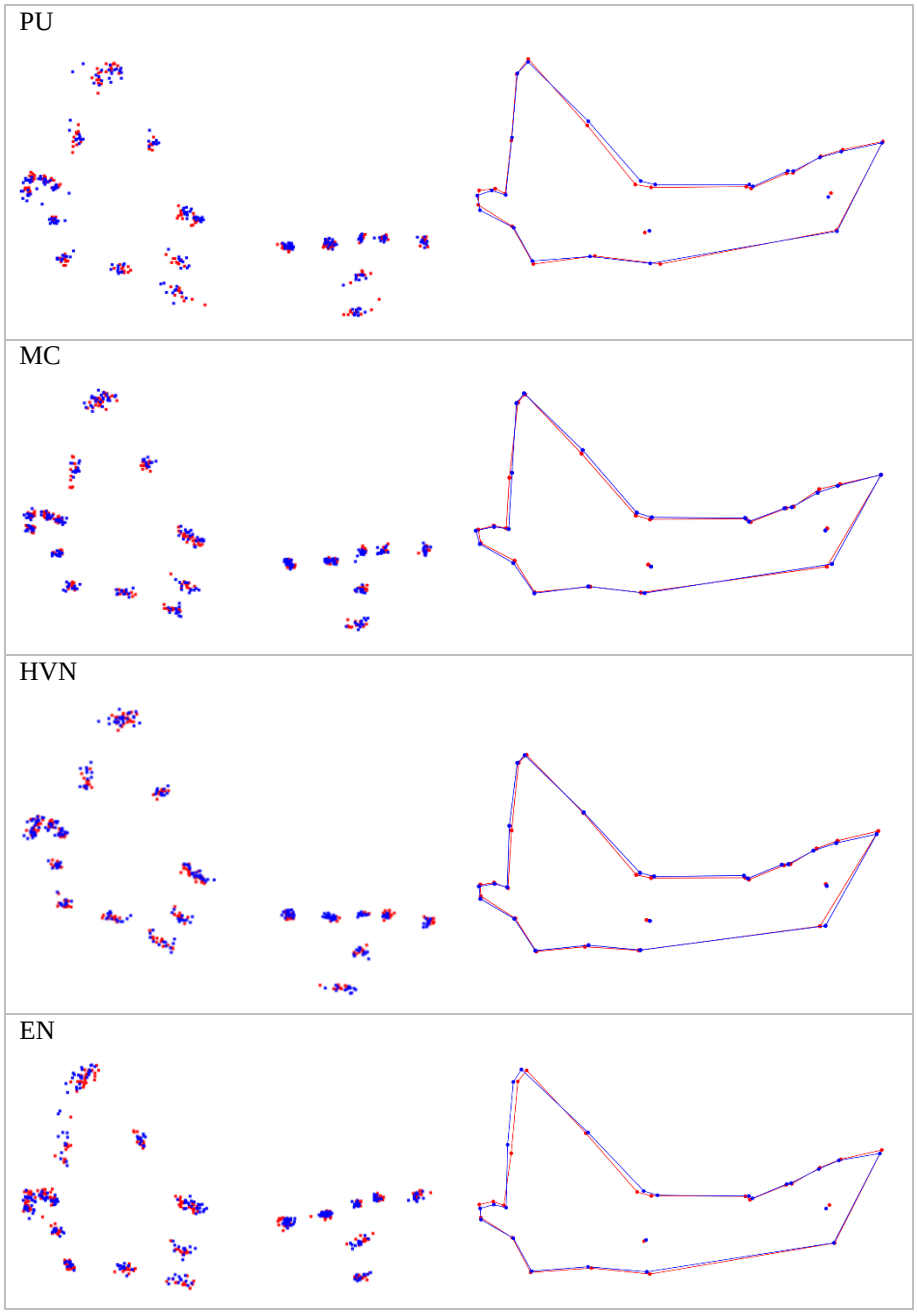


Figure 4.5. Composite figure of Procrustes superimpositions and Procrustes mean shapes of male and female stoats from 4 of 11 locations (3 New Zealand, 1 England). Males: blue, females: red.

The discriminant function analyses produced some conflicting results (Table 23). At some locations there were significant distances between mean shape of each sex ($p<0.05$), either Procrustes or Mahalanobis, but most of these groups did not separate the sexes effectively in the classification/ misclassification tables. Three groups that did separate the sexes were: Pureora Forest Park (PU), Mount Cook National Park (MC) and the English (EN) stoats. The EN stoats separated most effectively as less than five percent of individuals were misclassified. The separation was most likely where differences were exaggerated, probably because

of the marginally thicker coronoid processes in the males. The difference in coronoid process thickness was not large as the PCA found no difference between male and females from PU, MC or EN. Therefore, these statistically significant differences were unlikely to be biologically significant and did not constitute a statistical trend seen in the other locations. This was because in locations with significant distances between means for sexes there was too much overall variation and cross-over in shape to allow clearly separate the sexes.

The allometric correction did have an effect on the results of the HVN sex comparison, because after the correction the Procrustes distances between means became statistically significant ($p<0.0001$), but the Mahalanobis distance was not significant in either data set, the Mahalanobis distance includes the shape variation of all individuals, the lack of significance indicates that more than one individual within each group is similar to individuals in other group. The classification/ misclassification tables also failed to separate the sexes. By, contrast, the PCA after allometric correction was able to separate the sexes along PC1.

Table 23. Within location discriminant function analysis of males and females, A) distances between means. B) Classification/ misclassification analysis, percentage of misclassified individuals calculated from the cross validation tables, significant values (p<0.05) are highlighted in bold. This table includes Hollyford valley results from both prior to and after allometric correction.

A)						B)
	Distance between means		Hotelling's T-square, parametric p-value	Permutation tests (10000 runs)		
	Procrustes	Mahalanobis		Procrustes	Hotelling's T-square	
PU	0.0285	3.7750	71.2544, 0.9530	**0.0056**	**0.0009**	10.0000 %
MC	0.0197	5.0815	154.9288, 0.9090	**0.0461**	**0.0034**	20.8333 %
HVN prior to allometric correction	0.0199	2.8559	48.9385, 0.9953	0.0974	0.3612	30.4348 %
HVN after allometric correction	0.0423	7.3436	323.5709, 0.4998	**<0.0001**	0.7643	56.5217 %
EN	0.0271	5.2554	165.7153, 0.8985	**0.0256**	**0.0031**	4.1667 %

4.2.2.4 Biomechanical advantage

The biomechanical advanatge of the mandible is a measure of mandible geometry and muscle efficiency used for bite force. The explanation on how this is done can be found in the Methods, Section 2.4.1.8. Kruskal-Wallis ANOVAs testing biomechanical advantage found no consistently significant differences between male and female stoats across the different locations. At Grebe valley (GV) males had a larger biomechanical advantage of the T/C, and on Resolution Island, (RI) males had greater efficiency of both T/C and T/M1. Figure 2.5 in the Methods (Chapter Two) shows the inlevers and outlevers.

Table 24. Within location Kruskal-Wallis ANOVAs of biomechanical advantage of male and female mandibles, significant values (p<0.05) are highlighted in bold (T: temporalis, M: masseter, C: canine, M1: carnassial).

	CP				PU				AP			
	H-value (1, N=22)	p-value	Average male	Average female	H-value (1, N=20)	p-value	Average male	Average female	H-value (1, N=24)	p-value	Average male	Average female
T/C	1.1130	0.2914	0.3629	0.3553	0.0229	0.8798	0.3671	0.3656	1.9200	0.1659	0.3584	0.3610
T/M1	0.0043	0.9474	0.6504	0.6492	0.8229	0.3643	0.6554	0.6661	1.2033	0.2727	0.6073	0.6302
M/C	2.9391	0.0865	0.3368	0.3278	0.0000	1.0000	0.3413	0.3378	2.0833	0.1489	0.3915	0.3273
M/M1	0.4348	0.5097	0.6038	0.5987	0.3657	0.5454	0.6091	0.6151	2.8033	0.0941	0.5081	0.5683
	WL				MC				HVN			
	H-value (1, N=36)	p-value	Average male	Average female	H-value (1, N=24)	p-value	Average male	Average female	H-value (1, N=24)	p-value	Average male	Average female
T/C	3.4845	0.0619	0.3731	0.3597	0.3333	0.5637	0.3743	0.3699	0.2700	0.6033	0.3615	0.3600
T/M1	2.0270	0.1545	0.3675	0.6503	0.0533	0.8174	0.6822	0.6846	0.2700	0.6033	0.6491	0.6552
M/C	1.0250	0.3113	0.3432	0.3364	0.0000	1.0000	0.3261	0.3266	0.6533	0.4189	0.3304	0.3231
M/M1	0.2563	0.6127	0.6150	0.6085	0.5633	0.4529	0.5941	0.6051	0.6533	0.4189	0.5997	0.5881
	EVN				GV				SI			
	H-value (1, N=24)	p-value	Average male	Average female	H-value (1, N=24)	p-value	Average male	Average female	H-value (1, N=18)	p-value	Average male	Average female
T/C	3.4133	0.0647	0.3725	0.3584	3.8533	**0.0496**	0.3824	0.3667	2.8500	0.0914	0.3803	0.3600
T/M1	3.0000	0.0833	0.6650	0.6364	1.2033	0.2727	0.6673	0.6545	3.8211	0.0506	0.6889	0.6480
M/C	2.0833	0.1489	0.3248	0.3130	2.6133	0.1060	0.3401	0.3279	0.1974	0.6569	0.3237	0.3257

M/M1	3.4133	0.0647	0.5800	0.5553	0.2133	0.6442	0.5936	0.5852	0.0316	0.8590	0.5868	0.5862

	RI				EN			
	H-value (1, N=21)	p-value	Average male	Average female	H-value (1, N=24)	p-value	Average male	Average female
T/C	6.0629	**0.0138**	0.3988	0.3761	2.0833	0.1489	0.3812	0.3695
T/M1	5.7115	**0.0169**	0.7105	0.6764	0.8533	0.3556	0.6848	0.6732
M/C	0.5245	0.4689	0.3220	0.3256	1.0800	0.2987	0.3241	0.3279
M/M1	0.4248	0.5145	0.5740	0.5856	1.7633	0.1842	0.5821	0.5974

4.2.3 Section summary

Mandibles of males were always larger than those of females, but there were differences in the degree of sexual dimorphism in mandible size between sites, largest in the sample from England (EN). This was consistent with the results from King et al. (1982c) and supports the hypothesis that size is affected by local conditions (King 1989, 1991b; Powell et al. 1997; Piontek et al. 2015) Statistically significant differences in shape (mean shape) were detected by discriminant function analyses at some locations, but they were not corroborated by the other analyses. The parsimonious conclusion is that there were no biologically significant differences in mandible shape between the sexes, and so the sexes were combined for the inter-location shape analyses. Allometry in mandible shape was found in only one New Zealand sample, HVN. The effects of birth year on size and shape of mandibles are considered separately in Section 1.3 below.

4.3 Did beech masting events and associated resource pulses in Fiordland National Park Nothofagaceae forests have any effect on the mandibles of *Mustela erminea*?

This section covers investigation of the effect of beech (*Nothofagus* spp.) seed masting events on the mandible morphology of stoats from two locations in Fiordland National Park: Hollyford Valley (HV) and Eglinton Valley (EV). Fiordland National Park, has large areas of beech (Nothofagaceae) forests, which have a beech overstorey and a sparse understorey (King 1983; Dilks et al. 2003).

These forest systems both pure stands and mixed stands with podocarp trees have a cycling masting event, usually once every three to five years. These masting events are a simultaneous release of flowers and then seed by the trees which pulses resources into the system (King 1983; Dilks et al. 2003). These pulses, are utilised by native birds and invasive pests, and cause irruptions of mice (King 1983). Stoat diets vary with this cycle, the proportion of mice in the diet increasing with the mice population and as diet has been correlated with mandible shape and size an increased proportion of mice during the growth period was expected to have an effect on mandible shape (King 1983; Renaud et al. 2015).

Powell et al. (1997) hypothesised that sexual dimorphism is directly affected by the diet in the short term. They investigated this in Eglinton valley stoats, which were used in the following analysis, where they experience beech seed food fall pulses. Male size should be affected by abundant food, likely from a greater phenotypic plasticity in genes related to size, whereas females are constrained by the energy requirements for reproduction and therefore size should be more stable (Powell et al. 1997). Powell et al. (1997) did not find support for this hypothesis, therefore I was expecting that both sexes will be affected by seed year birth.

Results have been presented in the same order as in the previous section: size, shape, and biomechanical advantage. Both locations were subjected to the same analyses, but results that include HVY males (n=3) must be treated with caution because small sample numbers greatly affect shape results.

4.3.1 Size

Stoats born during a seed year and collected from both the Hollyford and Eglinton Valleys were tested for significant differences in size and the average level of sexual dimorphism and then compared with those from non-seed years. HVY male stoats were significantly larger (8.54 %) (Table 19) than HVY females (p=0.0002) (Table 25 and Table 26), but there was a smaller degree of size sexual dimorphism compared to HVN and the Eglinton valley. In EV, EVY males were significantly larger than EVY females (p<0.0001), sexual dimorphism in seed years (EVY compared with EVN) was 13.52 %, the highest recorded size sexual dimorphism in any New Zealand location (Table 26). The normality plots showed that the ANOVA was a valid test, these can be found in Appendix two.

Table 25. The mean centroid size of mandibles from Eglinton (EV) and Hollyford (HV) valleys, stoats were separated into those not born during a seed masting year (N) and those that were (Y).

Site	Sex	N	Mean	Standard deviation
HVN	Female	12	40.3944	1.1077
	Male	12	45.4134	1.9770
HVY	Female	10	40.8566	1.1441
	Male	3	44.6736	0.5320
EVN	Female	13	41.1780	1.0817
	Male	12	46.6027	1.4103
EVY	Female	11	41.5406	1.3514
	Male	12	48.0376	1.2084

As expected from previous analyses of condylobasal length by Powell et al. (1997), two-way factorial ANOVAs confirmed the effect of sex, but not location, on mandible size in HV, but neither sex changed significantly in size under the effect of seed year (Figure 4.6, Table 25, Table 26, and Table 27). In EV both sex and location had significant effects on size (p<0.0001 and p=0.0179 respectively) but their interaction was not significant, this is likely because the males born in a seed year significantly increased in size (3.08 %) (p=0.0082), but the females did not.

Table 26. Results from ANOVAs of centroid sizes from Hollyford and Eglinton valleys. All birth years and sexes combined, followed by the Newman-Keuls test of the post-hoc analysis. Significant values (p<0.05) are highlighted in bold.

	Effect SS	Effect df	Effect MS	Error SS	Error df	Error MS	F-value	p-value
HVY	33.6208	1	33.6208	12.3464	11	1.1224	29.9544	**0.0002**
HV	196.2364	3	65.4122	68.8399	33	2.0861	31.3569	**<0.0001**
EVY	242.2533	1	242.2533	34.3269	21	1.6346	148.2023	**<0.0001**
EV	441.6821	3	147.2274	70.2458	44	1.5965	92.2191	**<0.0001**

Newman-Keuls test

		HVN		HVY	
		F	M	F	M
HVN	F				
	M	**0.0002**			
HVY	F	0.5631	**0.0001**		
	M	**0.0001**	0.3566	**0.0001**	

		EVN		EVY	
		F	M	F	M
EVN	F				
	M	**0.0001**			
EVY	F	0.4866	**0.0001**		
	M	**0.0002**	**0.0082**	**0.0001**	

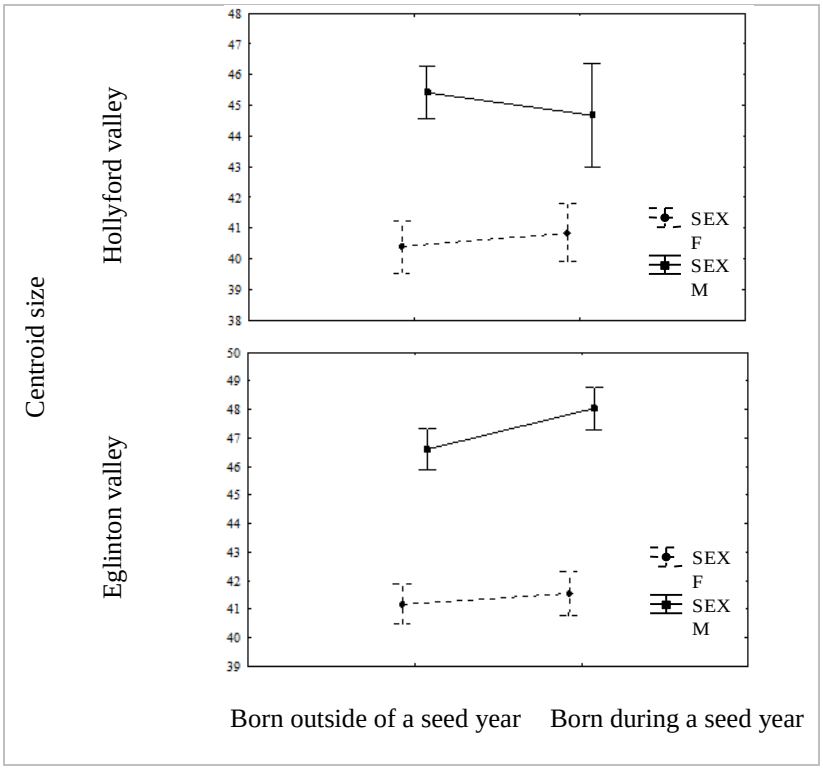


Figure 4.6. Graphical results from the factorial ANOVA comparing sex and the effect of a seed year birth in individuals from Hollyford and Eglinton valleys.

Table 27. Factorial two-way ANOVA results of Hollyford and Eglinton valleys centroid sizes. Investigating whether birth year and sex had a combined significant effect on centroid size. Sigma-restricted parameterization. Significant values (p<0.05) are highlighted in bold.

	Hollyford valley				
Effect	SS	df	MS	F	p-value
Intercept	48927.8649	1	48927.8649	23454.7226	**<0.0001**
Birth year	0.1284	1	0.1284	0.0616	0.8056
Sex	130.1233	1	130.1233	62.3777	**<0.0001**
Birth year*sex	2.4083	1	2.4083	1.1545	0.2904
Error	68.8398	33	2.0861		
	Eglinton valley				
Effect	SS	df	MS	F	p-value
Intercept	94039.6884	1	94039.6884	58903.8000	**<0.0001**
Birth year	9.6607	1	9.6607	6.0512	**0.0179**
Sex	424.8935	1	424.8935	266.1413	**<0.0001**
Birth year*sex	3.4374	1	3.4374	2.1531	0.1494
Error	70.2458	44	1.5965		

4.3.2 Shape

4.3.2.1 Regression and allometry

The pooled-within group regression was significant for the HVN stoats, but not for the HVY stoats, probably because only three adult HVY males were available (Figure 4.7 and Table 28). The HV analysis, where pools were organised by birth year and sex, found significant allometry (p=0.0093); size accounted for 6.01 % of shape. All following HV analyses were performed on both the raw and allometric corrected data.

The regression found significant allometry (p=0.0044) in EVY stoats; size accounted for 12.25 % of shape, but the regression for EVN stoats was not significant. All EVY male and female comparisons were conducted on both the raw and allometric corrected data. The EV regression was not significant and so analyses comparing EVN and EVY stoats were only performed on the raw data (Figure 4.7 and Table 28).

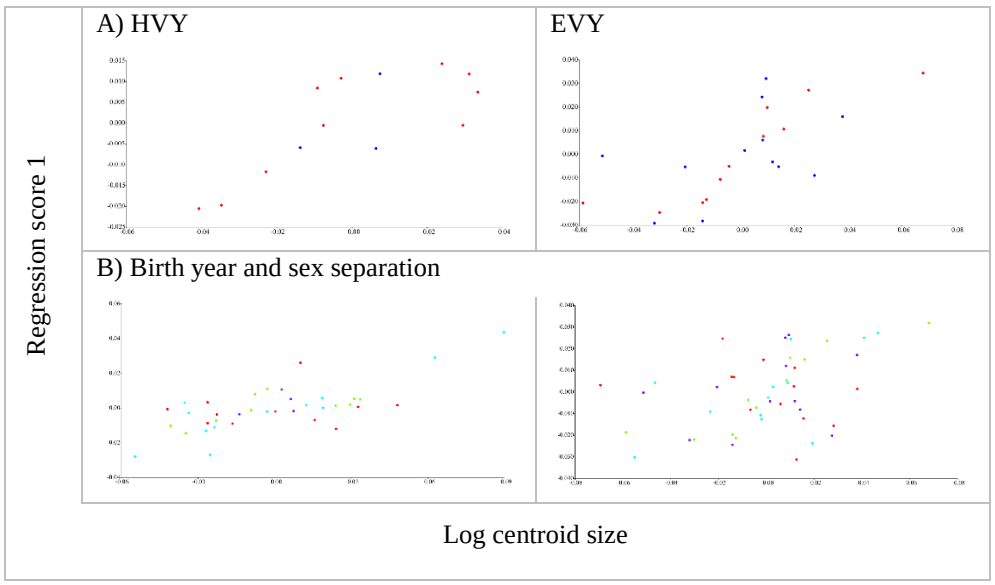


Figure 4.7. Pooled-within group regression analysis of shape on log centroid size, as a test for allometry on Hollyford and Eglinton valley stoats. A) On stoats born within a seed year, males: blue, females: red. B) On each valley separated by sex and birth year, males N: pale blue, females N: red, males Y: purple, females y: green. The associated statistics for these regressions can be found in Table 28.

Table 28. Pooled within-group regression analyses for the Hollyford and Eglinton valley samples. Significant values (p<0.05) are highlighted in bold.

		Total SS	Predicted SS	Residual SS	d.f.	Predicted (%)	Permutation test p-value (10000 runs)
HVY		0.0185	0.0010	0.0174	1, 11	5.6100	0.7966
EVY		0.0322	0.0039	0.0283	1, 21	12.2500	**0.0044**
Birth year and sex separation	HV	0.0521	0.0031	0.0490	1, 35	6.0100	**0.0093**
	EV	0.0712	0.0024	0.0689	1, 46	3.3300	0.1058

4.3.2.2 Principal components analysis

For HVY, PC1 accounted for 25.43 % of the variance in shape (Figure 4.8A) but there was no division along this axis, so the three male samples fell easily within the range for females. PC2 accounted for 22.69 % of the variance, but there was no division along this PC either. In theory the first six eigenvalues for HVY could have biological significance as they all account for more than five percent of the variance (Table 29). However, none of the PCs returned significant in chi-squared values, and there was also no identifiable inflection point on the percentage of variance plot (Figure 4.8), combining this with the lack of separation of the sexes, the eigenvalues are unlikely to have any biological significance.

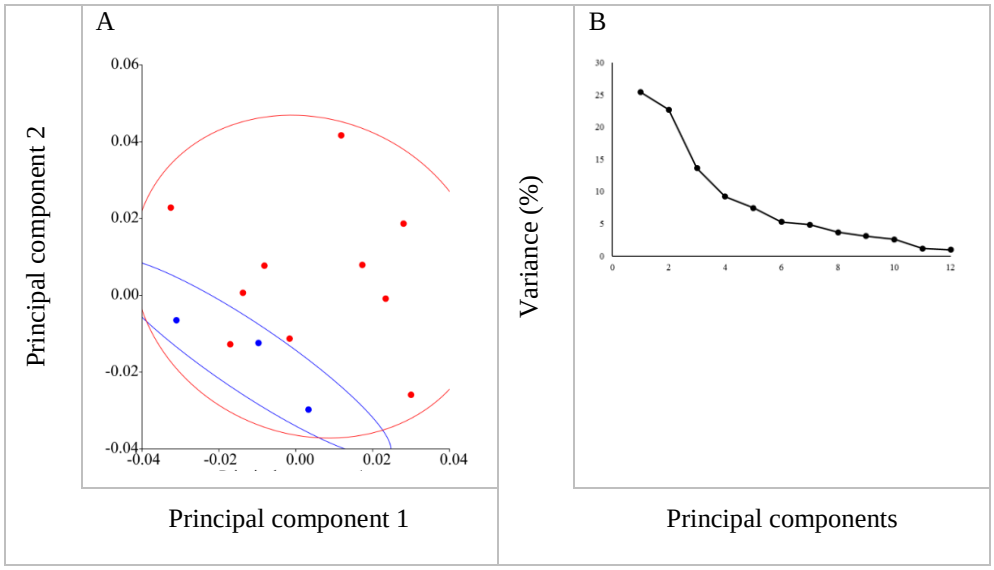


Figure 4.8. Hollyford valley stoats born during a seed year. A) PCA, males: blue, females: red. Circles indicate the 90 % confidence ellipses. B) Eigenvalue and percentage of variance scree plot from the Hollyford valley PCA.

In the HV data before allometric correction, PC1 accounted for 18.92 % of variance and PC2 15.79 %, but there was no group separation along any of the axes (Figure 4.9 and Table 29). The first six eigenvalues appeared to be above the inflection point, and all accounted for at least five percent of the variance, but the chi-squared statistical analysis was non-significant. After allometric correction PC1 accounted for 24.16 % of variance and there appeared to be some trend in the group shapes, females were slightly to the left and males to the right on the axis (Figure 4.9 and Table 29). This was similar to the HVN PCA after allometric correction. Eigenvalue one was separated slightly from the others on the scree plot, which indicated there may be some significance, the first three eigenvalues were above the inflection point.

Table 29. Eigenvalues relating to the PCAs, eigenvalues that account for five percent or more of the variance were reported, eigenvalues that correspond to the PC's above the inflection point on the scree graph were italicised, significant eigenvalues ($\chi 2<5.99$) are highlighted in bold.

		EV1	EV2	EV3	EV4	EV5	EV6
HVY	Eigenvalue	0.0004	0.0004	0.0002	0.0002	0.0001	0.0001
	Variance (%)	25.4310	22.6860	13.6120	9.2230	7.4510	5.2980
	Cumulative %	25.4310	48.1170	61.7290	70.9530	78.4040	83.7010
HV before allometric correction	Eigenvalue	*0.0003*	*0.0003*	*0.0002*	*0.0002*	*0.0001*	*0.0001*
	Variance (%)	*18.9200*	*15.7940*	*13.4570*	*9.1640*	*7.0040*	*6.5340*
	Cumulative %	*18.9200*	*34.7140*	*48.1710*	*57.3350*	*64.3390*	*70.8730*
HV after allometric correction	Eigenvalue	*0.0004*	*0.0003*	*0.0003*	0.0001	0.0001	0.0001
	Variance (%)	*24.1560*	*16.3750*	*13.9630*	8.0220	6.3140	5.4710
	Cumulative %	*24.1560*	*40.5310*	*54.4940*	62.5170	68.8310	74.3020
EVY before allometric correction	Eigenvalue	*0.0005*	0.0002	0.0002	0.0001	0.0001	0.0001
	Variance (%)	*29.2590*	15.6420	12.2060	8.8360	6.2770	5.5060
	Cumulative %	*29.2590*	44.9020	57.1080	65.9440	72.2210	77.7280
EVY after allometric correction	Eigenvalue	*0.0017*	0.0003	0.0002			
	Variance (%)	*60.8890*	9.3230	6.8150			
	Cumulative %	*60.8890*	70.2110	77.0260			
EV	Eigenvalue	*0.0004*	*0.0003*	*0.0002*	*0.0002*	0.0001	
	Variance (%)	*24.1130*	*15.9820*	*9.9640*	*9.4850*	7.0230	
	Cumulative %	*24.1130*	*40.0940*	*50.0580*	*59.5430*	66.5660	

The wireframe deformation for PC1 after allometric correction (Figure 4.10) was almost identical to the wireframe deformation from the PCA for HVN after allometric correction. In this case it is showing the general but exaggerated trend from female mandibles (pale blue) to male mandibles (royal blue), it was exaggerated because the pale blue shape is the average shape from -0.1 on the axis and the royal blue shape is the average shape from 0.1 on the axis. The mandible body of males is deeper, and the front of the mandible thicker. The coronoid process is tilted towards the rear and the condyle tilted up, reducing the angle between the coronoid process and the condyle. The angular process is further forward under the coronoid, and the arch where the masseter muscle joins the base of the mandible is deeper. This means that males would have a greater ability to transmit muscle force to the carnassial but less so to the canine.

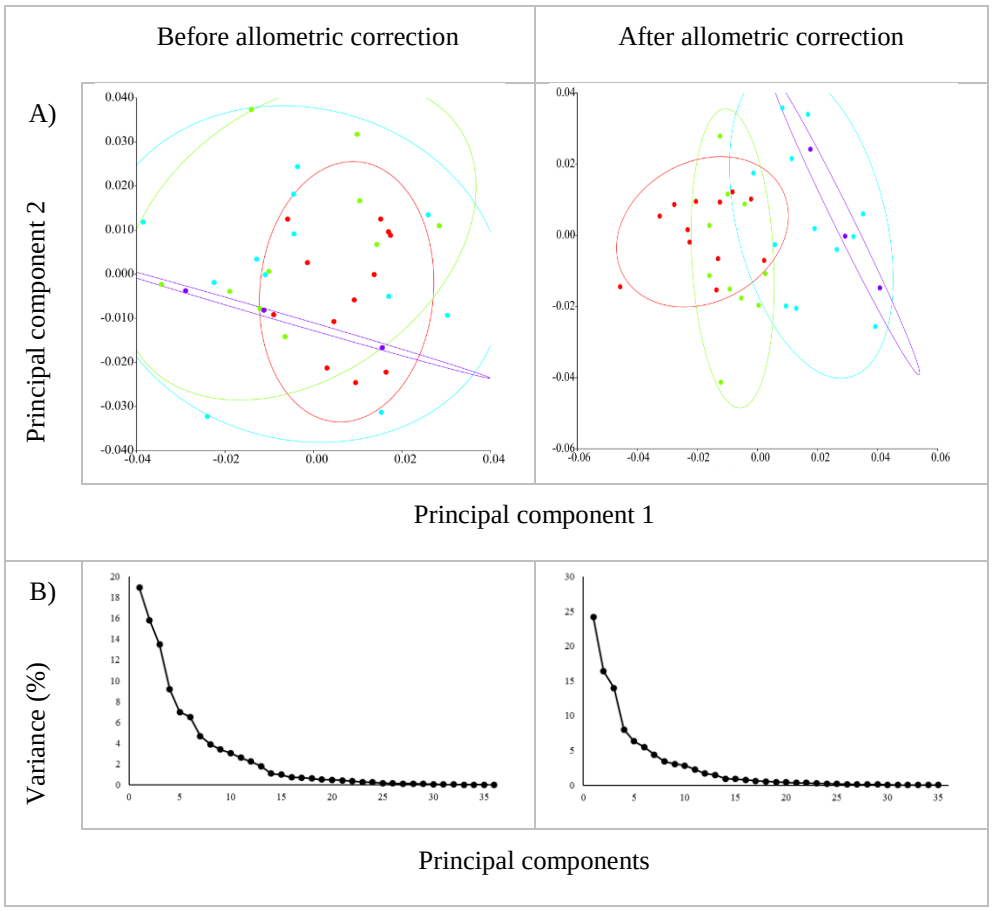


Figure 4.9. PCAs of all Hollyford valley stoats before (left column) and after (right column) allometric correction. Circles indicate the 90 % confidence ellipses. Males N: pale blue, females N: red, males Y: purple, females y: green. B) Eigenvalue and percentage of variance scree plot from the Hollyford valley PCAs.

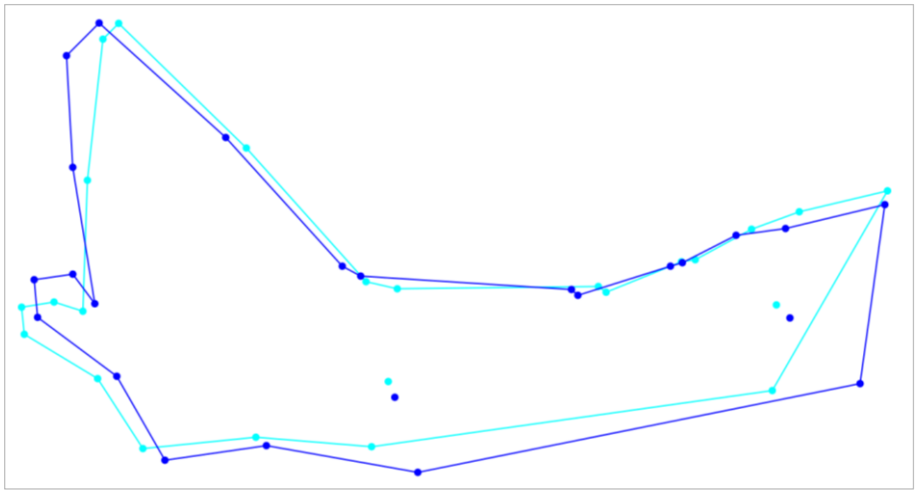


Figure 4.10. Procrustes deformation wireframe plot of all Hollyford valley stoat mandibles based on the deformation implied by PC1 from the PCA after allometric correction. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

In the EVY data before allometric correction, PC1 accounted for 29.26 % of variance and PC2 15.64 %, but there was no sex separation along any of the axes (Figure 4.9, Figure 4.11 and Table 29). Only the first eigenvalue appeared to fall above the inflection point, but the first six accounted for at least five percent of the variance, indicating biological significance. However, the chi-squared statistical analysis was non-significant. After the allometric correction, PC1 accounted for 60.89 % of variance and the sexes separated out, males on the left and females to the right (Figure 4.11 and Table 29). Eigenvalue one was separated from the others on the scree plot, and it was the only one above the inflection point, suggesting that it may be of some significance.

Figure 4.12 shows the wireframe deformation for PC1 after allometric correction. Once again the deformation plot is an exaggerated version of the true difference between males and females. Along the axis from males towards females the mandible body thinned, the masseter muscle attachment arch shortened, the coronoid process thinned and tilted back toward the condyle, which was tilted up and forward. The shadowed means overlain on the PCA also indicated this trend. This difference between males and females was different to the HVN and HV deformations, while males had thicker mandibles in all the significant analyses, in this analysis females had a ramus shape (vertical mandible section) similar to males from HVN and HV. This means female mandibles have less strength.

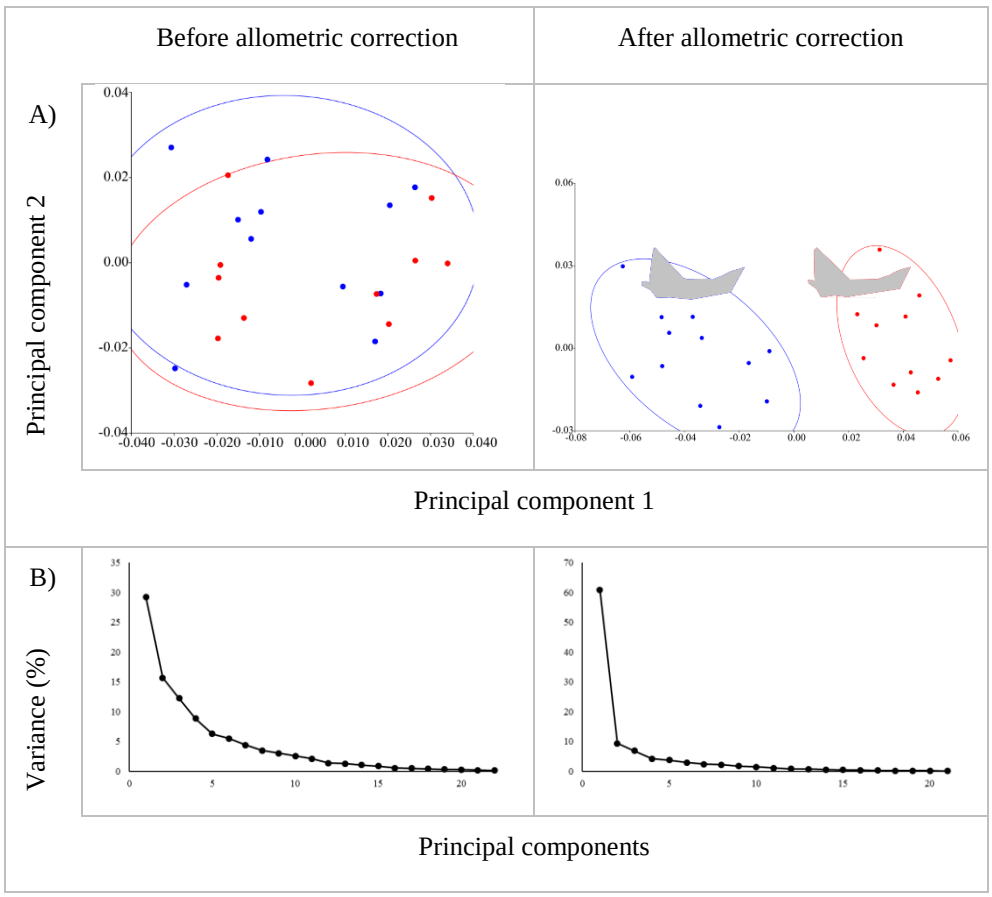


Figure 4.11. Eglinton valley stoats born during a seed year, before (left column) and after (right column) after allometric correction. A) PCA. Males: blue, females: red. Circles indicate the 90 % confidence ellipses. B) Eigenvalue and percentage of variance scree plot from the Eglinton valley PCA.

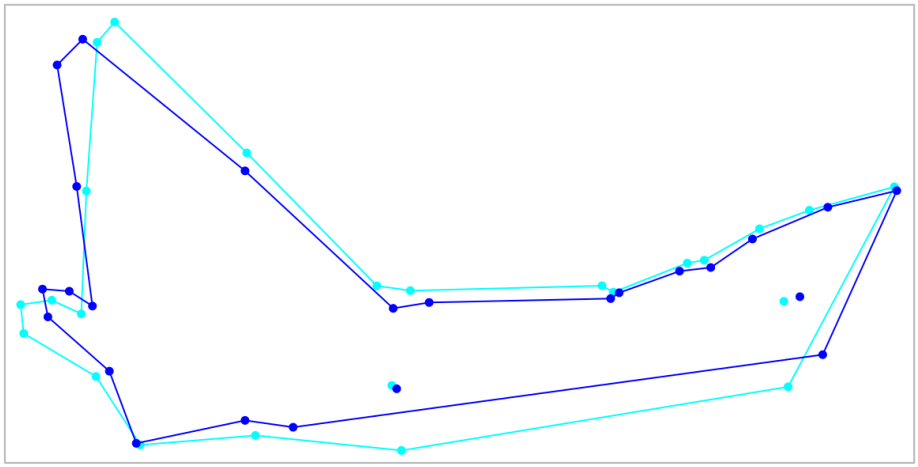


Figure 4.12. Procrustes deformation wireframe for mandibles from Eglinton valley stoats born during a seed year, based on PC1 from the PCA after allometric correction. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

On the PCA for EV, PC1 accounted for 24.11 % of the variance in shape (Figure 4.13A) but there was no division of groups along this axis. PC2 accounted for 15.98 %

of the variance, also without subdivision along this PC. The EV eigenvalues indicated the first five eigenvalues may have biological significance, although only four were above the inflection point (Table 29) the chi-squared statistical tests did not find any significant PCs (Figure 4.13) combining this information with the lack of separation of the groups either by birth year or sex, the eigenvalues are unlikely to have any biological significance.

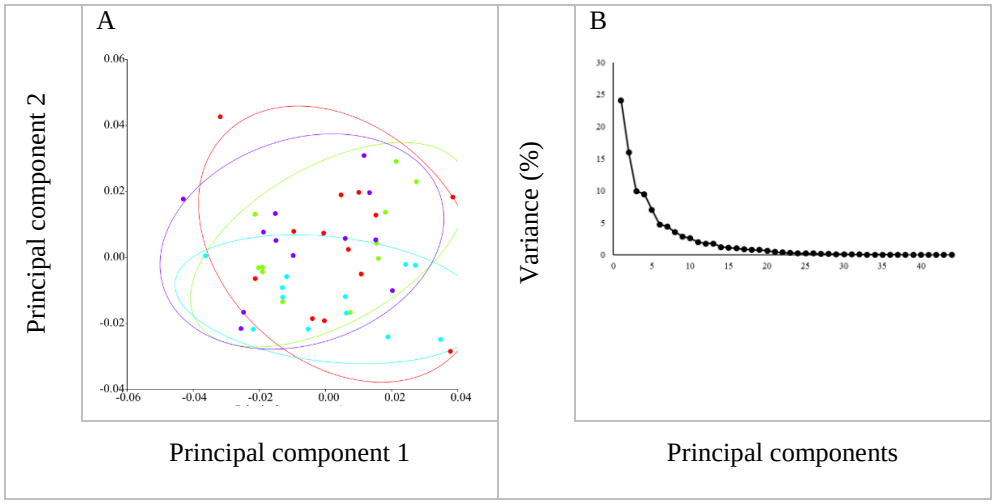


Figure 4.13. A) PCA of the Eglinton valley stoats. A) PCA showing PC1 (horizontal axis) and PC2 (vertical axis). Males N: pale blue, females N: red, males Y: purple, females y: green. Circles indicate the 90 % confidence ellipses. B) Eigenvalue and percentage of variance scree plot from the Eglinton valley PCA.

4.3.2.3 Discriminant function analysis

Procrustes-based superimpositions show the difference in shape between male and female mandibles of HV and EV stoats, comparing first males and females born within a seed year, then all four categories for each location (sex and seed year) (Figure 4.14). The left column in the Procrustes shows superimpositions of all individuals from each location, the right hand column shows the mean Procrustes superimposed mean shape of male and female stoats for each data set. In all locations there appeared to be greater variation in the ramus section compared with the mandible body, where the points are more tightly grouped.

The differences between HV stoats appeared to be mostly in the mandible body, whereas in EV stoats it was the ramus. The results of the discriminant function were mostly non-significant. EVY after allometric correction did have significant Procrustes distances between the means, but still did not separate out in the classification/ misclassification tables (Table 30).

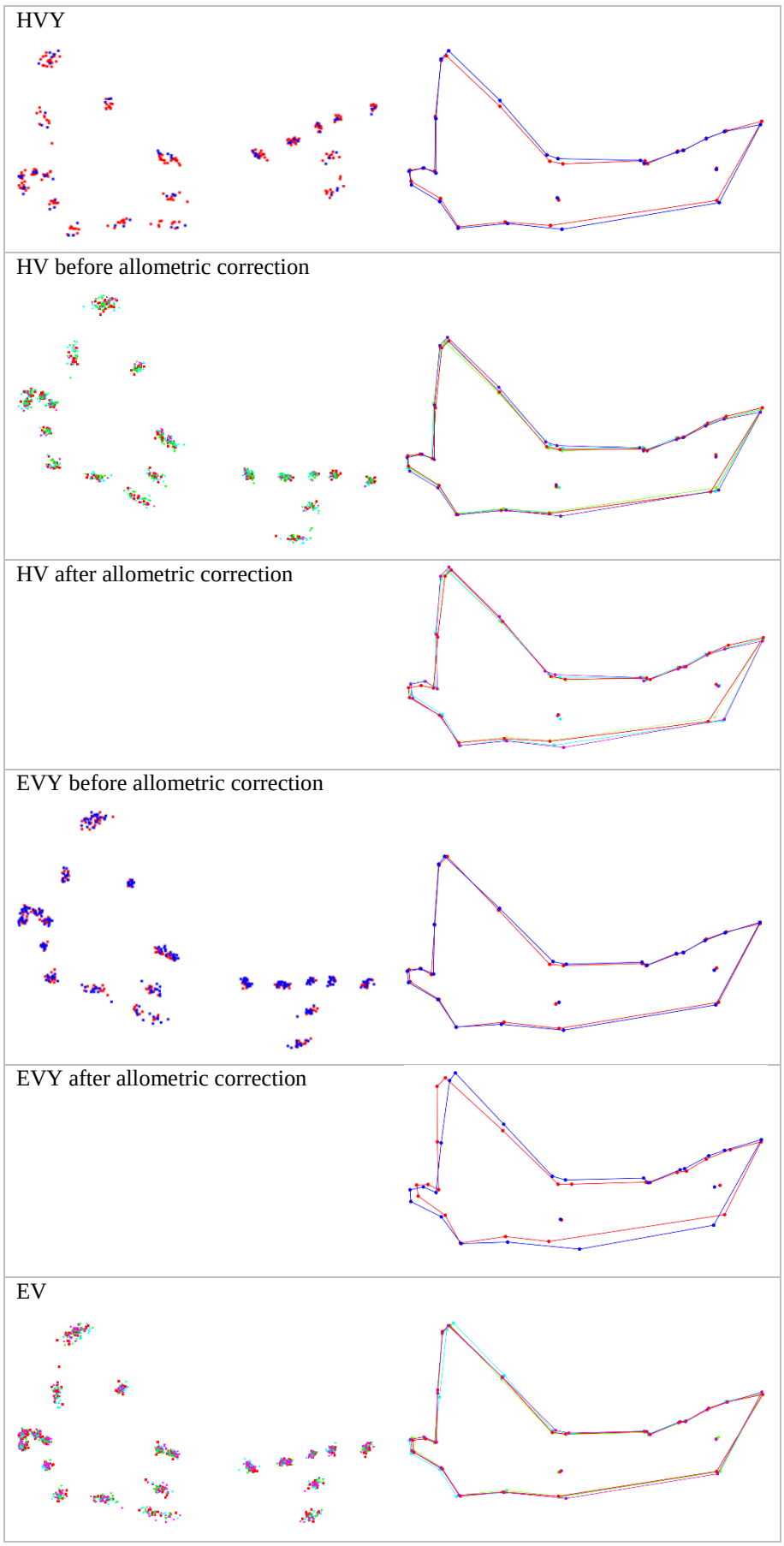


Figure 4.14. Composite figure of Procrustes superimpositions and Procrustes mean shapes of male and female stoats from Hollyford and Eglinton Valleys. HVY and EVY stoats are coded by sex, males: blue, females: red. HV and EV are split by sex and by birth year. Males N: pale blue, females N: red, males Y: purple, females y: green.

Table 30. Discriminant function analyses, distances between means, for males and females from HVY and EVY. The classification/ misclassification analyses were presented as the percentage of misclassified individuals calculated from the cross validation table. Significant values (p<0.05) are highlighted in bold.

	Distance between means		Hotelling's T-square, parametric p-value	Permutation tests (10000 runs)		Percentage of misclassified individuals from the classification/ misclassification tables
	Procrustes	Mahalanobis		Procrustes	Hotelling's T-square	
HVY	0.0333	2.7897	17.9593, 0.9751	0.1075	0.0667	38.4615 %
EVY	0.0190	3.2573	60.8906, 0.9863	0.1930	0.0978	39.1304 %
EVY after allometric correction	0.07547	10.1787	594.6033, 0.2933	**<0.0001**	0.5853	39.1304 %

As in the between-species analyses, the distances between three or more groups was reported as part of the CVA in the classification/ misclassification tables (Table 31). After the HV allometric correction, HVY males and females separated out with 92.31 % efficiency. Females had a thinner mandible body, a shorter arch where the masseter muscle connects to the base of the mandible, and the length of the base of the mandible was shorter. EVY females and EVN males separated out with 95.65 % accuracy, as the coronoid process of females was tilted back farther than that of the males.

Table 31. Classification/ misclassification analysis for HV and EV, individuals were separated by sex and birth year, percentage of misclassified individuals calculated from the cross validation table. The top (pale green) triangle: data before allometric correction, the bottom (white) triangle: data after allometric correction.

HV				
	MN	MY	FN	FY
MN		38.4615 %	29.1667 %	59.0909 %
MY	46.1538 %		46.6667 %	38.4615 %
FN	25.0000 %	30.7692 %		40.9091 %
FY	22.7273 %	7.6923 %	40.9091 %	
EV				
	MN	MY	FN	FY
MN		25.0000 %	32.0000 %	4.3478 %
MY			32.0000 %	39.1304 %
FN				37.5000 %
FY				

4.3.2.4 Canonical variates analysis

CVA maximises the differences between groups to better distinguish between them. Like the previous discriminant function analysis and PCA, this analysis was done both on the original data and on the data with the allometric correction, where required. The CVAs were performed on the valleys only when grouped by sex and birth year.

In the HV samples before allometric correction, the Mahalanobis distances showed HVY males to have the most distinct mandibles, and HVN males and HVY females the most similar, as the distance between HVN and HVY females was very small (Table 32). The Procrustes distances, for differences between the group means, were for the most part not significant. After allometric correction, the distance measures increased but the trends stayed the same, and the Procrustes distances became significant. See the CVA graphs (Figure 4.15).

Table 32. Results from the CVA. A) Eigenvalue analysis, eigenvalues that account for greater than five percent of variance was reported. B) The calculated Procrustes and Mahalanobis distances between the groups (bottom white triangles) and their associated p-values (top green values). Significant values (p<0.05) are highlighted in bold.

A)

		EV1	EV2
HV before allometric correction	Eigenvalue	106.8920	8.8416
	Variance (%)	92.3600	7.6400
	Cumulative %	92.3600	100.0000
HV after allometric correction	Eigenvalue	148.5010	20.6959
	Variance (%)	87.7680	12.2320
	Cumulative %	87.7680	100.0000
EV	Eigenvalue	129.9794	31.0491
	Variance (%)	76.8590	18.3600
	Cumulative %	76.8590	95.2190

B)

	Mahalanobis distances					Procrustes distances				
HV before allometric correction		MN	MY	FN	FY		MN	MY	FN	FY
	MN		**0.0018**	**<0.0001**	**<0.0001**	MN		0.3847	0.0973	0.8716
	MY	9.7615		**<0.0001**	**0.0020**	MY	0.0287		**0.0036**	0.1134
	FN	7.1649	13.0822		**<0.0001**	FN	0.0199	0.0355		0.0870
	FY	3.9710	10.2354	5.4404		FY	0.0137	0.0333	0.0200	
		MN	MY	FN	FY		MN	MY	FN	FY
	MN		**0.0003**	**<0.0001**	**<0.0001**	MN		0.3029	**<0.0001**	**0.0003**

HV after allometric correction	MY	8.9957		**0.0004**	**<0.0001**	MY	0.0274		**0.0013**	**0.0023**
	FN	12.7270	19.7986		**<0.0001**	FN	0.0378	0.0513		0.0078
	FY	9.7295	17.0652	5.5424		FY	0.0313	0.0471	0.0203	
EV		MN	MY	FN	FY		MN	MY	FN	FY
	MN		**<0.0001**	**<0.0001**	**<0.0001**	MN		**0.0478**	**0.0318**	**0.0399**
	MY	12.5883		**<0.0001**	**<0.0001**	MY	0.0223		0.0877	0.1852
	FN	10.6322	15.1148		**<0.0001**	FN	0.0234	0.0211		0.2825
	FY	29.3634	23.4337	25.7447		FY	0.0227	0.0190	0.0181	

The four groups were spread out across CV1, which accounted for 67.5 % prior to allometric correction and 88.89 % after allometric correction (Table 32). There was less variation along CV2, and no separation of the groups along CV3.

The wireframe deformations for the analysis before allometric correction showed very little difference between the groups even along CV1 (Figure 4.16). The most noticeable difference was that the mandible body of females was not as deep as that of males. Along CV2 the mandible body and the coronoid process became thinner. The wireframe deformations for the analysis after allometric correction were more exaggerated, which was expected from the results of the PCA. The deformations for CV3 were not presented because there was no separation along the axis. The CVA grouped the four classifications separately even though PCA did not, probably because the CVA exaggerated the differences between groups.

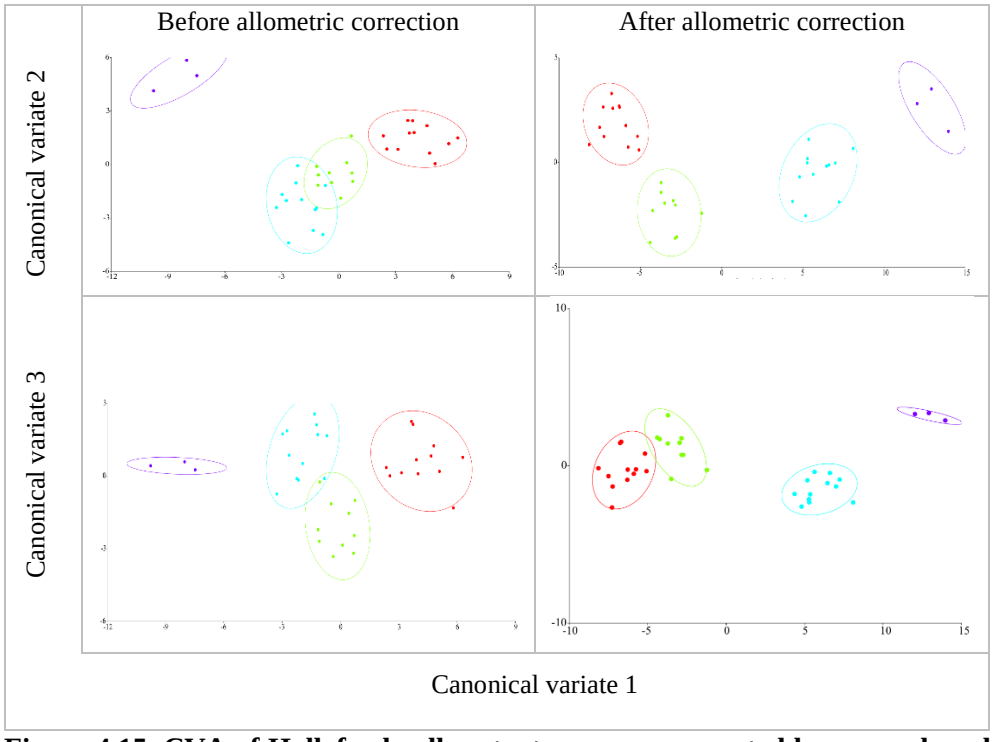


Figure 4.15. CVA of Hollyford valley stoats, groups separated by sex and seed year. Left column: before allometric correction, right column: after allometric correction. Males N: pale blue, females N: red, males Y: purple, females y: green. Associated statistics can be found in Table 32.

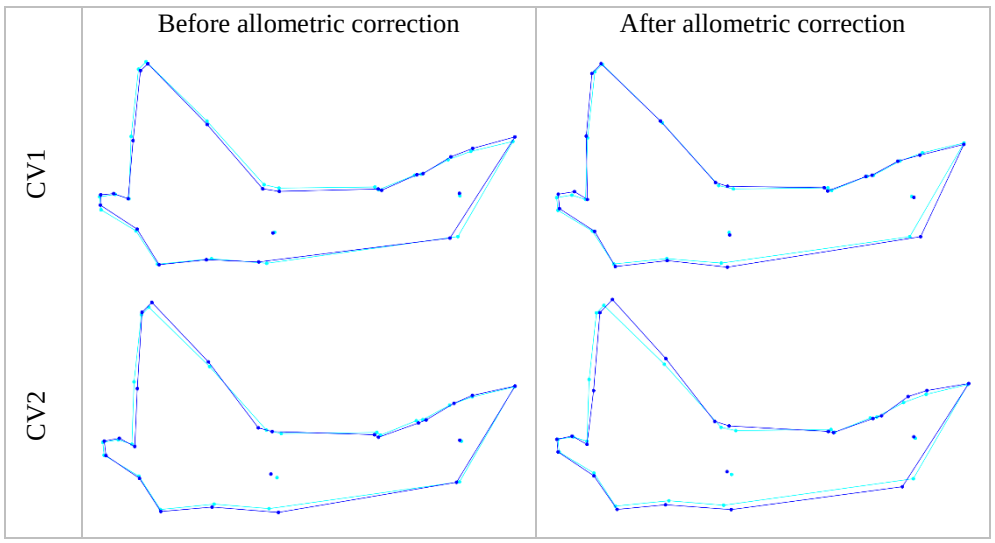


Figure 4.16. CVA wireframe deformation plots from the Hollyford valley CVA analyses. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

In the EV analysis the Mahalanobis distances were all significant and the Procrustes distances of the EVN males were significant against the other three groups (Table 32). According to the Mahalanobis distances, EVY females were the most distinct, and the largest distance was between EVY females and EVN males, whereas for the Procrustes distances it was between EVN females and males.

CV1 accounted for 76.86 % of the variation the EVY females, the most separate of the four groups (Figure 4.17), and the other three groups overlapped to some extent (Table 32). CV2 accounted for 18.36 % of the variance, and there was some separation along this axis, mostly between EVN females and EVY males, which were the most similar along CV1. There was almost no separation of the groups along CV3.

The wireframe deformations indicate that there is almost no perceptible difference between the groups (Figure 4.18), although the ramus was thicker at the EVY female end of the axis of CV1, and along CV2 the mandible body shortened and thickened.

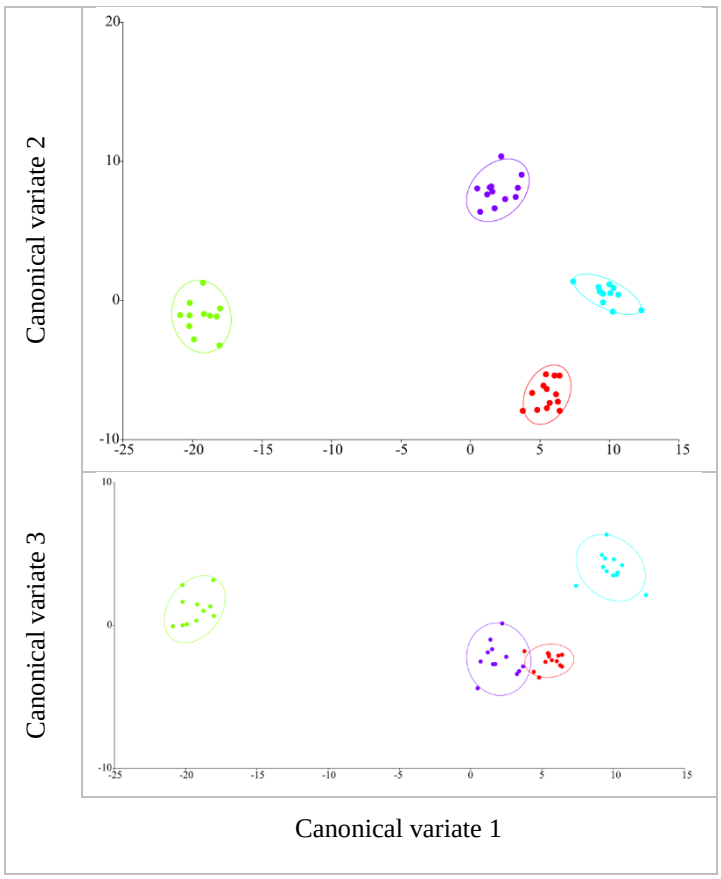


Figure 4.17. CVA of Eglinton valley stoats, groups separated by sex and seed year. Males N: pale blue, females N: red, males Y: purple, females y: green. Associated statistics can be found in Table 32.

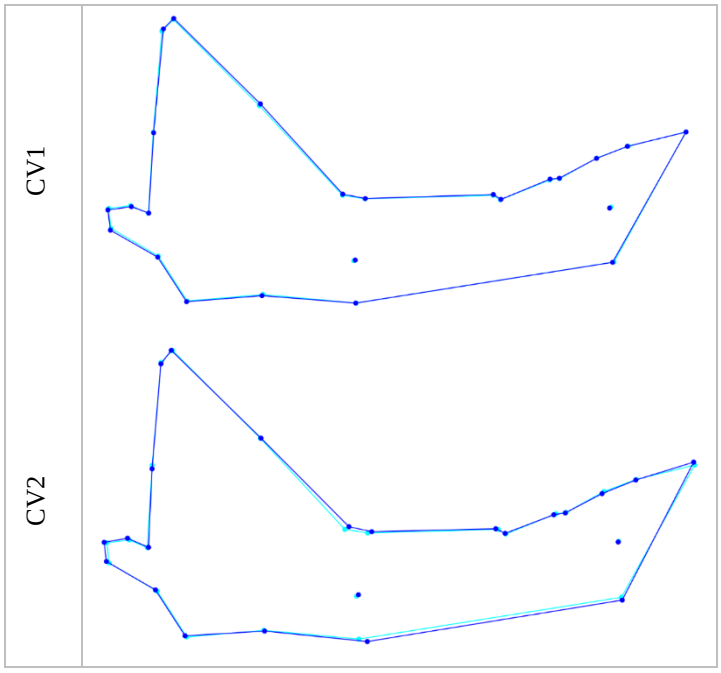


Figure 4.18. CVA wireframe deformation plots from the Eglinton valley CVA analyses. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

4.3.2.5 Biomechanical advantage

The results of the biomechanical advantage Kruskal-Wallis ANOVAs showed that for most of the comparisons there was no difference between groups (Table 34). The HVY males and females are tentatively different in T/C efficiency. The EVY males when compared with EVN males had lower efficiency of the T/C and T/M1.

Table 33. The mean biomechanical advantage of mandibles from Eglinton (EV) and Hollyford (HV) valleys, stoats were separated into those not born during a seed masting year (N) and those that were (Y) (T: temporalis, M: masseter, C: canine, M1: carnassial).

	EVN		EVY		HVN	HVY
	Female	Male	Female	Male	Female	Female
T/C	0.3584	0.3725	0.3561	0.3561	0.3600	0.3553
T/M1	0.6364	0.6650	0.6435	0.6338	0.6552	0.6442
M/C	0.3130	0.3248	0.3233	0.3197	0.3231	0.3249
M/M1	0.5553	0.5800	0.5844	0.5687	0.5881	0.5889

Table 34. Within sex across birth year (non-seed year and seed year) Kruskal-Wallis ANOVAs of biomechanical advantage of the mandible, significant values ($p<0.05$) are highlighted in bold (T: temporalis, M: masseter, C: canine, M1: carnassial).

		T/C	T/M1	M/C	M/M1
HVY males v. females	H-value (1, N=13)	6.4286	0.7143	1.0286	0.7143
	p-value	**0.0112**	0.3980	0.3105	0.3980
HVF seed v. non-seed	H-value (1, N=22)	0.4348	0.5261	0.2130	0.0391
	p-value	0.5097	0.4683	0.6444	0.8432
EVY males v. females	H-value (1, N=23)	0.0038	0.0947	0.2424	0.3068
	p-value	0.9509	0.7583	0.6225	0.5796
EVF seed v. non-seed	H-value (1, N=23)	0.2424	0.0947	1.3674	2.9697
	p-value	0.6225	0.7583	0.2423	0.0848
EVM seed v. non-seed	H-value (1, N=24)	3.8533	5.0700	0.6533	0.9633
	p-value	**0.0496**	**0.0243**	0.4189	0.3263

4.3.3 Section summary

Regardless of the birth year food supplies, female stoats from EV and HV did not show any change in size, shape, or biomechanical advantage of the mandibles. The logical conclusion that beech seed masting events have no impact on the bone morphology or developmental plasticity of females in that area of Fiordland National Park.

By contrast, birth year food supplies do have a statistical effect on EV males. EVY males were significantly larger than EVN males as expected from the results of Powell et al. (1997), and demonstrated a significant decrease in the biomechanical advantage of the T/C and T/M1. These results were consistent with the predictions of sexual dimorphism theory explored by Powell & King 1997; males would be affected by food availability during the growth phase but females would not be. However, this was not consistent with the results from Powell et al. (1997), which indicated females increased significantly in size and therefore sexual dimorphism did not change significantly between seed year and non-seed year born stoats (Powell et al. 1997).

While the CVA easily differentiated between males and females and their birth year, the deformations for those CVAs and the PCA did not show any differences within the sexes and between the birth years. There may be a small difference in mandible shape between the four groups, most likely a shortening of the arch where the masseter muscle connects to the base of the mandible body, and a shortening of the coronoid process in females compared with males. This would indicate that females have an advantage in capturing prey due to increased relative strength, but this difference is unlikely to be statistically or biologically significant.

4.4 What external factors affect the size or shape of the mandibles of Mustela erminea?

The following section covers the results of investigating the impact of various habitats and the genetics of the mandibles of stoats from 10 different locations from New Zealand and one location from Warwickshire, England. Size analyses was conducted first, followed by shape and biomechanical advantage. Because there was no difference in mandible shape between stoats born during beech seed years and those who were not, the shape analyses for these were combined, although they were kept separate for analyses of size and biomechanical advantage.

4.4.1 Size

The ANOVA results showed that there was limited variation in female stoat mandible size across locations, and EN females were not significantly different in size compared to New Zealand females (Table 19, Table 35, and Table 36). HVY females and RI females were typically smaller than females from the other locations. RI females were not only the smallest but also had the most differences with the other locations. Females from Arthurs Pass NP (AP) were significantly larger than those from Westland NP (WL).

By contrast, male stoats had a larger range of mandible sizes, and therefore there were more locations that were significantly different from each other. EN males were also larger than males from most other locations except EVY males, which were the largest of the New Zealand males. AP males were also quite large, though not to the same extent as the EVY and EN stoats; the smallest male stoats were those from RI and WL.

Table 35. Centroid size ANOVAs comparing female stoats from locations across New Zealand and one location from Warwickshire, England. P-values are in the top triangle (green) and f-values are in the bottom triangle (white). Significant values (p<0.05) are highlighted in bold.

	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.9203	0.1178	0.8933	0.6636	0.2771	0.8828	0.6062	0.2836	0.1703	0.1595	**0.0119**	0.9435
PU	0.0103		0.1191	0.7896	0.7180	0.2116	0.7972	0.6627	0.3008	0.1744	0.1662	0.0815	0.8731
AP	2.6717	2.6482		**0.0360**	0.2974	**0.0068**	0.0861	0.2388	0.7733	0.7036	0.6809	**0.0021**	0.1461
WL	0.0183	0.0726	**4.8546**		0.4914	0.2153	0.9582	0.4238	0.1352	0.0644	0.0541	0.0571	0.9769
MC	0.1949	0.1342	1.1392	0.4861		0.1351	0.5648	0.9873	0.5154	0.4273	0.2681	**0.0483**	0.6375
HVN	1.2483	1.6654	**8.9089**	1.6077	2.4062		0.3484	0.0868	**0.0366**	**0.0094**	**0.0245**	0.5189	0.3700
HVY	0.0224	0.0680	3.2597	0.0028	0.3427	0.9221		0.4984	0.2283	0.1237	0.1314	0.1464	0.9532
EVN	0.2740	0.1957	1.4624	0.6582	0.0003	3.1997	0.4746		0.4728	0.3656	**<0.0001**	**0.0267**	0.5900
EVY	1.2175	1.1313	0.0852	2.3716	0.4378	**4.9855**	1.5498	0.5337		0.9963	0.5726	**0.0127**	0.2931
GV	2.0233	1.9831	0.1486	3.7076	0.6540	**8.0899**	2.5824	0.8517	<0.0001		0.5038	**0.0029**	0.2109
SI	2.1532	2.0826	0.1742	4.0695	1.2977	**5.9134**	4.4657	**45.3909**	0.3297	0.4636		**0.0094**	0.1582
RI	**2.9572**	3.3494	**11.9590**	3.9249	**4.3494**	0.4291	2.2749	**5.5719**	**7.3626**	**11.0638**	**8.1636**		0.1612
EN	0.0051	0.0262	2.2708	0.0009	0.2283	0.8376	0.0035	0.2987	1.1629	1.6607	2.1493	2.0957	

Table 36. Centroid size ANOVAs comparing male stoats from locations across New Zealand and one location from Warwickshire, England. P-values are in the top triangle (green) and f-values are in the bottom triangle (white). Significant values (p<0.05) are highlighted in bold.

	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.8087	0.0752	0.2621	0.6607	0.5812	0.2990	0.2667	**0.0021**	0.0772	0.5656	0.1105	**0.0219**
PU	0.0602		0.0878	0.1205	0.8150	0.4247	0.1355	0.3402	**0.0016**	0.0884	0.6836	0.0589	**0.0317**
AP	3.4883	3.2213		**0.0005**	0.2381	**0.0258**	**0.0112**	0.4212	0.0753	0.9532	0.1942	**0.0021**	0.2796
WL	1.3100	2.5776	**15.5777**		0.1105	0.7420	0.4494	**0.0073**	**<0.0001**	**0.0005**	**0.0478**	0.2027	**0.0002**
MC	0.1980	0.0562	1.4706	2.7159		0.3511	0.2384	0.5832	**0.0144**	0.2480	0.9288	0.0694	0.0665
HVN	0.3136	0.6641	**5.7176**	0.1106	0.9075		0.5421	0.1039	**0.0007**	**0.0261**	0.2855	0.2794	**0.0087**
HVY	1.1704	2.5950	**8.7192**	0.5966	1.5274	0.3920		**0.0405**	**0.0005**	**0.0089**	0.0549	0.8278	**0.0315**
EVN	1.2993	0.9548	0.6718	**8.3742**	0.3102	2.8777	**5.1731**		**0.0138**	0.4420	0.5934	**0.0095**	0.1038
EVY	**12.1347**	**13.4023**	3.4845	**39.8682**	**7.0622**	**15.3917**	**21.2323**	**7.1638**		0.0597	**0.0047**	**<0.0001**	0.8538
GV	3.4365	3.2092	0.0035	**15.7945**	1.4084	**5.6882**	**9.4519**	0.6131	3.9436		0.1962	**0.0019**	0.2557
SI	0.3426	0.1723	1.8184	**4.3512**	0.0082	1.2117	4.8617	0.2954	**10.4045**	1.8018		**0.0383**	0.0773
RI	2.8172	4.1368	**12.8602**	1.7156	3.7291	1.2437	0.0502	**8.4310**	**26.1061**	**13.1308**	5.2301		**0.0022**
EN	**6.0846**	**5.3324**	1.2291	**18.2253**	3.7273	**8.3028**	**5.8107**	2.8792	0.0347	1.3617	3.5114	**12.7248**	

4.4.2 Shape

4.4.2.1 Regression and allometry

The pooled within-locations regression analysis was significant (Figure 4.19 and Table 37). The regression detected a small allometric component of mandible shape, as 3.16 % of shape was accounted for by size ($p<0.0001$). Therefore, all further analyses were conducted on the data before and after allometric correction, although such a small percentage was unlikely to create a large difference between the two sets of results. The two groupings along the log centroid size axis of the regression graph is probably due to the size difference between males and females.

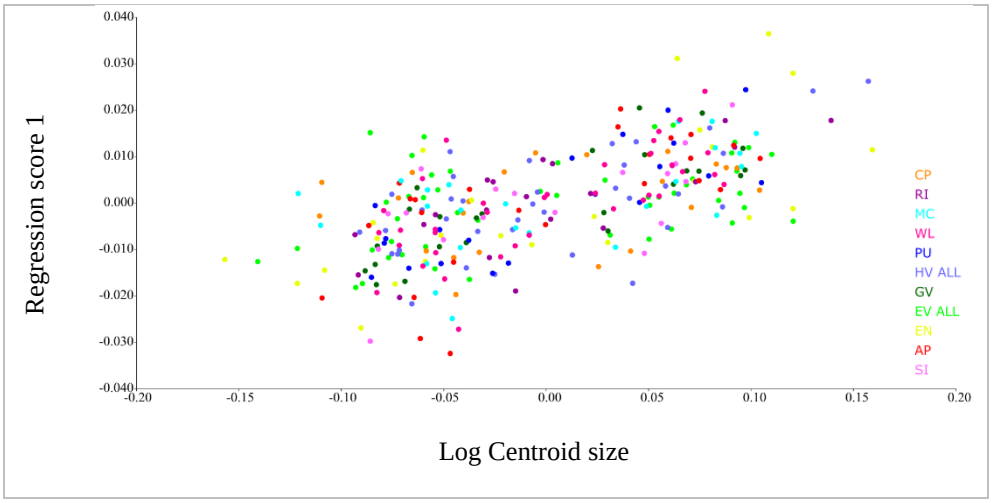


Figure 4.19. Pooled-within group regression analysis of shape and log centroid size, as a test for allometry on all sites. The associated statistics for this regression can be found in Table 37.

Table 37. Pooled within-group (site) regression analyses of mandible shape and centroid size as a test for allometry, The permutation test p-value is against the null-hypothesis, significant values ($p<0.05$) are highlighted in bold.

Total SS	Predicted SS	Residual SS	d.f.	Predicted (%)	Permutation test p-value (10000 runs)
0.4619	0.0146	0.4473	10, 288	3.1600	**<0.0001**

4.4.2.2 Principal components analyses

PC1 (χ^2=0.2392), accounted for 15.28 % of the variance, and PC2 accounted for 14.44 % of the variance (Figure 4.20). Most of the locations along both PCAs overlapped with each other, although the spread of points for each group varied. RI had one of the smallest variances, and very little overlap with SI or WL.

The first seven eigenvalues in the PCA before allometric correction, and the first three in the PCA after allometric correction, were likely to be biologically significant, but only three were above the inflection point, shown in Figure 4.21 (Table 38). None of the PCs were significant in chi-squared statistical tests in the PCA before correction.

However, after allometric correction the variance of the groups seemed to overlap further, and there was less distinction between the groups. The percentage variance scree graph also did not change significantly, most likely because the level of allometry was small.

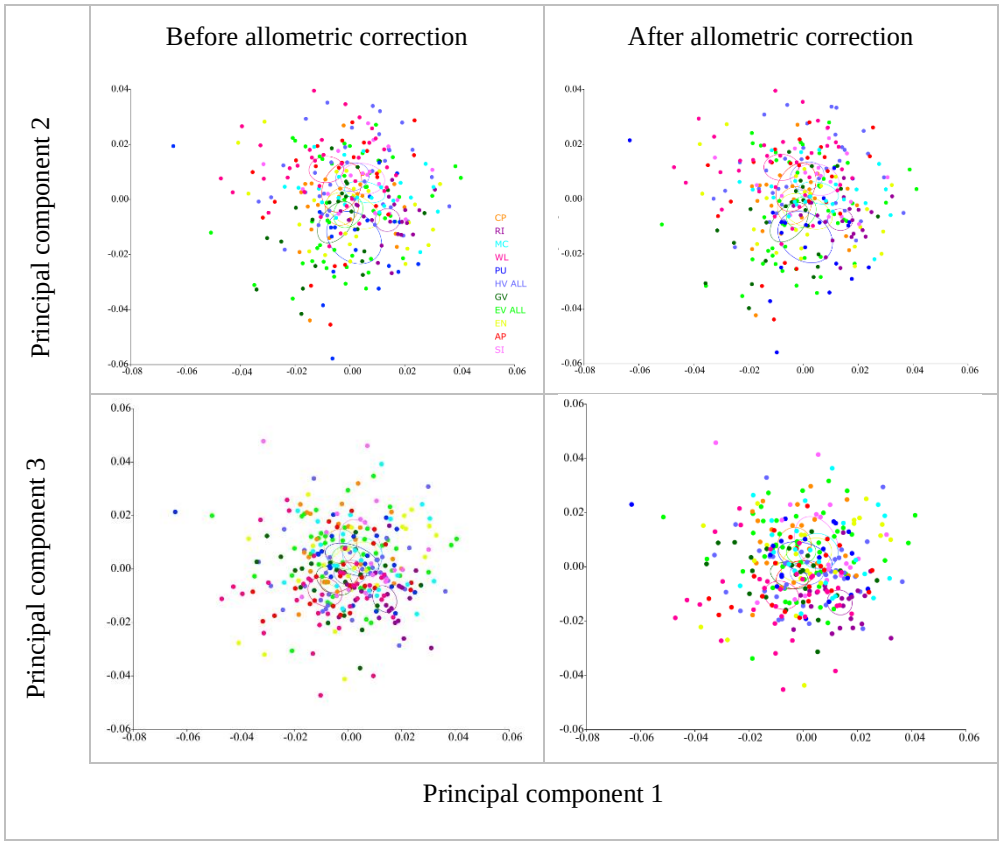


Figure 4.20. PCAs of all stoats grouped by location, before (left column) and after (right column) allometric correction. Circles indicate the mean shape confidence ellipses.

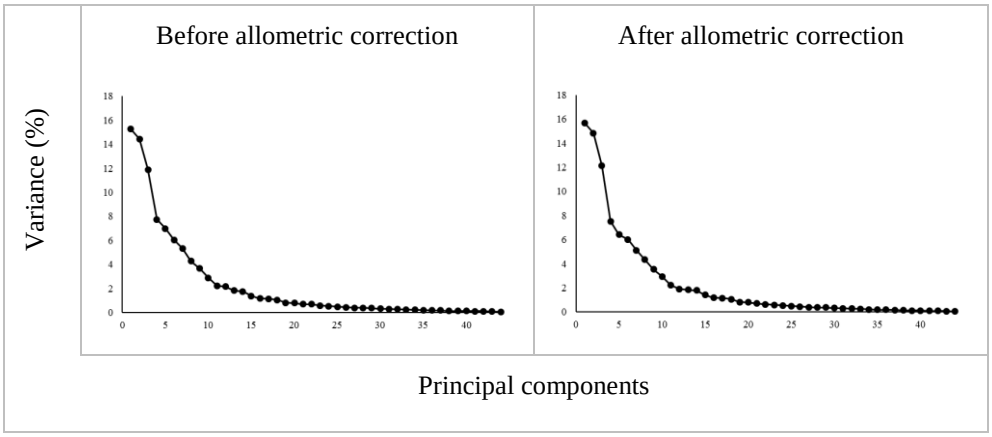


Figure 4.21. Eigenvalue and percentage of variance scree plot from the PCAs of all stoats grouped by location, before (left column) and after (right column) allometric correction.

Stoats on Secretary and Resolution Islands probably share the same haplotype, based on data from the rest of Fiordland National Park (A. Veale, personal communication, November 17, 2016), although they can be distinguished by other genetic markers (P. McMurtrie, unpublished data, 2016). The two Islands are similar in almost all respects except the food resources for stoats, since Resolution Island has mice while Secretary Island has not. There are therefore significant differences in diet between the two stoat populations, described by Murphy et al. (2016). This difference created a specific interest in how diet affected mandible shape, so a PCA was conducted on mandibles from the two islands. A. Veale was of the opinion that any morphological differences would be more likely due to the environment, than to genetics or founder effects (A. Veale, personal communication, November 17, 2016).

The PCA plot (Figure 4.22A) shows a separation along PC1 which accounts for 26.12 % of the variance (χ^2=4.7061). It is the only PC above the inflection point (Figure 4.22B), and while not quite significant according to the chi-squared test, it confirms other indicators suggesting a difference. There were a total of six PCs that accounted for more than five percent of variance but were not separated from the other PCs (Table 38).

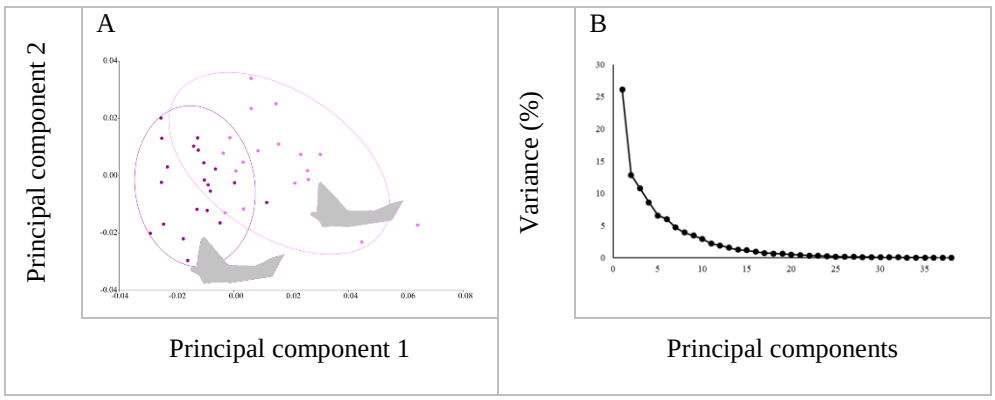


Figure 4.22. A) PCA of Secretary and Resolution Islands stoats. Circles indicate the 90 % confidence ellipses, SI: pale pink, RI: purple. B) Eigenvalue and percentage of variance scree plot from the PCA.

The wireframe deformation plot was again an exaggeration of the true difference between groups, it was exaggerated because the pale blue shape is the average shape from -0.1 on the axis and the royal blue shape is the average shape from 0.1 on the axis. but there seemed to be differences across all aspects of the mandible shape (Figure 4.23). Along the axis towards the SI stoats the mandible body was thinner and the coronoid process tilted back. The distance between the condyle and the angular process was also smaller. This indicates that a mammal free diet results in reduced muscle attachment, and the mandible body does not need to be as thick to support the force of the teeth, bird bones and insect exoskeletons are not as hard as solid mammalian bones. There was a greater variation in SI mandible shape that crossed over with the RI stoats.

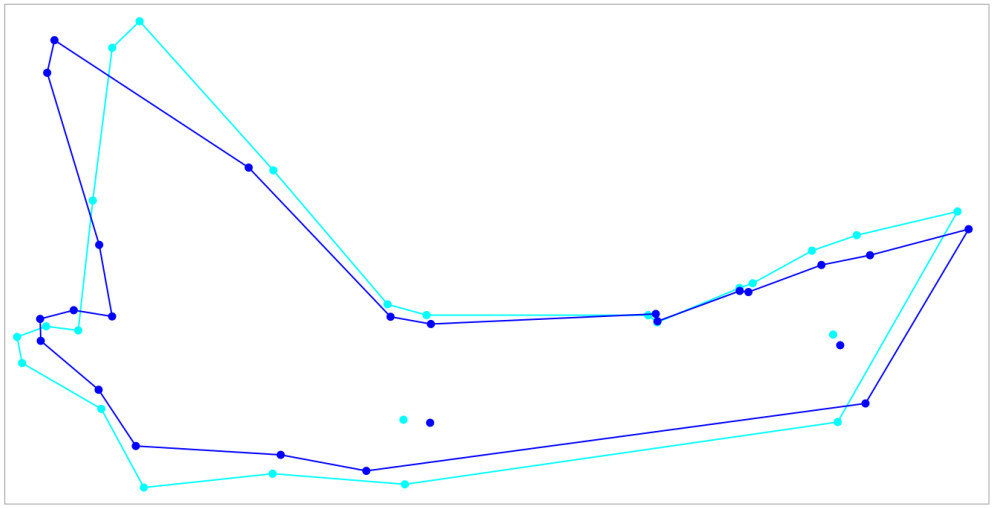


Figure 4.23. Procrustes deformation wireframe plot of the Secretary and Resolution Islands stoat mandibles based on the deformation implied by PC1. Starting shape (negative end of axis) closer to RI stoats: pale blue, end shape (positive end of axis) closer to SI stoats: royal blue.

Table 38. Eigenvalues relating to the PCAs, eigenvalues that account for five percent or more of the variance were reported, eigenvalues that correspond to the PC's above the inflection point on the scree graph were italicised, significant eigenvalues ($\chi 2<5.99$) are highlighted in bold.

		EV1	EV2	EV3	EV4	EV5	EV6	EV7
Before allometric correction	Eigenvalue	*0.0003*	*0.0003*	*0.0002*	0.0001	0.0001	0.0001	0.0001
	Variance (%)	*15.2770*	*14.4370*	*11.8830*	7.7530	6.9820	6.0560	5.3490
	Cumulative %	*15.2770*	*29.7140*	*41.5960*	49.3490	56.3310	62.3870	67.7360
After allometric correction	Eigenvalue	*0.0003*	*0.0003*	*0.0002*	0.0001	0.0001	0.0001	0.0001
	Variance (%)	*15.6810*	*14.8440*	*12.1220*	7.5330	6.4250	6.0210	5.1280
	Cumulative %	*15.6810*	*30.5250*	*42.6470*	50.1800	56.6050	62.6270	67.7540
SI v. RI	Eigenvalue	*0.0004*	0.0002	0.0002	0.0001	0.0001	0.0001	
	Variance (%)	*26.1180*	12.8360	10.8140	8.6050	6.5730	5.9960	
	Cumulative %	*26.1180*	38.9540	49.7670	58.3730	64.9450	70.9410	

4.4.2.3 Discriminant function analyses

Procrustes- based superimpositions show the differences in shape between stoat mandibles from eleven different locations (Figure 4.24). The Procrustes superimposed mean shapes for each location, obtained from the discriminant function analyses, have been presented separately, not overlaid, as that made the image too hard to read. The discriminant function analysis of the data before allometric correction, and the data after allometric correction, indicated that there were some statistical differences between some of the groups.

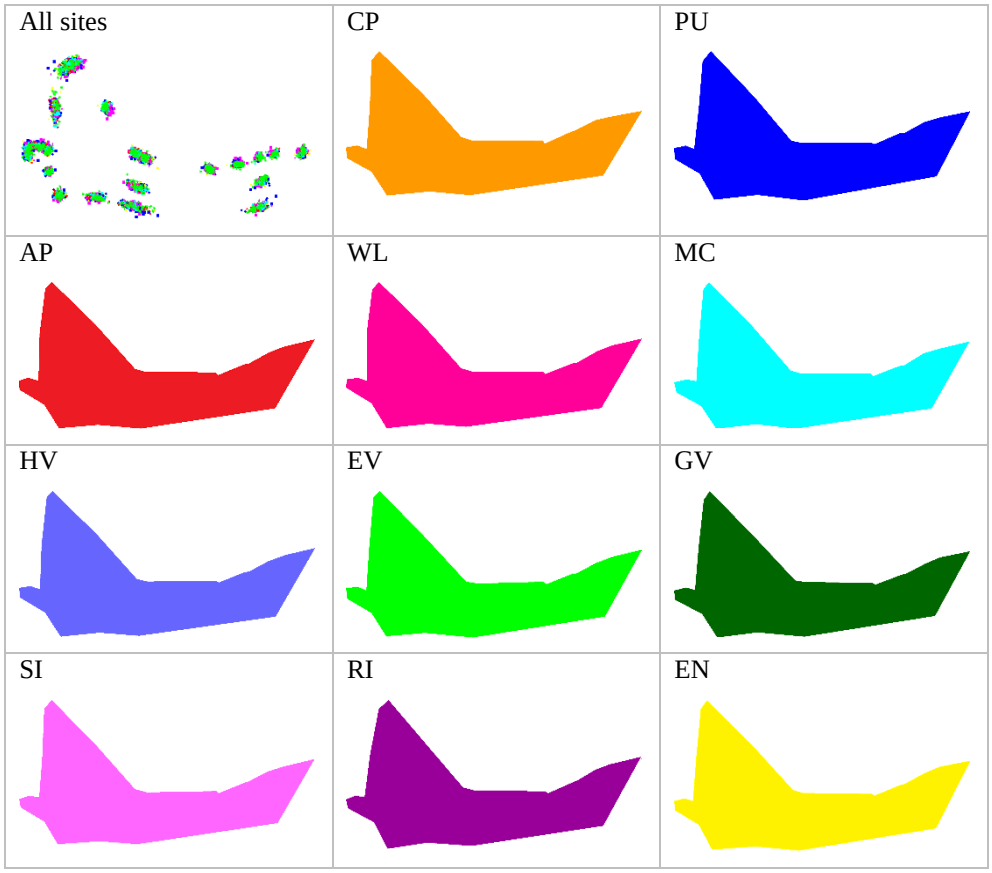


Figure 4.24. Composite image of the all locations Procrustes superimpositions and the Procrustes mean shapes of each location. The mean shape colours match the colours used in the other analyses of the locations.

Before allometric correction the classification/ misclassification tables separated out only one pair effectively: PU and EN (Figure 4.25 and Table 39). The condyle of the EN stoats was smaller than that of the PU stoats, the coronoid was slightly larger and the angular process was slightly shorter. The SI versus RI discriminant function was also presented, due to the theoretical interest in the difference between the two. The classification/ misclassification table was able to discriminate with only 92.31 % accuracy, which was still better than the majority of the other

discriminations. The differences between the island mandible shapes produced a less exaggerated version of the PC1 wireframe: SI had a thinner mandible, and smaller features overall, with a tilted back condyle (Figure 4.25). There were greater differences between the SI and RI means than between the PU and EN means.

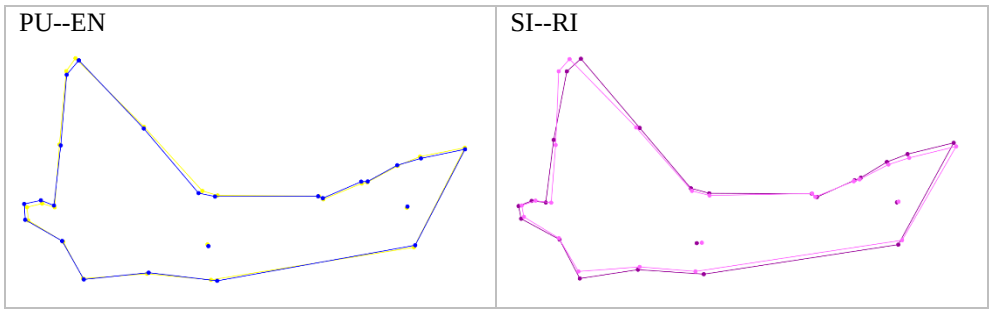


Figure 4.25. Procrustes mean shapes overlay from the discriminant function analysis before allometric correction. Pureora Forest Park (dark blue) and England (yellow). Secretary Island (pale pink) and Resolution Island (purple).

After allometric correction, the discriminant function analyses separated out three pairs: Coromandel Peninsula (CP) and MC, GV and EN, WL and RI (Figure 4.26). All of the differences were in the ramus end of the mandible, except for CP and MC where there was some difference in the canine area, as well as the difference in the tilt of the top portion of the coronoid process. When compared with GV the EN stoats had a thicker mandible and a shorter condyle. The coronoid process of WL stoats was tilted further back than RI, and there was a deeper notch between the coronoid process and the condyle. The differences between SI and RI after the allometric correction were the same as those before the correction. Some were smaller in magnitude and some had no change, therefore the classification/ misclassification tables did not significantly change and still misclassified 7.69 % of individuals.

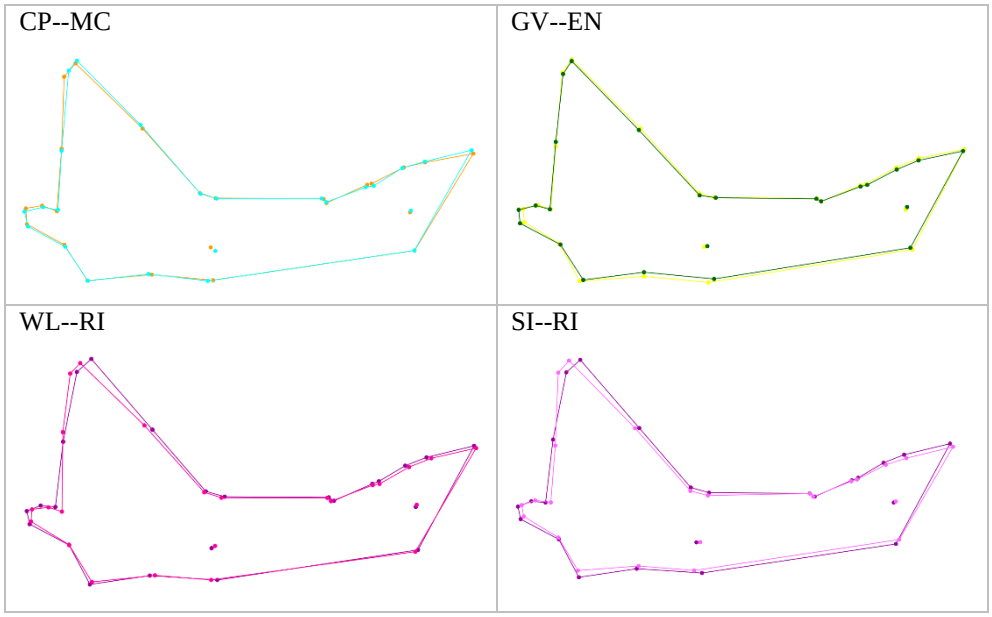


Figure 4.26. Procrustes mean shapes overlays from the discriminant function analysis after allometric correction. Coromandel Peninsula (orange) and Mount Cook National Park (light blue). Grebe valley (dark green) and England (yellow). Westland National Park (dark pink) and Resolution Island (purple).

Table 39. Discriminant function analysis from between species analyses, classification/ misclassification analysis percentage of misclassified individuals calculated from the cross validation table. The top (pale green) triangle was for data prior to allometric correction, the bottom (white) triangle was for data after allometric correction

	CP	PU	AP	WL	MC	HV	EV	GV	SI	RI	EN
CP		45.2381	52.1739	27.5862	30.4348	28.8136	21.4286	39.1304	25.0000	13.9535	69.5652
PU	35.7143		36.3636	28.5714	34.0909	21.0526	23.5294	65.9091	28.9474	17.0732	**2.2727**
AP	43.4783	27.2727		33.3333	47.9167	32.7869	12.5000	22.9167	45.2381	35.5556	52.0833
WL	31.0345	26.7857	31.6667		18.3333	17.8082	11.9048	21.6667	33.3333	5.2632	44.0000
MC	**4.3478**	36.3636	39.5833	21.6667		29.5082	25.0000	39.5833	45.2381	26.6667	37.5000
HV	33.8983	21.0526	29.5082	21.9178	27.8689		27.0588	29.5082	27.2727	15.5172	29.5082
EV	20.0000	22.0588	11.1111	11.9048	31.9444	29.4118		13.8889	15.1515	5.7971	28.5714
GV	43.4783	47.7273	29.1667	23.3333	45.8333	32.7869	13.8889		30.9524	46.6667	20.8333
SI	25.0000	18.4211	26.1905	22.2222	47.6190	32.7273	19.6970	28.5714		7.6920	42.8571
RI	11.6279	19.5122	53.3333	**1.7543**	24.4444	17.2414	8.6957	37.7778	7.6923		26.6667
EN	50.0000	13.0435	47.9267	36.6667	37.5000	27.8689	13.8889	**4.1667**	42.8571	31.1111	

4.4.2.4 Canonical variates analysis

The CVA on the data before allometric correction all had significant ($p<0.05$) Mahalanobis distances, RI stoats had the most distinct mandibles, and HV and AP had the most similar mandibles (Table 40). The Procrustes distances were for the most part significant, and followed the same trends as the Mahalanobis distances, indicating that while the variance among the groups was large the means were different. Unlike the discriminant function, which showed differences between PU and EN in the classification/ misclassification tables, the Mahalanobis distances were not significant. After the allometric correction, the distance measures increased but the trends stayed the same, including the lack of significance (Table 41).

The graphs from before and after allometric correction were similar; both had six CVs that accounted for more than five percent of the variance. The locations were grouped into two sections along CV1, which accounted for 30.41 % of variation before correction and 29.87 % after correction (Figure 4.27, Table 40, and Table 41). The wireframe deformation along CV1, from the cluster on the negative side of the axis to the cluster on the positive side of the axis, indicated that the coronoid process was tilted back and the angle between the coronoid process and the condyle smaller (Figure 4.28).

Along CV2, each location slightly overlapped with the one next to it, and fully overlapped with the one in the other cluster on CV1 (Figure 4.27). CV2 accounted for 19.06 % of variance before allometric correction and 18.48 % after allometric correction (Table 40, and Table 41). Along CV2 the coronoid process shortened and fattened, the angular process got bigger, and the condyle smaller (Figure 4.28). There were no clear clusters along the other CVs and so they were not presented.

Table 40. Results from the CVA before allometric correction. A) Eigenvalue analysis, eigenvalues that account for greater than five percent of variance was reported. B) The calculated Mahalanobis distances between the groups (top green values) and their associated p-values (bottom white triangles). C) The calculated Mahalanobis distances between the groups (top green values) and their associated p-values (bottom white triangles). Significant values (p<0.05) are highlighted in bold.

A)

	EV1	EV2	EV3	EV4	EV5	EV6					
Eigenvalues	2.5467	1.5964	1.0698	0.8062	0.6015	0.4898					
% Variance	30.4120	19.0640	12.7750	9.6270	7.1830	5.8490					
Cumulative %	30.4120	49.4770	62.2520	71.8790	79.0610	84.9100					

B)

	CP	PU	AP	WL	MC	HV	EV	GV	SI	RI	EN
CP		3.7022	4.2626	4.5562	4.2783	4.0826	4.1685	4.0093	3.9242	5.4001	3.9256
PU	**<0.0001**		4.6592	5.0187	4.7401	4.2956	3.9255	3.5908	4.5516	4.9659	3.4729
AP	**<0.0001**	**<0.0001**		2.9618	3.6056	2.5621	3.3057	4.2950	4.2978	5.4006	4.3598
WL	**<0.0001**	**<0.0001**	**<0.0001**		4.0493	3.1975	4.3820	4.7651	4.7706	5.9051	4.7910
MC	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		2.9707	3.1706	4.0644	4.2383	5.8669	5.0324
HV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		2.6502	3.9773	4.2233	5.6430	4.2801
EV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		3.6822	4.3618	5.8239	4.1426
GV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.0874	4.7642	3.8302
SI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.5073	4.3270
RI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.9926
EN	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

C)

	CP	PU	AP	WL	MC	HV	EV	GV	SI	RI	EN
CP		0.0201	0.0210	0.0271	0.0198	0.0224	0.0232	0.0226	0.0205	0.0320	0.0193

PU	**0.0066**		0.0261	0.0344	0.0270	0.0279	0.0220	0.0195	0.0267	0.0267	0.0195
AP	**0.0006**	**0.0002**		0.0163	0.0186	0.0110	0.0197	0.0208	0.0219	0.0280	0.0191
WL	**<0.0001**	**<0.0001**	**0.0074**		0.0289	0.0200	0.0318	0.0291	0.0296	0.0341	0.0285
MC	**<0.0001**	**<0.0001**	**0.0021**	**<0.0001**		0.0165	0.0213	0.0254	0.0195	0.0319	0.0209
HV	**<0.0001**	**<0.0001**	0.3713	**<0.0001**	**0.0026**		0.0184	0.0254	0.0193	0.0302	0.0198
EV	**<0.0001**	**0.0001**	**0.0005**	**<0.0001**	**<0.0001**	**0.0001**		0.0172	0.0215	0.0319	0.0179
GV	**<0.0001**	**0.0042**	**0.0014**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0022**		0.0255	0.0277	0.0187
SI	**0.0015**	**0.0001**	**0.0006**	**<0.0001**	**0.0016**	**0.0006**	**0.0004**	**<0.0001**		0.0314	0.0206
RI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		0.0263
EN	**0.0067**	0.0816	**0.0159**	**<0.0001**	**0.0011**	**0.0002**	**0.0028**	**0.0071**	**0.0113**	**<0.0001**	

Table 41. Results from the CVA after allometric correction. A) Eigenvalue analysis, eigenvalues that account for greater than five percent of variance was reported. B) The calculated Mahalanobis distances between the groups (top green values) and their associated p-values (bottom white triangles). C) The calculated Mahalanobis distances between the groups (top green values) and their associated p-values (bottom white triangles). Significant values (p<0.05) are highlighted in bold.

A)											
	EV1	EV2	EV3	EV4	EV5	EV6					
Eigenvalues	2.5480	1.5762	1.1120	0.8562	0.6081	0.5100					
% Variance	29.8680	18.4760	13.0350	10.0370	7.1280	5.9780					
Cumulative %	29.8680	48.3440	61.3790	71.4160	78.5440	84.5220					
B)											
	CP	PU	AP	WL	MC	HV	EV	GV	SI	RI	EN
CP		3.7067	4.2556	4.5786	4.3514	4.3188	4.2303	4.1658	3.9468	5.5294	4.0623
PU	**<0.0001**		4.6518	5.0257	4.7787	4.4672	3.9578	3.7098	4.5565	5.0647	3.5738

AP	**<0.0001**	**<0.0001**		2.9603	3.6716	2.8480	3.3773	4.4307	4.3105	5.4794	4.4744
WL	**<0.0001**	**<0.0001**	**<0.0001**		4.0558	3.3358	4.3708	4.7943	4.7632	5.9501	4.8085
MC	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		3.0180	3.1639	4.0751	4.2532	5.8559	5.0355
HV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		2.6734	3.9437	4.3211	5.6378	4.2541
EV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		3.7034	4.3716	5.7888	4.1545
GV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.1484	4.6893	3.8311
SI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.5415	4.3716
RI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		4.9232
EN	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

C)

	CP	PU	AP	WL	MC	HV	EV	GV	SI	RI	EN
CP		0.0201	0.0206	0.0273	0.0198	0.0233	0.0229	0.0219	0.0205	0.0327	0.0187
PU	**0.0051**		0.0258	0.0345	0.0318	0.0286	0.0215	0.0186	0.0266	0.0274	0.0164
AP	**0.0003**	**<0.0001**		0.0161	0.0270	0.0124	0.0197	0.0209	0.0218	0.0282	0.0191
WL	**<0.0001**	**<0.0001**	**0.0074**		0.0287	0.0203	0.0316	0.0285	0.0296	0.0343	0.0281
MC	**0.0001**	**<0.0001**	**0.0011**	**<0.0001**		0.0168	0.0213	0.0253	0.0196	0.0318	0.0209
HV	**<0.0001**	**<0.0001**	0.1487	**<0.0001**	**0.0015**		0.0190	0.0227	0.0201	0.0301	0.0201
EV	**<0.0001**	**0.0002**	**0.0005**	**<0.0001**	**<0.0001**	**<0.0001**		0.0172	0.0214	0.0318	0.0179
GV	**<0.0001**	**0.0052**	**0.0010**	**<0.0001**	**<0.0001**	**0.0001**	**0.0028**		0.0253	0.0268	0.0187
SI	**0.0009**	**<0.0001**	**0.0006**	**<0.0001**	**0.0014**	**0.0003**	**0.0002**	**<0.0001**		0.0318	0.0259
RI	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		0.0204
EN	**0.0079**	0.0941	**0.0069**	**<0.0001**	**0.0011**	**0.0002**	**0.0010**	**0.0045**	**0.0069**	**<0.0001**	

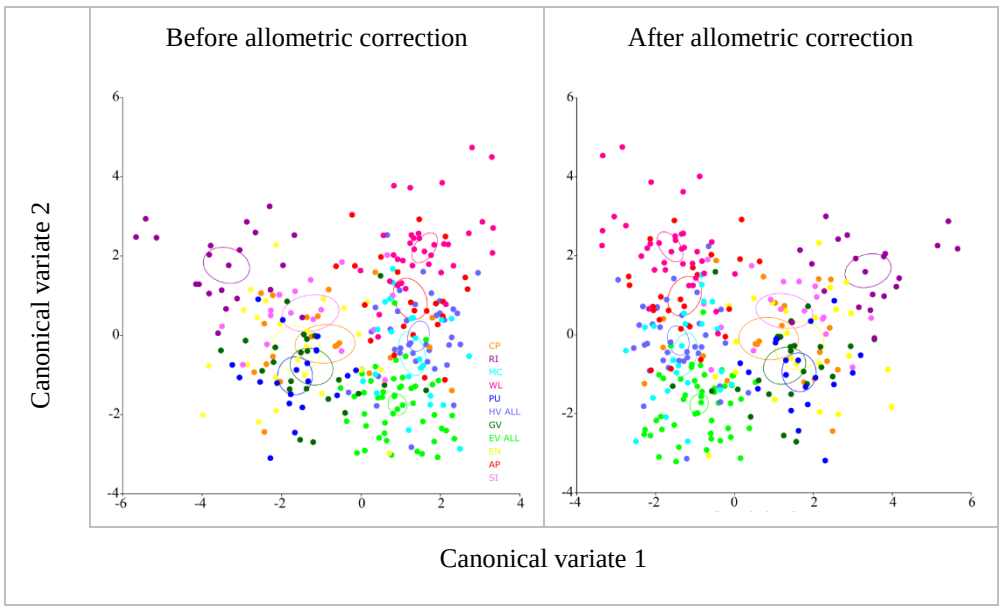


Figure 4.27. CVA of all stoats grouped by site. Left column: before allometric correction, right column: after allometric correction. Associated statistics can be found in Table 40 and Table 41 respectively.

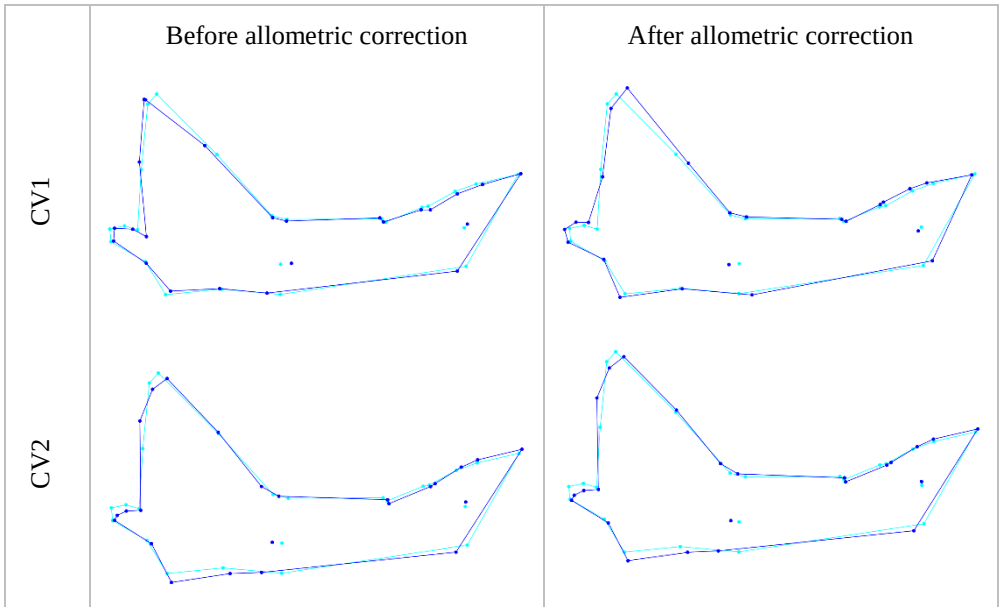


Figure 4.28. CVA wireframe deformation plots of stoat mandibles grouped by location based on the deformation implied by the analysis A) before allometric correction; B) after allometric correction. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

4.4.2.5 Modularity analysis

The hypothesis of developmental modularity was not supported (p-value=0.4237) and neither was the functional modularity hypothesis (p-value=0.4622), therefore no further testing was needed.

4.4.2.6 Biomechanical advantage

Kruskal-Wallis ANOVAs found some consistency in differences of biomechanical advantage across locations. For biomechanical advantage measurements that include the action of the temporalis muscle (T/C and T/M1), the MC, RI, and EN stoats all had more efficient muscle lever systems than the majority, but not all, of the other locations ($p<0.05$) (Table 42 and Table 43). The T/M1 comparisons did have more variation between locations than the T/C comparisons. There was less consistency in the biomechanical advantage of the masseter muscle ratios M/C and M/M1 measurements, although for both M/C and M/M1, CP, PU, and WL stoat mandibles typically had greater relative strength than the other locations. For the M/C measurement GV was significantly higher than three other locations. The M/M1 measure also found that two locations, AP and EVY, had significantly smaller relative strength than most other locations, EVN was also significantly lower than three other locations.

Table 42. Average biomechanical advantage values for each location (T: temporalis, M: masseter, C: canine, M1: carnassial).

	T/C	T/M1	M/C	M/M1
CP	0.3594	0.6499	0.3327	0.6015
PU	0.3664	0.6607	0.3396	0.6121
AP	0.3510	0.6187	0.3063	0.5382
WL	0.3582	0.6563	0.3296	0.6042
MC	0.3721	0.6834	0.3263	0.5996
HVN	0.3616	0.6571	0.3268	0.5939
HVY	0.3607	0.6262	0.3268	0.5695
EVN	0.3655	0.6507	0.3189	0.5676
EVY	0.3561	0.6384	0.3214	0.5762
GV	0.3745	0.6609	0.3340	0.5894
SI	0.3690	0.6661	0.3248	0.5865
RI	0.3847	0.6894	0.3242	0.5812
EN	0.3753	0.6790	0.3260	0.5897

Table 43. Between location Kruskal-Wallis ANOVAs of the biomechanical advantage of mandibles, only h-values and p-values are presented (p-values in the top pale green triangle, h-values in the lower white triangle), all h-values corresponding with a significant p-value (p<0.05) are highlighted in bold (T: temporalis, M: masseter, C: canine, M1: carnassial).

T/C	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.1373	0.1798	0.6285	**0.0060**	0.6130	0.7848	0.2350	0.6174	**0.0073**	0.1148	**0.0001**	**0.0043**
PU	2.2078		0.8875	0.5245	0.0897	0.3339	0.3570	1.0000	0.0608	0.1198	0.5201	**0.0006**	0.0593
AP	1.7993	0.0200		0.7415	0.1078	0.4579	0.3903	0.9835	0.0773	0.1489	0.4458	**0.0011**	0.0870
WL	0.2340	0.4050	0.1088		**0.0392**	0.8206	1.0000	0.4455	0.4436	0.0526	0.2974	**0.0011**	**0.0274**
MC	**7.5556**	2.8800	2.5867	**4.2517**		**0.0221**	**0.0486**	0.1735	**0.0014**	0.8852	0.8389	**0.0203**	0.6062
HVN	0.2558	0.9339	0.5510	0.0514	**5.2385**		0.9240	0.4213	0.2594	**0.0177**	0.1863	**0.0002**	**0.0141**
HVY	0.0746	0.8484	0.7379	0.0000	**3.8907**	0.0091		0.4451	0.4587	0.0523	0.2146	**0.0006**	**0.0305**
EVN	1.4101	0.0000	0.0004	0.5821	1.8520	0.6467	0.5830		0.1105	0.1735	0.4767	**0.0014**	0.0990
EVY	0.2495	3.5152	3.1200	0.5870	**10.1902**	1.2722	0.5491	2.5476		**0.0014**	0.0586	**<0.0001**	**0.0011**
GV	**7.1973**	2.4200	2.0833	3.7568	0.0208	**5.6229**	3.7662	1.8520	**10.1902**		0.6657	**0.0384**	0.7415
SI	2.4863	0.4137	0.5814	1.0859	0.0413	1.7468	1.5401	0.5065	3.5776	0.1867		0.0671	0.6843
RI	**15.6842**	**11.6742**	**10.7329**	**10.5844**	**5.3851**	**13.9213**	**11.6951**	**10.1449**	**18.5906**	**4.2862**	3.3532		0.0923
EN	**8.1721**	3.5556	2.9290	**4.8678**	0.2657	**6.0208**	**4.6802**	2.7211	**10.6019**	0.1088	0.1654	2.8344	

T/M1	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.1373	0.7582	0.4547	**0.0011**	0.3224	0.9456	0.6922	0.2288	0.1659	0.0919	**0.0006**	**0.0089**
PU	2.2078		0.2116	0.6543	**0.0067**	0.6543	0.3766	0.3832	**0.0047**	0.9249	0.4130	**0.0006**	0.0547
AP	0.0948	1.5606		0.5227	**0.0009**	0.5094	0.8987	0.9179	0.0773	0.2745	0.0839	**0.0004**	**0.0078**
WL	0.5590	0.2006	0.4086		**0.0119**	0.9671	0.5886	0.7728	0.0969	0.5362	0.3093	**0.0059**	**0.0412**
MC	**10.7355**	**7.3472**	**11.0208**	**6.3282**		**0.0065**	**0.0100**	**0.0017**	**<0.0001**	**0.0126**	0.1780	0.4666	0.5094
HVN	0.9792	0.2006	0.4354	0.0017	**7.4082**		0.5041	0.6501	**0.0432**	0.6207	0.3470	**0.0020**	**0.0288**
HVY	0.0047	0.7819	0.0162	0.2925	**6.6407**	0.4464		0.7264	0.4996	0.3087	0.2298	**0.0039**	**0.0259**
EVN	0.1567	0.7606	0.0106	0.0833	**9.8231**	0.2058	0.1225		0.1013	0.4213	0.1621	**0.0007**	**0.0137**
EVY	1.4482	**7.9779**	3.1200	2.7554	**19.4063**	**4.0874**	0.4558	2.6852		**0.0204**	**0.0068**	**<0.0001**	**0.0003**
GV	1.9192	0.0089	1.1943	0.3827	**6.2249**	0.2449	1.0364	0.6467	**5.3809**		0.5935	**0.0063**	0.0578
SI	2.8411	0.6701	2.9871	1.0336	1.8146	0.8844	1.4423	1.9541	**7.3216**	0.2849		0.0910	0.4016
RI	**11.9032**	**11.9032**	**12.5963**	**7.5782**	0.5300	**9.5735**	**8.3419**	**11.4912**	**19.4099**	**7.4534**	2.8571		0.4016
EN	**6.8477**	3.6904	**7.0753**	**4.1671**	0.4354	**4.7772**	**4.9595**	**6.0719**	**13.2432**	3.5986	0.7035	1.2428	

M/C	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.2678	**0.0078**	0.6285	0.1407	0.2655	0.1945	**0.0078**	**0.0171**	0.7415	0.0607	**0.0463**	0.1593
PU	1.2279		**0.0089**	0.2579	**0.0162**	0.0593	**0.0466**	**0.0020**	**0.0099**	0.4094	**0.0244**	**0.0091**	**0.0251**
AP	**7.0798**	**6.8450**		**0.0133**	0.1323	0.2655	0.1117	0.9835	0.7496	**0.0274**	0.1863	0.1392	0.1323
WL	0.2340	1.2800	**6.1224**		0.1432	0.3025	0.4840	**0.0133**	**0.0411**	0.6207	0.1546	0.1062	0.2317
MC	2.1707	**5.7800**	2.2657	2.1433		0.7415	1.0000	0.1489	0.2685	0.1802	0.5761	0.5390	0.8046
HVN	1.2398	3.5556	1.2398	1.0629	0.1088		0.9746	0.2011	0.3070	0.2655	0.9190	0.9275	0.8366
HVY	1.6830	**3.9584**	2.5304	0.4899	0.0000	0.0010		0.1522	0.2844	0.2521	0.6597	0.7904	0.9493
EVN	**7.0798**	**9.5339**	0.0004	**6.1224**	2.0833	1.6344	2.0496		0.8315	**0.0100**	0.2323	0.2953	0.1609
EVY	**5.6840**	**6.6617**	0.1019	**4.1739**	1.2246	1.0435	1.1457	0.0453		**0.0351**	0.4306	0.4882	0.2594
GV	0.1088	0.6806	**4.8678**	0.2449	1.7963	1.2398	1.3117	**6.6433**	**4.4389**		0.1151	0.0880	0.1735
SI	3.5188	**5.0675**	1.7468	2.0258	0.3127	0.0103	0.1939	1.4270	0.6211	2.4832		0.7142	0.4611
RI	**3.9693**	**6.8027**	2.1869	2.6092	0.3773	0.0083	0.0706	1.0952	0.4805	2.9115	0.1341		0.4128
EN	1.9807	**5.0139**	2.2657	1.4303	0.0612	0.0425	0.0040	1.9660	1.2722	1.8520	0.5433	0.6708	

M/M1	CP	PU	AP	WL	MC	HVN	HVY	EVN	EVY	GV	SI	RI	EN
CP		0.3646	**0.0005**	0.8950	0.4816	0.4547	0.1830	**0.0008**	**0.0039**	0.1907	**0.0328**	**0.0087**	0.1407
PU	0.8220		**0.0002**	0.3832	0.1791	0.1090	0.0710	**0.0004**	**0.0027**	**0.0451**	**0.0141**	**0.0053**	**0.0320**
AP	**12.2249**	**14.2222**		**0.0001**	**0.0011**	**0.0094**	**0.0330**	0.7415	0.3172	**0.0246**	**0.0307**	0.0798	**0.0078**
WL	0.0174	0.7606	**15.1875**		0.5094	0.2482	0.2391	**0.0002**	**0.0020**	0.1171	**0.0288**	**0.0078**	0.0870
MC	0.4952	1.8050	**10.6140**	0.4354		0.6501	0.4840	**0.0018**	**0.0078**	0.3535	0.1038	0.0531	0.3325
HVN	0.5590	2.5689	**6.7500**	1.3333	0.2058		0.8486	**0.0187**	0.1308	0.6801	0.5252	0.1870	0.7571
HVY	1.7727	3.2593	**4.5435**	1.3856	0.4899	0.0364		0.0698	0.0963	0.9493	0.3169	0.3298	0.9493
EVN	**11.1722**	**12.5000**	0.1088	**13.6229**	**9.6943**	**5.5255**	3.2885		0.5513	**0.0433**	0.0839	0.1332	**0.0187**
EVY	**8.3153**	**8.9698**	1.0005	**9.5222**	**7.0765**	2.2831	2.7662	0.3551		0.1800	0.2269	0.3295	0.0705
GV	1.7120	**4.0139**	**5.0514**	2.4558	0.8610	0.1701	0.0040	**4.0833**	1.7976		0.7125	0.4128	0.8690
SI	**4.5549**	**6.0308**	**4.6673**	**4.7778**	2.6460	0.4037	1.0016	2.9871	1.4603	0.1358		0.9775	0.4767
RI	**6.8855**	**7.7884**	3.0688	**7.0854**	3.7396	1.7412	0.9498	2.2547	0.9509	0.6708	0.0008		0.2953
EN	2.1707	**4.6006**	**7.0753**	2.9290	0.9392	0.0957	0.0040	**5.5255**	3.2722	0.0272	0.5065	1.0952	

4.4.2.7 Multi-block partial least-squares analyses

The following section covers the results of the last two partial least-squares analyses. The first one investigated the correlation between mandible shape and environmental variables, and the second one looked at the correlation between mandible shape and stoat diet.

4.4.2.7.1 Mandible shape and its correlation with environmental variables

The RV coefficient for the two block PLS analysis (block one: mandible shape, block two: environmental variables) was 0.1212, indicating that the strength of the block correlation was statistically significant (p-value <0.0001).

Only PLS1 explained more than five percent of the total covariation between the blocks (Table 44). The permutations for the singular value (13.2899) and the correlation (0.5554) were significant, and the correlation strength was moderately positive, see Figure 4.28. The variable with the greatest negative loading was annual mean rainfall (-0.9894). High altitude had the biggest positive loading (0.1424), but this was a smaller effect than rainfall. Haplotype and habitat type did not appear to have any significant correlation with shape.

Table 44. Results of a partial least-squares analysis of the covariation between the environmental attributes of the 11 locations and the shape data of the mandibles. Only axes that had greater than five percent total covariation were presented. Significant values (p<0.05) were highlighted in bold.

Environmental variable	PLS1
Latitude	0.0044
Lowest altitude from (m asl)	0.0295
Highest altitude (m asl)	0.1424
Annual Mean Rainfall (mm)	-0.9894
Habitat dummy code	0.0003
Temperature from (°C)	-0.0003
Temperature to (°C)	-0.0004
Major haplotype	0.0001
Minor haplotype	-0.0004
Singular value	13.2899
Singular p-value	**<0.0001**
Correlation	0.5554
Correlation p-value	**<0.0001**
Total covariation (%)	**95.2930 %**

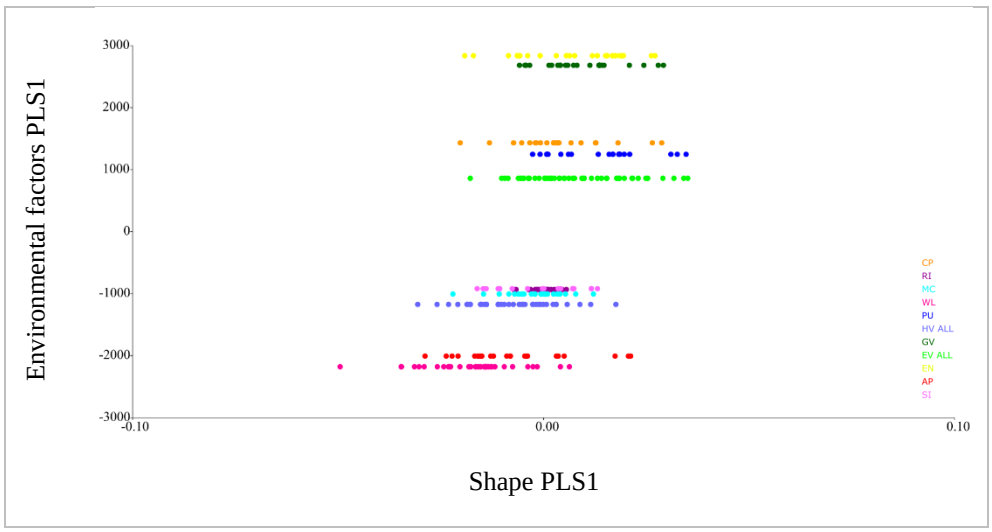


Figure 4.29. Partial least-squares analysis of mandible shape and environmental variables. The correlation for PLS 1 is 0.5554. The two axes were not plotted to the same scale because they are in different units. Individuals were coded by habitat like the previous analyses. The RV coefficient between the two blocks was 0.1212, p-value <0.0001.

Along mandible shape PLS1, in the direction of decreasing annual mean rainfall, the mandible body was thicker and shorter, and the carnassial shorter, correlated with a jaw marginally more efficient for catching prey, as there was a reduced length for the muscle force transmit across. The ramus lengthened, the coronoid process tilted back and became taller, the condyle also got bigger. As in PCA wireframes this was an exaggerated view of the trend, as the samples weren't spread over the whole length of the axis (Figure 4.30).

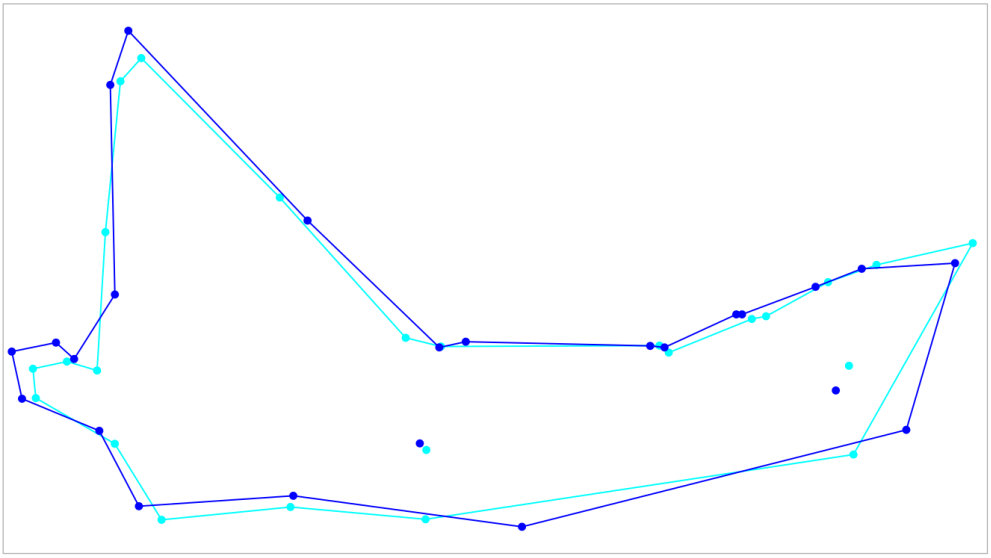


Figure 4.30. The wireframe deformation that corresponds to the shape variables along PLS1. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue.

4.4.2.7.2 Mandible shape and its correlation with variation in diet

The RV coefficient for the two block PLS analysis (block one: mandible shape, block two: diet variables) was 0.0770, indicating that the correlation strength between the blocks was statistically significant (p-value<0.0001).

The first four axes explained more than five percent of the total covariation between the blocks (Table 45). The singular values for the diet variables were not as high as those found for the environmental data, though the permutation tests for the singular values and for the correlations were significant (Table 45). On the first axis the diet variable with the greatest negative loading was insects, the positive loadings were large mammals, followed by lizards and rats.

Table 45. Results of a partial least-squares analysis of the covariation between the diet variation of the 11 locations and the shape data of the mandibles. Only axes that had greater than five percent total covariation were presented. Significant values (p<0.05) were highlighted in bold.

Diet variables	PLS1	PLS2	PLS3	PLS4
Large mammal	0.5702	-0.3594	0.1537	-0.2443
Rat	0.3400	0.0440	0.4505	0.7994
Bird	0.0985	-0.2917	-0.7467	0.2771
Mouse	0.0494	-0.3249	-0.3045	0.3576
Insects	-0.6352	0.1169	0.0310	0.3073
Lizards	0.3791	0.8152	-0.3496	0.0467
Singular value	0.0079	0.0053	0.0045	0.0030
Singular p-value	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**
Correlation	0.4096	0.4239	0.5704	0.3111
Correlation p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Total covariation (%)	**49.0930 %**	**22.1170 %**	**16.0340 %**	**7.0740 %**

The highest positive loading along PLS2 was for lizards; the highest negative loading along PLS3 was for birds; the positive loading on PLS4 was for rats. There were no clear differences between each PLS correlation, and no a large difference in the covariance they accounted for. The correlation values and the PLS graphs (Figure 4.31) confirmed the weak to moderate relationship between mandible shape and the diet variables.

The small RV coefficient and correlation values made it unlikely that the wireframe deformations would have any biological meaning (Figure 4.31), although there was a minor trend suggesting that a high proportion of larger mammals and rats, i.e: “hard food”, corresponded with a coronoid process with a backwards tilt, and a

deeper mandible body. Because the RV coefficient, and the singular and correlation values were much higher for the Environmental factors PLS analysis, the variations in diet were unlikely to have had much of a biologically significant effect on mandible shape.

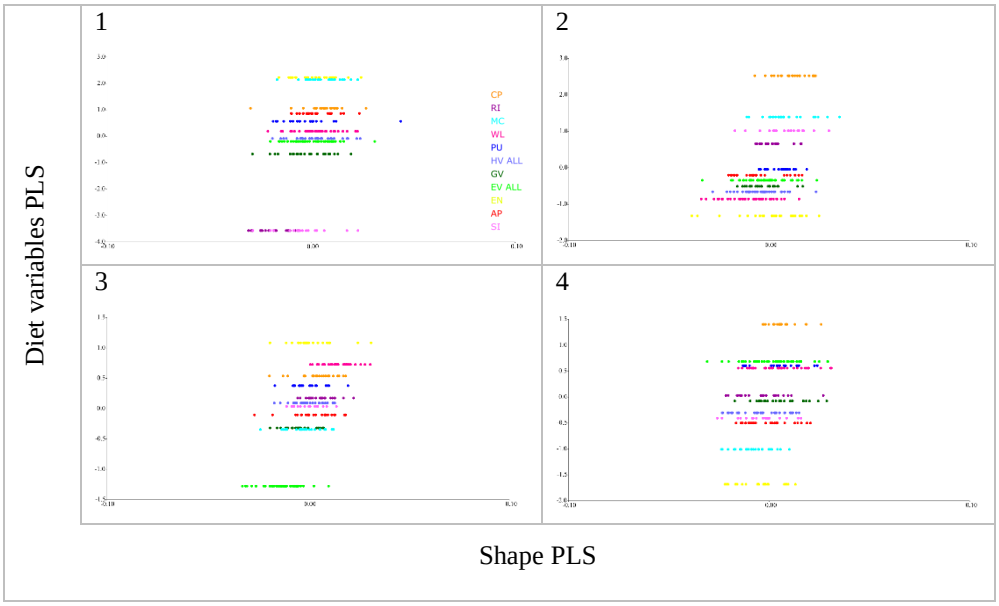


Figure 4.31. Plot of the four columns of mandible shape variables against the four columns of diet variables. The two axes were not plotted to the same scale because they are in different units. Individuals were coded by habitat in the same way as the previous analyses. The RV coefficient between the two blocks was 0.0770, p-value <0.0001. The singular values and correlations can be found in Table 45.

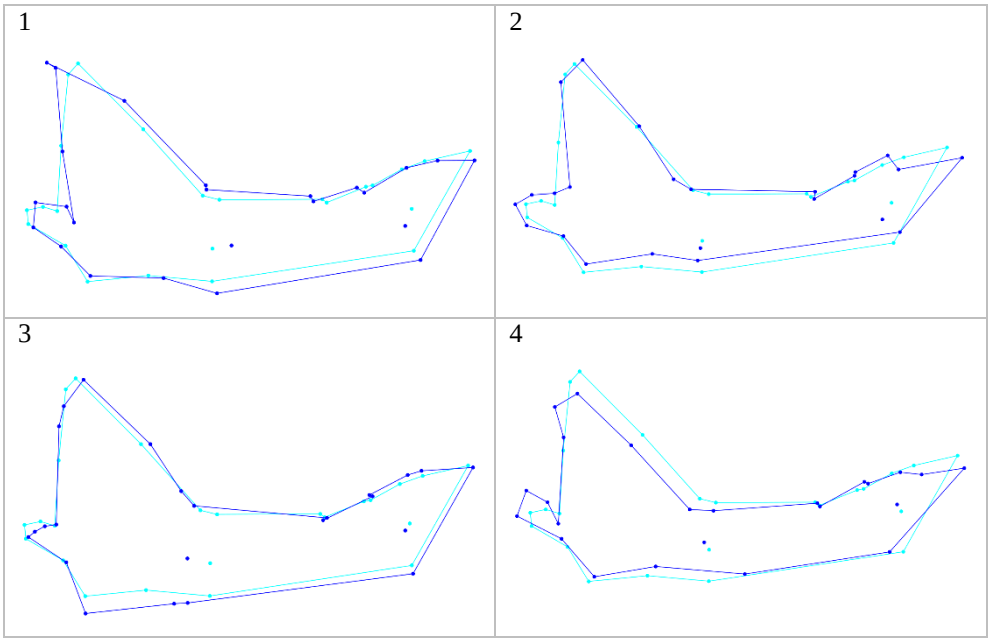


Figure 4.32. The wireframe deformation that corresponds to the shape variables along the four PLS axis that accounts for more than five percent of the covariation. Starting shape (negative end of axis): pale blue, end shape (positive end of axis): royal blue

4.4.3 Section summary

A brief summary of statistical tests and their results can be found in Table 46. The biggest differences between locations was in the size of the mandibles, particularly in males. The male EN mandibles were only smaller than EVY males, which was much larger than expected there was a similar trend, although the differences weren't as significant, with the EN females.

Allometry was not a large component of mandible shape, and there were few differences in mandible shape between locations. Although biomechanical advantage of the temporalis measurements tended to increase with mammalian prey, EN and MC have the highest components of rabbits, although this doesn't explain RI, which has high proportions of insects in their diet. This difference could be from the age of the RI stoats, while mandible length of the males was within the adult male range from (King et al. 1982c) the age was not determined by cementum layers, so there could be a chance males were in their sub-adult phase, just very large. Masseter measurements, which are related to food processing increased with a diet that had more even concentrations of each prey type. Mandible shape had a higher correlation with environmental factors than diet, in particular with rainfall, there is an inverse relationship of rainfall and mice populations (King 1991a).

Table 46. Stoats across locations comparisons section summary results table.

Question	Material	Test	Test	Test	Test
Effect masting on size, sexual dimorphism, and shape	HV separated by sex and birth year	ANOVAs. Males were bigger than females regardless of birth year. p=**0.0002** and **0.0001**	2-way factorial ANOVA of sex and birth year. Only sex was significant p<**0.0001** Birth year: p=0.8056	PCA. No significant differences in mandible shape between any of the groups either by sex or birth year. Males could have a greater muscle efficiency system.	CVA. No significant differences in mandible shape between any of the groups either by sex or birth year.
	EV separated by sex and birth year	Males were bigger than females regardless of birth year p=**0.0002** and **0.0001**. EVY males were larger than EVN males p=**0.0082**.	2-way factorial ANOVA of sex and birth year. Sex (p<**0.0001)** and birth year had a significant effect on size p= **0.0172**	PCA. No significant differences in mandible shape between any of the groups either by sex or birth year.	CVA. No significant differences in mandible shape between any of the groups either by sex or birth year.
Size variation between locations	13 samples separated by sex: 1 England, 10 New Zealand. EV and HV separated by birth year	Males: Individual ANOVAs. EN, EVY, and AP males larger than other locations p=**<0.0001-0.0478**. RI and WL males smaller than most other locations p=**<0.0001-0.0478**.		Females: ANOVAs. HVY, RI smaller than most locations p=**0.0021-0.0483**. AP were larger than WL p=**0.0360**	
Shape variation between locations	11 samples	Regression p**<0.0001**. Shape accounted for 3.16 % of shape.	PCA. No PCs were significant (χ^2<5.99) but locations were grouped and had different means on the PCA.	Discriminant analysis. CP and MC (4.35 %), GV and EN (4.17 %), WL and RI	CVA. RI stoats had the most distinct mandibles (p<**0.0001**), and HV and AP the most similar (p=0.1487). There

				(1.75 %), RI and SI (7.69 %) separated effectively. Percentages are the % of misclassified individuals. Differences were mostly in the ramus.	were groupings along CV1 and 2. Differences were in the ramus.	
Biomechanical advantage	13 samples: 1 England, 10 New Zealand. EV and HV separated by birth year	Kruskal-Wallis ANOVAs	T/C. MC, RI, and EN all had greater efficiency of the kill bite muscle and teeth system, p=<**0.0001-0.0384**.	T/M1. MC, RI, and EN all had greater efficiency of this muscle lever system p=<**0.0001-0.0412**.	M/C. CP, PU, WL, and GV all had greater relative strength than most locations p=**0.0078-0.0466**.	M/M1. CP, PU, WL, and GV all had greater relative strength than most locations p=**0.0001-0.0328**. AP and EVY had less relative efficiency than most locations p=**0.0011-0.0307**.
Effects environmental variables on shape	11 samples	Two block PLS. RV coefficient= 0.1212, p<**0.0001**, PLS1 explained 95.29 % of covariation. Rainfall had the highest loading (-0.9894). Less rainfall was correlated with a jaw that had more efficient muscle lever systems, and therefore greater relative strength.				
Effects diet on shape	11 samples	Two block PLS. RV coefficient= 0.0770, p<**0.0001**, PLS1 explained 49.09 % of covariation. Insects and then mammals had the highest loadings (-0.6352 and 0.5702 respectively). This PLS correlation was not as strong as the environmental one.				

Chapter Five

Discussion

Drawing 6. Pencil drawing of a stoat carrying a dead mouse. Artist: C. Hill.

5.1 Introduction

In this chapter I will discuss my results in the context of other studies and what they might mean, in the same order used in the results. First I will discuss the results from the results of the interspecific morphological differences of New Zealand mustelid mandibles: size and sexual dimorphism, and then shape. I will discuss the habitat differences of stoat mandibles: size, sexual dimorphism, the effect of beech seed years and environmental variables. Then I will summarise my results following the format of questions proposed at the beginning of the results chapters and end with the questions that have arisen from my results and possible future studies that could answer them.

5.2 Objective one: Interspecific morphological differences of New Zealand mustelid mandibles

5.2.1 Sexual dimorphism: size and shape

There are several hypotheses on why the degree of sexual dimorphism changes across populations and related species: (1) short term effects of local variations in diet or (2) long term effects of resource partitioning of the sexes (Lynch et al. 1993; Powell et al. 1997; Abramov et al. 2003; Piontek et al. 2015). (3) The degree of sexual selection, which in mustelids depends on opportunities for polygyny (long term), less dimorphism means less competition for mates (Lynch et al. 1993; Powell et al. 1997; Abramov et al. 2003; Piontek et al. 2015).

The resource partitioning hypothesis states that sexual dimorphism arises from different trophic niches for each sex, which reduces competition (Lynch et al. 1993; Piontek et al. 2015). Lynch et al. (1993) found that male otters (*Lutra lutra*) have smaller post orbital widths allowing for larger temporalis muscles which would confer greater bite forces, in turn allowing them to take on larger prey, but support for the resource partitioning hypothesis appears to be waning (Piontek et al. 2015).

The degree of mandible size sexual dimorphism in New Zealand ferrets (15.38 %) was close to the sexual dimorphism reported for skull length of the two most closely related *Mustela* spp. Males of the European polecat (*M. putorius*) and Steppe polecat (*M. eversmanii*) were typically 16 % larger than females (Abramov et al.

2003; Sato et al. 2012). Variations of bone size sexual dimorphism do not seem to rely on diet in these three closely related species because the diets of these three closely related species are varied, even from similar habitat types. The diet of *M. putorius* from a mixed land of open grassland and forests was typically half small mammals and a third birds, with vegetation, fish, and insects making up the rest (Lanszki et al. 2007). The diet of *M. eversmanii* from similar habitats was typically 73 % small mammals, 1.4 % *Lepus spp.*, 21 % birds, fish and vegetation make up the remainder of the diet (Lanszki et al. 2007). Ferret diets vary from open grasslands and forests in New Zealand depending on where in the country they are located (King 2005). The majority of the diet is generally lagomorphs (~60 %); secondary prey are typically small mammals, invertebrates and frogs or fish (Norbury et al. 1996; Jones 2002; King 2005).

Stoat size sexual dimorphism across countries varies when compared to the three polecat species. Piontek et al. (2015) summarised the body weight sexual dimorphism from a range of different studies from seven European countries and New Zealand. New Zealand stoats were much larger than their European counterparts, though body weight is typically more variable than other body measurements as it depends on the recent condition of the animal (Powell et al. 1997; Piontek et al. 2015). The sexual dimorphism ranged from 32-57 % (males larger than females) this sexual dimorphism is much larger than that found in mandible size but expected based on results from King et al. (1982c) and the discussion from Powell et al. (1997) (Piontek et al. 2015).

Yom-Tov et al. (2010) studied stoats and weasels in Sweden and the correlation between skull length and environmental factors. They found that female stoats were 50 % of male body weight but didn't report skull length dimorphism, despite that being what they studied. Powell et al. (1997) looked at the sexual dimorphism of New Zealand stoats condylobasal length, this was about eight percent, which is much lower than the body weight sexual dimorphism mentioned earlier, the corresponding body dimorphism was 36 % which was at the low end of those reported by Piontek et al. (2015). The mandible length dimorphism of New Zealand stoats grouped across habitats was about 12 %, which was still larger than those of the Westland National Park stoats (9.6 %) used here (King et al. 1982c).

The sexual dimorphism of weasels also varies; Zub et al. (2012) found an average weight dimorphism of 50 % (males larger than females) but Yom-Tov et al. (2010) found male weasels to be 35 % bigger than females. Dayan et al. (1994) measured condylobasal length of male and female weasels from Britain and found males were 13.5 % larger than females, this degree of sexual dimorphism is quite a bit less than the sexual dimorphism found here. Although, size sexual dimorphism of New Zealand weasels was 18 % based on head and body length from King (2005), which was closer to the results of this study, which made sense because many of the specimens were the same in both results.

As mentioned in the introduction the mandible is much more plastic than the skull, probably because it is used for very few functions compared to the skull. There is a chance that the mandible is more related to diet and environment than the skull (Cardini et al. 2008; Cornette et al. 2013; Klingenberg 2013a). Therefore, the two measurements, skull and mandible size, may not be as correlated as assumed and could explain why my size sexual dimorphism is so large in New Zealand weasel mandibles, compared to British weasel skulls, but there are other possible explanations. It was recently discovered that some of the female weasels here may not have been full grown which may have exaggerated the size sexual dimorphism (C. M. King, personal communication, January 27, 2017).

As discussed before diet does not seem to be a factor in changing the degree of sexual dimorphism when comparing ferrets from New Zealand with the polecat species, indicating that they have a similar degree of sexual competition. Adding to the support for the sexual competition and the energetic requirements of females for reproduction is that the two polecat species can live sympatrically and the sexual dimorphism remains the same despite different niches between sexes and across species (Abramov et al. 2003). Out of the New Zealand species, weasels would appear to have the largest competition for mates in the long term.

Powell et al. (1997) hypothesised that sexual dimorphism can be directly affected by the diet in the short term. They investigated this in Eglinton valley stoats, which experience beech seed fall food pulses. Male size should be affected by abundant food, likely from a greater phenotypic plasticity in genes related to size, whereas females are constrained by the energy requirements for reproduction and therefore

size should be more stable (Powell et al. 1997). Greater available food, allowing male weasels to reach full size potential may be contributing to the large size sexual dimorphism. Powell et al. (1997) did not find support for the hypothesis, but I did and this will be discussed further down in this discussion in the section on stoats, habitats, and the effects of beech seed masting on mandible shape. There is also the option that sexual dimorphism comes from a combination of hypotheses. Gittleman et al. (1997) found sexual dimorphism in the teeth is affected by different factors; the canine relates more to sexual competition and the carnassial to diet. Sexual dimorphism is likely affected by a combination of diet and sexual competition (Powell et al. 1997; Abramov et al. 2003; Yom-Tov et al. 2010).

I found no mandible shape sexual dimorphism in any of the three species. Some mandible shape sexual dimorphism would be expected if the trophic niches of the sexes affected their shape sexual dimorphism. In contrast to my results, Lynch et al. (1993) found cranial shape sexual dimorphism in otters (*Lutra lutra*). Suzuki et al. (2011) also found cranial shape sexual dimorphism in *M. sibirica*, allometry between the sexes accounted for these differences. Both Lynch et al. (1993) and Suzuki et al. (2011) used linear measurements of the cranium; there is a chance there was allometry and sexual dimorphism within ferrets, stoats, and weasels, but only in the cranium. Loy et al. (2004) also found shape sexual dimorphism in *Martes foina* and *M. martes* skulls, using landmark based geometric morphometrics, and this correlated to differences in diets. The shape of the mandible is known to exhibit greater phenotypic plasticity than the skull, which may have reduced any shape sexual dimorphism to non-significant (Cardini et al. 2008).

If I had used skull shape instead of the mandible there may have been shape sexual dimorphism. Although in greater white-toothed shrews (*Crocidura russula*) there was a high covariance between skull and mandible shape (Cornette et al. 2013). This was largely at the muscle attachment sites on both bones (Cornette et al. 2013).

5.2.2 Shape across species

There was some allometry in mandible shape across ferrets, stoats and weasels, but it did not account for all the variation in shape between the species. Mandibles of stoats and weasels were most alike, but the Mustelidae genetic tree produced by Sato et al. (2012) showed ferrets and weasels are the most closely related. The large

phenotypic plasticity enables the animal to adapt to environmental conditions over their own life time. Plasticity itself is a trait, shapes with plasticity in the favourable direction is heritable, allowing the species to adapt to available diet over time (plasticity shapes what there and then natural selection acts upon it) (Renaud et al. 2010; Scott et al. 2014; Renaud et al. 2015).

Two species, *Mustela itatsi* and *M. sibirica* showed allometric functional differentiation, most pronounced at sites of muscle attachments used for eating, reflecting diet differences (Suzuki et al. 2011), which is what I found in my results. Caumul et al. (2005) also found support for diet contributing to mandible shape in other species, they estimated 35 % of marmot mandible shape was caused by the diet, and only seven percent to the differences in mtDNA.

The analyses used in this thesis easily discriminated between ferrets, stoats, and weasels based on mandible shape, but an analysis of British mustelids by Lee et al. (2004) could not. This was likely because I used whole structure shape whereas Lee et al. (2004) used a series of linear measurements. Lee et al. (2004) was able to separate out ferrets/ polecats from the other two, but was not able to distinguish between stoats and weasels. This supports the results reported here, ferrets were the most distinct in terms of shape of the three species. Stoats and weasels have larger coronoid processes (temporalis attachment) relative to rest of mandible shape. Lee et al. (2004) suggested that as weasels and stoats require proportionally larger muscle attachments to acquire enough power to perform a killing blow because they will take on prey same size as ferrets, for example rabbits. The differences in relative muscle attachment size were more visible when allometry was accounted for. In the discriminant function after allometric correction weasels do have slightly larger coronoid processes than stoats, which was further support for this idea.

These differences can also be matched with the biomechanical advantage results. Ferrets had the most efficient system, with the shorter coronoid process, which was also not tilted as far back as stoats and weasels. Anthwal et al. (2015) demonstrated how bone growth in part comes from the requirements of support from the muscle. Therefore, the more efficient systems require less muscle attachment areas, this was in terms of lever and muscle efficiency for providing force to the teeth (Anthwal et al. 2015). The coronoid processes of stoats were likely tilted back because the

muscle put more force on the bone in that direction therefore causing the bone to respond and grow more in that direction. Ferrets had the most efficient muscle systems according to the biomechanical advantage which supports the idea that the size of the prey and requirements for killing helped to shape the mandible of these species (Lee et al. 2004; Anthwal et al. 2015). Although weasels had greater biomechanical efficiency than stoats in the measures of the muscle shearing and bone crushing movements of the carnassial, not the killing blow of the canine.

Christiansen (2008) studied the bite forces of sabre-tooth and extant and extinct felines. An increase in gape size was reciprocal with muscle in-force levers and therefore bite force. This matches my results and the previous statements on the size of the coronoid processes. To kill prey that is proportionally large to themselves stoats and weasels require a large gape, which matches with the lower biomechanical advantage when compared with ferrets, which must have smaller gapes. Gape size should be tested to confirm. Rabbits do not typically make up as large a component of weasel diets when compared with British stoats and ferrets, because they usually go for smaller prey (McDonald et al. 2000; King 2005; McDonald et al. 2008). This may explain why the coronoid processes of weasels were not much larger than stoats.

There was no evidence to support the presence of modularity within or between these three species. This was not expected because modularity was found by Meloro et al. (2011a); Meloro et al. (2011b) in their studies of carnivores and mandible modularity; which included the three species studied here. The difference in results could have been a lack of diversity in the shape across ferrets, stoats, and weasels. The mandible shapes of ferrets, stoats and weasels were quite similar to each other compared to those used by Meloro et al. (2011a); Meloro et al. (2011b), because their modularity was found despite the small numbers of landmarks on the mandible, 14 total.

Although Anderson et al. (2014); Renaud et al. (2015) did find modularity in mice, the difference between groups was largely diet composition. The mouse diets ranged in their degree of herbivory to omnivory which had a large effect on mandible shape (Anderson et al. 2014; Renaud et al. 2015). This range in diets was greater than that between ferrets, stoats, and weasels. Modularity was also not found

when all the stoats from the second results chapter were combined. Modularity might have been found if three dimensional methods had been used (Cornette et al. 2013). Particularly because the bones showed different angles where the functional modules interacted, for example; the angle at which the angular process and the condyle intersected was different for each species. I noticed this when I was handling the bones to prepare for the photographs.

One slight issue with my analyses was the lack of female weasels, they are relatively harder to catch than males, due to their small size, this is also why they were sourced from more than one location (King 2005). I am not the only one who has had trouble with finding enough females. Suzuki et al. (2011) had trouble with collecting any females from a similar species and Zub et al. (2012) had also had trouble collecting the *M. nivalis* females. Renaud et al. (2010) had difficulties with low numbers of mice in some samples, this may have affected their results. I did use more female weasels than specimens per group used by Caumul et al. (2005). The analyses may have been adversely affected as there were meant to be at least ten or more specimens in each group. For example, having lower numbers of the smallest species may have affected the between species regression, and the level of allometry detected (Klingenberg 2016).

5.3 Objective two: Mandible plasticity and adaptations of New Zealand Mustela erminea mandibles across locations

5.3.1 Size and sexual dimorphism

Males varied more in size compared with females, which could be explained by Powell et al. (1997) who hypothesised males are affected more than females are during in their growth period by food abundance. Females reach adult size six months before males and are thought to be constrained by energy requirements for reproduction whereas males are not (King et al. 1982c; Powell et al. 1997). Yom-Tov et al. (2010) found a correlation in the size of male stoats and weasels in Sweden with latitude and net primary productivity. Net primary productivity has been used as a proxy for prey density because prey density increases with net primary productivity (Yom-Tov et al. 2010). The size of females was not correlated

with any of the measures which supports the hypothesis: male size is enhanced by food availability during their growth period but females are not, from Powell et al. (1997) (Yom-Tov et al. 2010).

In weasel species, larger males typically have greater reproductive success (Powell et al. 1997; Canady et al. 2016). And as shown in the EV comparisons of males and their birth year, seed year males are larger, this was also found by Powell et al. (1997), so it would be expected that they would have a greater reproductive success. However, this is a short term advantage because the smaller males, like those not born during a seed year, have a longer life and therefore more opportunities to breed over a long term than larger males during a short term (King et al. 1982c; Powell et al. 1997). Large size is only a short-term response to the environments and is not a stable adaptation, as there is no long-term increase in male body size (Powell et al. 1997).

The two-way EV ANOVA analysing the significance of birth year and sex on mandible size, found each effect was significant, but the interaction was not. This was likely because females did not significantly change in size across birth years, which is a confirmation of the sexual dimorphism theory discussed in (Powell et al. 1997). But this was not consistent with their own results which used condylobasal length (Powell et al. 1997). Possibly because evolution acts on characteristics to increase survival in the long term, plasticity in mandibles allows them to adjust more than skulls to environmental pressures such as dietary changes (Freeman et al. 2007).

Because there were not many HV males born in a seed year there was not sufficient data to see if the mixed beech forest stoats followed the same size patterns as the full beech forest stoats, when comparing seed year and non-seed year births. For the sake of completeness ideally more specimens would be collected and then the comparison rerun. Some European forests also have masting years where rodent species have similar peaks and crashes, such as: England (Harmer et al. 2005; Packham et al. 2012), Sweden (Sjoberg et al. 2007), Poland (Pucek et al. 1993), and Italy (Salmaso et al. 2009). It would be interesting to see if the same size patterns are seen in their stoat populations, provided trapping and measuring the stoats was allowed as they are protected in many areas (Piontek et al. 2015).

Out of the stable populations of large males (i.e., non-seed year samples), mandibles of EN males were larger than those of the New Zealand stoats, and of the New Zealand stoats AP males were the largest. This indicated that EN and AP stoats had greater access to food during their growth (Powell et al. 1997). The GV stoats used in this study were also born during a seed year but the males were not as large as EV males and the sexual dimorphism also wasn't as high; reasons for this should be investigated. Although the habitats were similar, they did come from a lower latitude, the temperature range was warmer, and the altitude did not go as high, these may all be contributors, rainfall data was also recorded as different for these two habitats.

King (1991b) tested a hypothesis from Erlinge (1987) comparing the average size British stoats and New Zealand stoats, New Zealand stoats should be larger than British stoats as the average body size of mammalian prey is larger in New Zealand. I did not find this but their results supported the hypothesis, even though it was acknowledged that invertebrates were excluded, despite being eaten with high frequency by many New Zealand stoats (King 1991b).

Piontek et al. (2015) summarised the body weight of stoats from a range of different studies from seven European countries and New Zealand. New Zealand stoats were much larger than their European counterparts, but body weight is typically more variable than other body measurements as it depends on the size of recent meals before death. The mandibles of New Zealand stoats are really not that much different in size from the English ones, and the differences were not in the expected direction, so either New Zealand has some fat stoats or there is something else affecting mandible size.

English stoat mandibles were larger than expected based on condylobasal length comparisons with New Zealand stoats from King et al. (1982c). The English stoat sample used here was trapped between 1977-1989, this was after the crash of rabbit populations and subsequently of stoat populations due to Myxomatosis, during the mid-recovery stage (Sumption et al. 1985; McDonald et al. 2008). English rabbit populations were still recovering at this point and did not feature as highly in stoat diets as they did before the crash and as they do today (Sumption et al. 1985;

McDonald et al. 2008). However, rabbits still featured more in the diet of my English sample, particularly males, than they did in male New Zealand stoat diets, and rabbits are much larger than the common prey of male New Zealand stoats: rats and birds (King et al. 1982b; McDonald et al. 2008).

Female British stoats typically eat smaller prey such as voles, which are smaller than the rats many female New Zealand stoats prey upon (King et al. 1982b; McDonald et al. 2008). These diet differences combined with the plasticity of mandibles could explain why male English stoats were larger than expected but females were not. The larger possums in the diet of New Zealand stoats are likely to be carrion and do not require high force applied by the mandible for a killing blow (King et al. 1982b; McDonald et al. 2000). One contradiction to this hypothesis is MC males. They typically eat rabbits as a large proportion of their diet, but the males are smaller than EN males, though this difference was not quite significant (King et al. 1982b).

This leads to the question; what is the correlation between skull size and mandible size? And how are they related to prey size (King 1991b)? Previously it has been assumed there is a high correlation between the two measurements, and so there has not been many reports of mandible size (King 1991b). As mentioned in the introduction and the previous chapter the mandible is much more plastic than the skull (Klingenberg 2013a). Because the number of functions it is used for is much less than the skull there is a chance that the mandible has a higher correlation with diet and environment than the skull (Klingenberg 2013a). Therefore, studies relating skull size to prey size may in fact have had better success if they had used mandible size. Particularly when my results are taken into consideration and the comparisons I have made here with respect to the average kill prey of each sex.

It is not a new concept to study the correlation between prey size and a feature other than the size of the skull. Dayan et al. (1994) suggested that canine diameter would be a good indicator of prey size. However Gittleman et al. (1997) later found that canine size had a greater correlation with sexual dimorphism. The size of the carnassial did have a high correlation with prey size. Testing the correlation between skull, carnassial, and mandible size and their correlation with prey size would be a future topic to investigate further. Correlating carnassial size to diet

would be easier than mandible size if the only option for specimens were live animals. The animal could be sedated for teeth measurements, or photos could be taken with a scale in the background to analyse away from the field. However, with trapped and dead animals we have access to the whole mandible, and adding this to teeth data would provide more information.

Cornette et al. (2013) studied the shape covariation of the mandible and the skull in shrews and found the highest shape covariation was in the muscle attachments as expected. However, they did not study the size covariation which could be as important, this is a topic which could be explored more in the future, particularly because there is such as large collection of samples stored from the NPSS (King et al. 1982a).

The size sexual dimorphism of mandibles in my results (11 %) was smaller than mandible size sexual dimorphism from King et al. (1982c) (13 %). The size sexual dimorphism of condylobasal length from King et al. (1982c) was only 9.5 %, which prompts more consideration of the correlation between the two structures in shape and size.

The reason for the difference between the mandible size sexual dimorphism results King et al. (1982c) and I found were likely from the way the specimens were grouped. Unlike the groupings I used King et al. (1982c) did not separate out the individuals born during a seed year. However, all results agreed that English stoats have greater size sexual dimorphism than New Zealand stoats. As the degree of size sexual dimorphism changed with location this provides more support for the hypothesis tested in Powell et al. (1997), which they themselves did not find support for. This difference could be because they used condylobasal length, which provides more evidence to question the degree of correlation between mandible and skull size.

There was no shape sexual dimorphism in the New Zealand or English stoat mandibles. However, King et al. (1982c) and Powell et al. (1997) found allometry in the stoat skull between the sexes, but, Powell et al. (1997) found that other body measurements of weight and body length were isometric. Only when all locations were used (and pooled via location) did the mandible shape show a small degree of

allometry (3.16 %). The difference in the amount of allometry in the skulls between sexes and the mandibles between sexes could be because the skull and mandible have different functions (Cardini et al. 2008).

5.3.2 Shape across locations

There was very little allometry between locations, therefore size had a very small effect on the variation in mandible shape. Not all the habitats had significantly different mandible shapes, although CP and MC, GV and EN, WL and RI, and SI and RI were different enough to show up in statistical tests. These differences were small, and usually at the muscle attachments. The differences in shape were not as pronounced as the shape differences found in mice (Anderson et al. 2014; Renaud et al. 2015). This could be because mice have a greater range in diet, both in terms of hardness and composition, and unlike the mice which belonged to different sub-species, the stoats here were all one species with no hybrid zones (King et al. 1982a; Anderson et al. 2014; Renaud et al. 2015). While stoats in New Zealand do exhibit a range of haplotypes, they were unlikely to have any effect on mandible shape as the differences between the haplotype genes were very small, and more the one haplotype was usually found at all South Island locations (Veale et al. 2015) (A. Veale, personal communication, November 17, 2016).

In the different analyses (PCA and CVA) the locations were only slightly separated and grouped from each other. The wireframe deformations showed that the biggest differences between any of the groups was the ramus for the muscle attachments. This indicates that muscle action on the bone was affecting the shape at least to some degree (Klingenberg 2010; Anderson et al. 2014).

Despite the overall mandible shape showing very small differences there were many more differences in the biomechanical advantage, which reinforced the idea that the slight differences between the mandibles could be real. The differences are likely to be the consequence of natural selection rather than just plasticity (Freeman et al. 2007). The results of the beech masting effect on mandible shape provided evidence for this (Freeman et al. 2007). We know that the size is plastic and not heritable, as the results of Powell et al. (1997) showed that the increase in size seen with beech masting was not passed on to future generations (Freeman et al. 2007). The

mandible shape was not significantly different between the two groups, and if shape plasticity had a significant effect on mandible shape across these growth years it would have been visible in the beech mast cohorts (Freeman et al. 2007). This indicates that any differences between locations was a result of natural selection, not variable growth rates.

Resolution Island has only birds, insects, and mice without any larger prey, yet had one of the highest efficiencies of transmission of muscle force to teeth force particularly of the temporalis measurements (Murphy et al. 2016). RI coronoid processes were tilted forward compared to the other locations, in particular SI and WL. Ferrets compared to stoats and weasels also had coronoid processes that were tilted forwards, associated with higher biomechanical efficiencies. The other two locations with high efficiencies of the temporalis measurements, in particular the T/C were MC and EN. Both of these locations typically have a high proportion of rabbits as part of their diet, and need high efficiencies for the killing blow (King et al. 1982b; Biknevicius et al. 1996; McDonald et al. 2000; Christiansen 2008; McDonald et al. 2008).

The locations with the highest masseter muscle biomechanical advantage measurements, related more to mastication than killing blows, were CP, PU, and WL. All of these location were podocarp forests, although PU also has exotic forest and some farmland mixed in (Nicholls 1976; King et al. 1982a; King et al. 1996a). However, the diets of these were more varied than the high temporalis stoats (King et al. 1982b; King et al. 1996a; Gillies 2016).

In contrast to the stoats I used, a different pest mustelid (*Mustela vison*), had visible, inherited, and very significant morphological changes in the cranium, when comparing ancestral populations with the ex-domesticated populations (Kruska et al. 2003). This has happened in America and Europe since 1860, which was a similar time frame since the New Zealand stoats were released (Kruska et al. 2003). However, the New Zealand stoat mandibles were not all significantly different from each other or England. One of the reasons for this difference could be that most of the pest *M. vison* were domesticated before they were released (Kruska et al. 2003). Domestication has a predictable effect on the cranium, for example the size of the

brain case decreases, which did not reverse after they were released into the wild (Kruska et al. 2003).

The two partial least-squares analyses indicated that rainfall had the highest correlation to mandible shape over any other factor, including the diet. The correlation between rainfall and mandible shape may be the correlation with habitat type and also indirectly with diet. Less rainfall can equal more mice and other herbivorous prey mammals; more mammals may require greater killing efficiency. Ideally a three block partial least-squares analyses should be done to test the correlations between mandible shape with environmental data and diet composition. The software to run this analysis is not yet available, PLSmaker7 is going to be upgraded soon so the analysis could be run then (Sheets 2001).

One reason that diet did not have as high a correlation with shape could be because rainfall is easier and more accurate to measure and compare across sites than diet. This is because a weather station can constantly measure rain whereas diet data is reliant on how soon before being trapped the animal ate, and what type of trap was used to kill the animal (King et al. 1982b). King et al. (1982b) acknowledged that the type of trap used could have an effect on gut analysis, also a gut analysis is only a snapshot the diet of each individual, just what was recently caught, which may or may not be representative of entire diet. A large sample may provide an accurate estimate of what is being eaten in an area but it also may not.

Other studies have used environmental data as proxies for prey and prey density (Yom-Tov et al. 2010). As mentioned above Yom-Tov et al. (2010) used Net Primary Productivity (NPP) as a proxy for prey densities and this is similar to mice populations and the correlation with rainfall (King 1991a). Yom-Tov et al. (2010) found areas with higher NPP and therefore higher prey densities had larger males, but not females, which was a similar pattern seen for size in this study. Beech seed falls are correlated with higher mice densities (Powell et al. 1997).

Mice have been proposed as the tentative secondary host for the parasite *Skrjabingylus nasicola* before it infects stoats (King et al. 1982f; King 1991a). It is unlikely that the parasite had a negative effect on mandible shape, because an increase in mice, while correlated with an increase in infection as well as decreasing

rainfall, is also correlated with a more efficient mandible muscle lever system which provides greater relative strength (King et al. 1982f; King 1991a).

Differences in diet hardness do result in differences in mandible shape if the differences are large enough (Anderson et al. 2014). It was possible that the diet PLS was not as significant as the environmental PLS because there was not enough variation in diet hardness across habitats. Mice typically have more variable diet, from vegetarian to insectivorous to omnivorous, therefore the differences in mandible shape were more pronounced (Renaud et al. 2010; Anderson et al. 2014; Renaud et al. 2015). Comparing species, like many of the diet and shape correlation studies, would likely have shown something different (Caumul et al. 2005; Nogueira et al. 2009; Galindo-Gil et al. 2015). For the three species studied in the first results chapter, there was not enough diet data for a cross-species PLS.

The GV rainfall figure was an issue with the environmental PLS because it was probably much lower than the actual rainfall for the valley. This was because the weather station was on the Canterbury side of the Southern Alps and not in the alps themselves which typically have a higher annual rainfall than Canterbury. GV is closer to the flat farmland on the lee side of the Southern Alps, which does have less rainfall than the centre of the Alps (Sturman et al. 2001).This would explain why GV is up with EN on the environmental PLS (Sturman et al. 2001).

As for the between species analyses, there were some problems with obtaining enough specimens. Unlike the previous analyses, the largest issue was with the males. HVY only had three adult male specimens, and both SI and RI only had eight males each, yet there were plenty of females. This could have been because the trapping period when my specimens were caught was October through to May. Based on the slower growth and later puberty of males compared to females, more females caught towards the end of the trapping period would have been adults, while many of the males were still sub-adults (King et al. 1982c). The females in the available pool of individuals for this study could range from several years old to only six to nine months (King et al. 1982c). However, males must be over a year old before they reach adult size, reducing the overall pool of available adult individuals (King et al. 1982c).

Adding in more habitat types and more representative of each habitat type, and obtaining stoats from all five haplotypes could aid in determining whether habitat type really does make a difference to mandible shape. It would also help determine whether haplotype really does have an effect, but that is hard to determine because with only three haplotypes are represented. The Department of Conservation has in its stores: Long Island, Coal Island, and also Northland samples (A. Veale, personal communication, November 18, 2016; C. Gillies, personal communication, November 25, 2016). Ideally stoats would also be obtained from: Waitakere ranges (North Island, kauri podocarp forest) (McKelvey et al. 1959), farmland stoats from both the North and South islands, Tongariro National park (scrub and podocarp forests) (Nicholls 1976) and also Mount Taranaki stoats (Podocarp forest with a high altitude range) (Clarkson 1985). To sample the full complement of haplotypes, stoats from Wellington and Dunedin would also need to be obtained (Veale et al. 2015). An attempt was made to collect some of these stoats, but trapping rates were low and several seasons could be required to collect enough for analyses.

Chapter Six

Conclusions

Drawing 7. Stoat standing in grass, done in watercolour paints. Artist: C. Hill.

Objective one conclusions

I identified six questions to be answered in the investigation on interspecific morphological differences of New Zealand mustelid mandibles.

1) Was there sexual dimorphism in mandible shape as well as in size? No shape sexual dimorphism was found, in any of the species, supporting the null hypothesis.

2) Did the degree of mandible sexual dimorphism differ between species? Yes, it did. All three species had different degrees of sexual dimorphism. Ferrets had a similar degree of sexual dimorphism as two other polecat species. Stoats and weasels had different degrees of sexual dimorphism compared with populations of the same species overseas. The results indicated that the weasels had the greatest degree of sexual competition and stoats the smallest.

3) and 4) Were any detectable differences in mandible shape across the species isometric or allometric with size? If allometric variation in shape was found, did size account for all or just a component of it? Only 5.94 % of shape variation between the species' mandibles was accounted for by size. However, there was variation particularly in the ramus end of the mandible that allometry did not account for.

5) How did shape differ between the species? The largest differences were along the ramus end of the mandible, used for muscle attachment. The ferret coronoid processes were proportionally smaller, and the angular processes larger than stoats and weasels. These indicated that ferrets had a stronger and more efficient muscle lever system, in particular for the killing bite and so required less muscle area. There was no support for the hypothesis of modularity either within or between species. This result could be because the species mandibles were too similar in shape or because they varied differently in three dimensions, not the two dimensional plane I photographed.

6) Did the bite force efficiency of the mandibles differ between the species? Yes, it did. This results reinforced the conclusions from the mandible shape differences, ferrets had a more efficient muscle to mandible force bite efficiency, and that stoats

and weasels required more muscle area to create extra force required for killing proportionally larger prey.

6.1 Objective two conclusions

I identified eight questions to be answered in the investigation on mandible plasticity and adaptations of New Zealand stoat mandibles across locations.

1) Was there sexual dimorphism in mandible shape as well as in size, which has been previously identified, *within* different locations from New Zealand? The resulted supported the null hypothesis. There was no shape sexual dimorphism between the sexes, only size sexual dimorphism.

2) Did the degree of size sexual dimorphism differ *between* locations? The results supported the alternative hypothesis. There was differences in the degree of sexual dimorphism between locations males ranged from 8.54-14.63 % larger than females. This could be because males vary more in size than do females because males are more affected by good supplies of food during growth years.

3) Were any detectable differences in mandible shape isometric or allometric with size? There was no consistent within location-between sex effects of size on shape supporting the null hypothesis. There was a small effect of size on shape (3.16 %) between locations, supporting the alternative hypothesis of allometry, though this was very small.

4) If any allometric variation in shape was found, did size account for all of it, or just a component of it? Size did not account for all of the shape variation between locations.

5) Was mandible morphology affected by the increased food available to young born during a beech (*Nothofagus* spp.) seed masting year? Mandible size changed with a beech seed year, EV males born during a seed year increased in size but females did not; mandible shape did not change (King et al. 1982b, c; King 1983, 2002). This supported the hypothesis from Powell et al. (1997); males are affected by short term food pulses.

6) How did shape differ *between* locations after the sexes were pooled for each location? There were some differences in mandible shape, these were mostly in the muscle attachment sites. This finding supported the alternative hypothesis that there was some difference in mandible shape across locations. RI was the most distinct shape and HV and AP were the most similar, while there were eight locations that were slightly different in shape: CP and MC, GV and EN, WL and RI, RI and SI. There was also no modularity detectable across the locations.

7) Did the bite force efficiency of the mandibles differ between the locations? Yes, there was support for the alternative hypothesis, differences in bite force efficiency of some locations. In some habitats the analysis showed greater relative strength of the temporalis muscle (MC, RI, and EN), in others of the masseter muscle (CP, PU, WL, and GV). There were no sites that had high efficiency of both muscle systems, it was either one or the other.

8) Was there any covariance between mandible shape and environmental or dietary factors? The results of the two PLS analyses indicate that rainfall had the highest correlation with mandible shape. Rainfall had a higher correlation with prey, particularly mice than did diet, probably because environmental data is easier to measure with greater accuracy than available diet.

6.2 Recommendations

I can suggest a few questions and ideas for furthering the results from my thesis.

1. A cross-species PLS on mandible shape and diet would help determine what factors of diet correlate most with changes in mandible shape between ferrets, stoats, and weasels. A gape width study of the three species could test the interesting hypothesis that stoats and weasels require a large gape to kill prey that is proportionally large to themselves, which would match their lower biomechanical advantage when compared with ferrets.

2. What is the correlation between skull size and mandible size? And how are they related to prey size? Previously it has been assumed there is a high correlation between the two measurements, and so there have been no reports of mandible size (King 1991b). The NPSS data of mandible length

and condylobasal length is extensive and should not require new specimens as all adults from the NPSS could be included.

3. More habitats could be added, and obtaining stoats representing all five haplotypes could aid in determining whether habitat type really does make a difference to mandible shape.

4. It would be interesting to see if the same size patterns are seen in European stoat populations to tree seed masting cycles, provided trapping and measuring the stoats was allowed as they are protected in many areas (Piontek et al. 2015).

5. An ontogenetic study of mandible shape and age would be a nice addition to the results of this study. Ideally more specimens of all ages should be collected from all habitats, in particular from EV, from both beech masting years and non-beech masting years to compare how the mandible grows under the different conditions. This was one of the original ideas for my thesis, but lack of samples distributed evenly across the large number of variables made it impractical. While the mandible shape ended up the same in adulthood from both birth conditions, the growth patterns may have differed. LaPoint et al. (2017) studied ontogenetic changes of skulls in stoats and weasels from the Northern Hemisphere. The size of the skull varied with season, and the variations depended on environmental conditions and sexes (LaPoint et al. 2017). It is likely that the development of the mandible may differ slightly among different growth conditions, either in shape or time taken to reach adult size (LaPoint et al. 2017).

Stoat (with apologies to D. H. Lawrence)

A stoat came to my water-trough
On a hot, hot day, and I in my shirtsleeves for the heat,
To drink there.

In the deep, un-scented shade of the great pohutukawa tree
I came down the steps with my pitcher
And must wait, must stand and wait, for there he was at the trough before me.

He looked out from a fissure in the earth-wall in the gloom
And leaned his chestnut-brown body down, jumped over the edge of the stone trough
And landed upon the stone bottom,
And where the water had dripped from the tap, in a small clearness,
He sipped with his small mouth,
Softly drank past his long whiskers, into his sleek long body,
Silently.

Someone was before me at my water-trough,
And I, like a second comer, waiting.

He lifted his head from his drinking, as cattle do,
And looked at me vaguely, as drinking cattle do,
And flickered his pink tongue round his lips, and mused a moment,
And stooped and drank a little more,
Being earth-brown, earth-golden from the secret tunnels of the earth
On a day of New Zealand January, with the sea shining.

The voice of my education said to me
He must be killed,
For in New Zealand the native species are innocent, the mustelids are introduced pests.

And voices in me said, If you were a man
You would take a stick and break him now, and finish him off.

But must I confess how I liked him,
How glad I was he had come like a guest in quiet, to drink at my water-trough
And depart peaceful, pacified, and thankless,
Into the secret tunnels of this earth?

Was it cowardice, that I dared not kill him? Was it perversity, that I longed to talk to him?
Was it humility, to feel so honoured?
I felt so honoured.

And yet those voices:
If you were not afraid, you would kill him!

And truly I was afraid, I was most afraid of his teeth, But even so, honoured still more
That he should seek my hospitality
From out the dark door of the tunnelled earth.

He drank enough
And lifted his head, dreamily, as one who has drunken,
And flickered his tongue as if to taste the air, so pink,
Seeming to lick his lips,
And looked around like a god, unseeing, into the air,
And calmly turned his head,
And then, as if he had all the time in the world,
He casually jumped up to the edge of the water trough
And climbed again the broken bank of my wall-face.

And as he put his head into that dreadful hole,
And as he climbed up, snake-easing his shoulders to enter the darkness farther,
A sort of horror, a sort of protest against his withdrawing into that horrid black hole,
Deliberately going into the blackness, assuming there could be nothing hurtful within,
Overcame me now his back was turned.

I looked round, I put down my pitcher,
I picked up a clumsy log
And threw it at the water-trough with a clatter.

I think it did not hit him,
But suddenly his hindquarters and tail convulsed in undignified haste.
Flicked like lightning, and was gone
Into the black hole, the earth-lipped fissure in the wall-front,
At which, in the intense still noon, I stared with fascination.

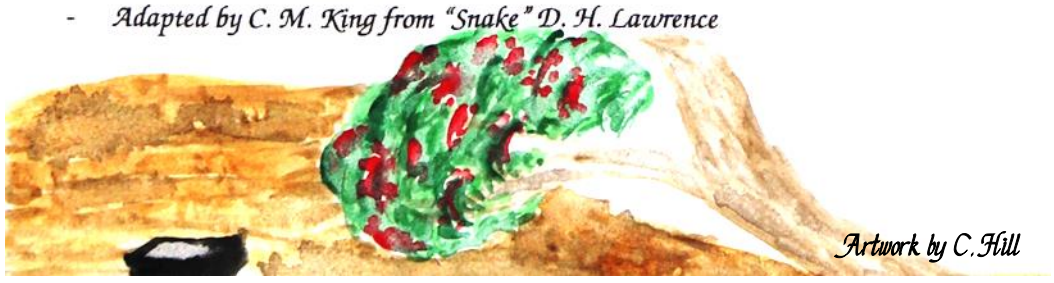
And immediately I regretted it.
I thought how paltry, how vulgar, what a mean act!
I despised myself and the voices of my accursed human education.

And I thought of the albatross
And I wished he would come back, my stoat.

For he seemed to me again like a king,
Like a king in exile, uncrowned in a strange land,
Never to be crowned again.

And so, I missed my chance with one of the lords
Of life.
And I have something to expiate:
A pettiness.

- *Adapted by C. M. King from "Snake" D. H. Lawrence*



Artwork by C.Hill

References

Abramov AV, Tumanov IL 2003. Sexual dimorphism in the skull of the European mink *Mustela lutreola* from NW part of Russia. Acta Theriologica 48: 239-246.

Adams DC, Cardini A, Monteiro LR, O'Higgins P, Rohlf FJ 2011. Morphometrics and phylogenetics: Principal components of shape from cranial modules are neither appropriate nor effective cladistic characters. Journal of Human Evolution 60: 240-243.

Adams DC, Rohlf FJ, Slice DE 2004. Geometric morphometrics: Ten years of progress following the 'revolution'. Italian Journal of Zoology 71: 5-16.

Aiello G, Barattolo F, Barra Det al. 2007. Fractal analysis of ostracod shell variability: A comparison with geometric and classic morphometrics. Acta Palaeontologica Polonica 52: 563-573.

Anderson PSL, Renaud S, Rayfield EJ 2014. Adaptive plasticity in the mouse mandible. Bmc Evolutionary Biology 14: 9.

Anthwal N, Peters H, Tucker AS 2015. Species-specific modifications of mandible shape reveal independent mechanisms for growth and initiation of the coronoid. Evodevo 6: 14.

Baker AJ, Moeed A 1979. Evolution in the introduced new zealand populations of the common myna, *Acridotheres tristis* (Aves: Sturnidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie 57: 570-584.

Biknevicius AR, Van Valkenburgh B 1996. Design for killing: Craniodental adaptations of predators. In: Gittleman JL ed. Carnivore Behavior, Ecology and Evolution. Ithaca, NY, Cornell University Press. Pp. 393-428.

Bower LM, Piller KR 2015. Shaping up: A geometric morphometric approach to assemblage ecomorphology. Journal of Fish Biology 87: 691-714.

Brombin C, Salmaso L 2009. Multi-aspect permutation tests in shape analysis with small sample size. Computational Statistics & Data Analysis 53: 3921-3931.

Canady A, Onderkova A 2016. Are size, variability and allometry of the baculum in relation to body length signals of a good condition in male weasels *Mustela nivalis*. Zoologischer Anzeiger 264: 29-33.

Canty and Associates LLC 2016. Weatherbase: Borland burn, New Zealand. Retrieved October 4 2016, from http://www.weatherbase.com/weather/weatherall.php3?s=596464&cityname=Borland+Burn%2C+Southland%2C+New+Zealand&units=

Cardini A, Elton S 2008. Does the skull carry a phylogenetic signal? Evolution and modularity in the guenons. Biological Journal of the Linnean Society 93: 813-834.

Cardini A, Seetah K, Barker G 2015. How many specimens do I need? Sampling error in geometric morphometrics: Testing the sensitivity of means and variances in simple randomized selection experiments. Zoomorphology 134: 149-163.

Catalano SA, Ercoli MD, Prevosti FJ 2015. The more, the better: The use of multiple landmark configurations to solve the phylogenetic relationships in musteloids. Systematic Biology 64: 294-306.

Caumul R, Polly PD 2005. Phylogenetic and environmental components of morphological variation: Skull, mandible, and molar shape in marmots (Marmota, Rodentia). Evolution 59: 2460-2472.

Christiansen P 2008. Evolution of skull and mandible shape in cats (Carnivora: Felidae). Plos One 3: 8.

Christiansen P, Wroe S 2007. Bite forces and evolutionary adaptations to feeding ecology in carnivores. Ecology 88: 347-358.

Clarkson BD 1985. The vegetation of the Kaitake range Egmont-National-Park, New-Zealand. New Zealand Journal of Botany 23: 15-31.

Clayton RI, Byrom A, Anderson Det al. 2011. Density estimates and detection models inform stoat (*Mustela erminea*) eradication on Resolution Island, New Zealand. In: Veitch CR, Clout MN, Towns DR ed. Island Invasives: Eradication and Management. Gland, Switzerland, IUCN. Pp. 413-417.

Cornette R, Baylac M, Souter T, Herrel A 2013. Does shape co-variation between the skull and the mandible have functional consequences? A 3D approach for a 3D problem. Journal of Anatomy 223: 329-336.

Cuthbert R, Sommer E, Davis LS 2000. Seasonal variation in the diet of stoats in a breeding colony of Hutton's shearwaters. New Zealand Journal of Zoology 27: 367-373.

Dayan T, Simberloff D 1994. Character displacement, sexual dimorphism, and morphological variation among British and Irish mustelids. Ecology 75: 1063-1073.

Dilks P, Willans M, Pryde M, Fraser I 2003. Large scale stoat control to protect mohua (*Mohoua ochrocephala*) and kaka (*Nestor meridionalis*) in the Eglinton Valley, Fiordland, New Zealand. New Zealand Journal of Ecology 27: 1-9.

Dumont M, Wall CE, Botton-Divet L, Goswami A, Peigne S, Fabre AC 2016. Do functional demands associated with locomotor habitat, diet, and activity pattern drive skull shape evolution in musteloid carnivorans? Biological Journal of the Linnean Society 117: 858-878.

Elsasser SC, Parker GH 2008. Morphometric criteria for distinguishing species and age-cohorts of Ermine (*Mustela erminea*) and long-tailed weasel (*M. frenata*). Acta Zoologica Academiae Scientiarum Hungaricae 54: 75-88.

Erlinge S 1987. Why do European stoats *Mustela erminea* not follow Bergmann's rule? Holarctic Ecology 10: 33-39.

Freeman S, Herron JC 2007. Evolutionary Analysis. 4 ed. San Francisco, CA, Pearon Benjamin Cummings.

Galindo-Gil S, Rodriguez-Romero FD, Velazquez-Rodriguez AS, Moreno-Barajas R 2015. Morphological correlations between head shape, diet and habitat use of some Sceloporus of Mexico: A quantitative analysis. International Journal of Morphology 33: 295-300.

Gasilin VV, Kosintsev PA 2013. Identification of the subgenus *Martes* str. species (*Martes zibellina, M. martes, M. foina, and M. melampus*; Carnivora, Mustelidae) according to the mandibular metric characteristics. Biology Bulletin 40: 708-717.

Gillies C 2016. Diet of stoats (*Mustela erminea*) from Northland Kauri (*Agathis australis*) Podocarp forests, New Zealand.

Gittleman JL, VanValkenburgh B 1997. Sexual dimorphism in the canines and skulls of carnivores: Effects of size, phylogeny, and behavioural ecology. Journal of Zoology 242: 97-117.

Harmer R, Boswell R, Robertson M 2005. Survival and growth of tree seedlings in relation to changes in the ground flora during natural regeneration of an oak shelterwood. Forestry 78: 21-32.

Herring SW 1993. Functional-morphology of mammalian mastication. American Zoologist 33: 289-299.

Hildebrand M, Goslow G 2001. Analysis of vertebrate structure. 5 ed. Hildebrand M, Goslow G ed. NY, NY, John Wiley & Sons, Inc.

Izenman AJ 2008. Modern Multivariate Statistical Techniques: Regression, classification, and manifold learning. Casella G, Fienberg S, Olkin I ed. NY, Springer.

Jones C 2002. A model for the conservation management of a 'secondary' prey: sooty shearwater (*Puffinus griseus*) colonies on mainland New Zealand as a case study. Biological Conservation 108: 1-12.

King CM 1983. The relationships between beech (*Nothofagus* sp) seedfall and populations of mice (*Mus musculus*), and the demographic and dietary responses of stoats (*Mustela erminea*), in 3 New-Zealand forests. Journal of Animal Ecology 52: 141-166.

King CM 1989. The advantages and disadvantages of small size to weasels. In: Gittleman JL ed. Carnivore Behavior, Ecology and Evolution. Ithaca, NY, Cornell University Press. Pp. 302-334.

King CM 1991a. Age-specific prevalence and a possible transmission route for *Skrjabingylosis* in New Zealand stoats, *Mustela erminea*. New Zealand Journal of Ecology 15: 23-30.

King CM 1991b. Body size - prey size relationships in european stoats *Mustela erminea* - a test case. Holarctic Ecology 14: 173-185.

King CM 2002. Cohort variation in the life-history parameters of stoats *Mustela erminea* in relation to fluctuating food resources: A challenge to boreal ecologists. Acta Theriologica 47: 225-244.

King CM ed. 2005. The Handbook of New Zealand Mammals. 2 ed. Melbourne, Australia, Oxford University Press.

King CM, Flux M, Innes JG, Fitzgerald BM 1996a. Population biology of small mammals in Pureora forest park .1. Carnivores (*Mustela erminea*, *M. furo*, *M. nivalis*, and *Felis catus*). New Zealand Journal of Ecology 20: 241-251.

King CM, Innes JG, Flux M, Kimberley MO 1996b. Population biology of small mammals in Pureora Forest Park .2. The feral house mouse (Mus musculus). New Zealand Journal of Ecology 20: 253-269.

King CM, Innes JG, Flux M, Kimberley MO, Leathwick JR, Williams DS 1996c. Distribution and abundance of small mammals in relation to habitat in Pureora Forest Park. New Zealand Journal of Ecology 20: 215-240.

King CM, McDonald RM, Martin RD, Tempero GW, Holmes SJ 2007a. A field experiment on selective baiting and bait preferences of pest mustelids (*Mustela* spp.). International Journal of Pest Management 53: 227-235.

King CM, Moody JE 1982a. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .1. General introduction. New Zealand Journal of Zoology 9: 49-56.

King CM, Moody JE 1982b. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .2. Food-habits. New Zealand Journal of Zoology 9: 57-80.

King CM, Moody JE 1982c. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .3. Morphometric variation in relation to growth, geographical-distribution, and colonization. New Zealand Journal of Zoology 9: 81-102.

King CM, Moody JE 1982d. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .4. Reproduction. New Zealand Journal of Zoology 9: 103-118.

King CM, Moody JE 1982e. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .5. Molt and color-change. New Zealand Journal of Zoology 9: 119-130.

King CM, Moody JE 1982f. The biology of the stoat (*Mustela erminea*) in the national-parks of New Zealand .6. Infestation with *Skrjabingylus nasicola*. New Zealand Journal of Zoology 9: 131-140.

King CM, Powell RA 2007b. The natural history of weasels and stoats: Ecology, behavior and management. 2 ed. NY, NY, Oxford University Press Inc.

Klingenberg CP 2009. Morphometric integration and modularity in configurations of landmarks: Tools for evaluating *a priori* hypotheses. Evolution & Development 11: 405-421.

Klingenberg CP 2010. Evolution and development of shape: Integrating quantitative approaches. Nature Reviews Genetics 11: 623-635.

Klingenberg CP 2011. MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353-357.

Klingenberg CP 2013a. Cranial integration and modularity: Insights into evolution and development from morphometric data. Hystrix-Italian Journal of Mammalogy 24: 43-58.

Klingenberg CP 2013b. Visualizations in geometric morphometrics: How to read and how to make graphs showing shape changes. Hystrix-Italian Journal of Mammalogy 24: 15-24.

Klingenberg CP 2014. MorphoJ user's guide. Retrieved March 2016, from http://www.flywings.org.uk/MorphoJ_guide/frameset.htm?index.htm

Klingenberg CP 2016. Size, shape, and form: Concepts of allometry in geometric morphometrics. Development Genes and Evolution 226: 113-137.

Klingenberg CP, Gidaszewski NA 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. Systematic Biology 59: 245-261.

Koepfli KP, Deere KA, Slater GJet al. 2008. Multigene phylogeny of the Mustelidae: Resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. Bmc Biology 6: 22.

Kruska DCT, Sidorovich VE 2003. Comparative allometric skull morphometrics in mink (*Mustela vison* Schreber, 1777) of Canadian and Belarus origin; taxonomic status. Mammalian Biology 68: 257-276.

Lanszki J, Heltai M 2007. Diet of the European polecat and the steppe polecat in Hungary. Mammalian Biology 72: 49-53.

LaPoint S, Keicher L, Wikelski M, Zub K, Dechmann DKN 2017. Growth overshoot and seasonal size changes in the skulls of two weasel species. Royal Society Open Science 4.

Lee S, Mill PJ 2004. Cranial variation in British mustelids. Journal of Morphology 260: 57-64.

Loy A, Spinosi O, Carlini R 2004. Cranial morphology of *Martes foina* and *M. martes* (Mammalia, Carnivora, Mustelidae): The role of size and shape in sexual dimorphism and interspecific differentiation. Italian Journal of Zoology 71: 27-34.

Lynch JM, Osullivan WM 1993. Cranial form and sexual dimorphism in the irish otter *Lutra lutra* L. Biology and Environment-Proceedings of the Royal Irish Academy 93B: 97-105.

Martin-Serra A, Figueirido B, Perez-Claros JA, Palmqvist P 2015. Patterns of morphological integration in the appendicular skeleton of mammalian carnivores. Evolution 69: 321-340.

McDonald RA, King CM 2008. Genus *Mustela*: Stoat *Mustela erminea*. In: Harris S, Yalden DW ed. Mammals of the British Isles: Handbook. 4 ed. Southhampton, UK, The Mammal Society. Pp. 455-467.

McDonald RA, Vaughan N 1999. An efficient way to prepare mammalian skulls and bones. Mammal Review 29: 265-266.

McDonald RA, Webbon C, Harris S 2000. The diet of stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) in Great Britain. Journal of Zoology 252: 363-371.

McKelvey PJ, Nicholls JL 1959. The indigenous forest types of North Auckland. New Zealand Journal of Forestry 8: 29-45.

McMurtrie P, Edge KA, Crouchley D, Gleeson D, Willans M, Veale A 2011. Eradication of stoats (*Mustela erminea*) from Secretary Island, New Zealand. In: Veitch CR, Clout MN, Towns DR ed. Island Invasives: Eradication and Management. Gland, Switzerland, IUCN. Pp. 455-460.

McNulty KP, Vinyard CJ 2015. Morphometry, geometry, function, and the future. Anatomical Record-Advances in Integrative Anatomy and Evolutionary Biology 298: 328-333.

Meloro C, O'Higgins P 2011a. Ecological adaptations of mandibular form in fissiped Carnivora. Journal of Mammalian Evolution 18: 185-200.

Meloro C, Raia P, Carotenuto F, Cobb SN 2011b. Phylogenetic signal, function and integration in the subunits of the carnivoran mandible. Evolutionary Biology 38: 465-475.

Met Office 2016. Sutton Bonington Historical Weather 1976-1982. Retrieved 28/11/2016 2016, from http://www.metoffice.gov.uk/pub/data/weather/uk/climate/stationdata/suttonboningtondata.txt

Meteorological Service of New Zealand Ltd 2008-2016 2016. Coromandel. Retrieved May, 17 2016, from http://www.metservice.com/rural/coromandel

Monteiro LR, Duarte LC, dos Reis SF 2003. Environmental correlates of geographical variation in skull and mandible shape of the punare rat *Thrichomys apereoides* (Rodentia : Echimyidae). Journal of Zoology 261: 47-57.

Mullin SK, Taylor PJ 2002. The effects of parallax on geometric morphometric data. Computers in Biology and Medicine 32: 455-464.

Murphy EC, Bradfield P 1992. Change in diet of stoats following poisoning of rats in a New-Zealand forest. New Zealand Journal of Ecology 16: 137-140.

Murphy EC, Clapperton BK, Bradfield PMF, Speed HJ 1998. Effects of rat-poisoning operations on abundance and diet of mustelids in New Zealand podocarp forests. New Zealand Journal of Zoology 25: 315-328.

Murphy EC, Gillies C, Maddigan Fet al. 2016. Flexibility of diet of stoats on Fiordland islands, New Zealand. New Zealand Journal of Ecology 40: 114-120.

Nicholls JL 1976. A revised classification of the North Island indigenous forests. New Zealand Journal of Forestry 21: 105-132.

NIWA 2016. The national climate database. Retrieved May, 17 2016, from http://cliflo.niwa.co.nz/

Nogueira MR, Peracchi AL, Monteiro LR 2009. Morphological correlates of bite force and diet in the skull and mandible of phyllostomid bats. Functional Ecology 23: 715-723.

Norbury G, McGlinchy A 1996. The impact of rabbit control on predator sightings in the semi-arid high country of the South Island, New Zealand. Wildlife Research 23: 93-97.

Olsen AM, Westneat MW 2015. StereoMorph: An R package for the collection of 3D landmarks and curves using a stereo camera set-up. Methods in Ecology and Evolution 6: 351-356.

Packham JR, Thomas PA, Atkinson MD, Degen T 2012. Biological flora of the British Isles: *Fagus sylvatica*. Journal of Ecology 100: 1557-1608.

Pallares LF, Turner LM, Tautz D 2016. Craniofacial shape transition across the house mouse hybrid zone: Implications for the genetic architecture and evolution of between-species differences. Development Genes and Evolution 226: 173-186.

Piontek AM, Wierzbowska IA, Bevanger K, Tokarz WM 2015. Comparison of the diet of stoat (*Mustela erminea*) in relation to sex and season in Norway. Mammal Research 60: 301-307.

Powell RA, King CM 1997. Variation in body size, sexual dimorphism and age-specific survival in stoats, *Mustela erminea* (Mammalia: Carnivora), with fluctuating food supplies. Biological Journal of the Linnean Society 62: 165-194.

Pucek Z, Jedrzejewski W, Jedrzejewska B, Pucek M 1993. Rodent population-dynamics in a primeval deciduous forest (Bialowieza National- Park) in relation to weather, seed crop, and predation. Acta Theriologica 38: 199-232.

Purdey DC, King CM, Lawrence B 2004. Age structure, dispersion and diet of a population of stoats (*Mustela erminea*) in southern Fiordland during the decline phase of the beechmast cycle. New Zealand Journal of Zoology 31: 205-225.

Renaud S, Auffray JC 2010. Adaptation and plasticity in insular evolution of the house mouse mandible. Journal of Zoological Systematics and Evolutionary Research 48: 138-150.

Renaud S, Rodrigues HG, Ledevin R, Pisanu B, Chapuis JL, Hardouin EA 2015. Fast evolutionary response of house mice to anthropogenic disturbance on a Sub-Antarctic island. Biological Journal of the Linnean Society 114: 513-526.

Rogers E 1986. Looking at vertebrates: A practical guide to vertebrate adaptations. NY, NY, Longman Group Limited.

Rohlf FJ 2015. The tps series of software. Hystrix-Italian Journal of Mammalogy 26: 9-12.

Salmaso F, Molinari A, Di Pierro Eet al. 2009. Estimating and comparing food availability for tree-seed predators in typical pulsed-resource systems: Alpine conifer forests. Plant Biosystems 143: 258-267.

Sato JJ, Wolsan M, Prevosti FJet al. 2012. Evolutionary and biogeographic history of weasel-like carnivorans (Musteloidea). Molecular Phylogenetics and Evolution 63: 745-757.

Schutz H, Guralnick RP 2007. Postcranial element shape and function: Assessing locomotor mode in extant and extinct mustelid carnivorans. Zoological Journal of the Linnean Society 150: 895-914.

Scott JE, McAbee KR, Eastman MM, Ravosa MJ 2014. Teaching an old jaw new tricks: Diet-induced plasticity in a model organism from weaning to adulthood. Journal of Experimental Biology 217: 4099-4107.

Sheets HD 2001. IMP series. Dept. of Physics, Canisius College, NY, http://www.canisius.edu/~sheets/morphsoft.html.

Siahsarvie R, Auffray JC, Darvish Jet al. 2012. Patterns of morphological evolution in the mandible of the house mouse *Mus musculus* (Rodentia: Muridae). Biological Journal of the Linnean Society 105: 635-647.

Siegel AF, Benson RH 1982. A robust comparison of biological shapes. Biometrics 38: 341-350.

Sjoberg K, Pettersson RB, Ball JP, Sundstrom T 2007. Seed crops of Norway spruce and winter habitat quality for boreal birds: Old-growth compared with managed forests. Annales Zoologici Fennici 44: 486-495.

Smith DHV, Moller H, Wilson DJ, Murphy EC 2010. Prey switching by stoats (*Mustela erminea*): A supplemental food experiment. Wildlife Research 37: 604-611.

Smith DHV, Wilson DJ, Moller H, Murphy EC, Pickerell G 2008. Stoat density, diet and survival compared between alpine grassland and beech forest habitats. New Zealand Journal of Ecology 32: 166-176.

StatSoft Inc. 2014. STATISTICA (data analysis software system). Version 12. www.statsoft.com, StatSoft Inc.

Sturman A, Wanner H 2001. A comparative review of the weather and climate of the Southern Alps of New Zealand and the European Alps. Mountain Research and Development 21: 359-369.

Sumption KJ, Flowerdew JR 1985. The ecological effects of the decline in rabbits (*Oryctolagus cuniculus* L.) due to myxomatosis. Mammal Review 15: 151-186.

Suzuki S, Abe M, Motokawa M 2011. Allometric comparison of skulls from two closely related weasels, *Mustela itatsi* and *M. sibirica*. Zoological Science 28: 676-688.

Veale AJ, Gleeson DM, Clout MN 2014. Measuring connectivity of invasive stoat populations to inform conservation management. Wildlife Research 41: 395-406.

Veale AJ, Holland OJ, McDonald RA, Clout MN, Gleeson DM 2015. An invasive non-native mammal population conserves genetic diversity lost from its native range. Molecular Ecology 24: 2156-2163.

Wojcik JM, Wojcik AM, Sikorski MD 2003. Morphometric variation of the common shrew, *Sorex araneus*, in different habitats. Mammalia 67: 225-231.

Wroe S, Chamoli U, Parr WCH, Clausen P, Ridgely R, Witmer L 2013. Comparative biomechanical modeling of metatherian and placental saber-tooths: A different kind of bite for an extreme pouched predator. Plos One 8: 9.

Yom-Tov Y, Yom-Tov S, Angerbjorn A 2010. Body size of the weasel *Mustela nivalis* and the stoat *M. erminea* in Sweden. Mammalian Biology 75: 420-426.

Zelditch ML, Swiderski DL, Sheets HD 2012a. Geometric morphometrics for biologists: A primer. Second ed. London, UK, Academic Press.

Zelditch ML, Swiderski DL, Sheets HD 2012b. A practical companion to geometric morphometrics for biologists: Running analyses in freely-available software. London, UK, Academic Press.

Zub K, Piertney S, Szafranska PA, Konarzewski M 2012. Environmental and genetic influences on body mass and resting metabolic rates (RMR) in a natural population of weasel *Mustela nivalis*. Molecular Ecology 21: 1283-1293.