An investigation into the quality of carrion crow *Corvus corone* feathers in England and Wales

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Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Abstract

Flight feathers are crucial for foraging, predator avoidance and large-scale movements in most avian populations. However, the structural integrity of these feathers can be compromised by growth defects, negatively impacting flight ability and survival. Poor feather condition is characterised by the presence of fault bars, which are weak areas displayed as translucent bands that appear through the width of the feather vane. Fault bars occur as a result of stressful or adverse environmental conditions during feather growth. The thesis investigated the macroscopic and microscopic characteristics of this growth defect in relation to current formation theories, assessed different feather quality measures and explored possible causes and consequences of fault bars. The study was carried out at RSPCA Stapeley Grange Wildlife Centre, which receive a large number of carrion crow *Corvus corone* admissions displaying poor feather condition each year.

Firstly, the macroscopic and microscopic characteristics of fault bars were observed, reviewing existing fault bar formation theories and imagery with the use of advanced technology. Unique observational evidence was presented from this, identifying a ‘squeezed’ appearance to the barbules within fault bars, supporting the hypothesis of muscular constriction around the growing feather pin. Moreover, for the first time, images of fault bar occurrence within the growing feather pin were presented.

Relationships between different measures of feather quality were then assessed, accounting for differences between feather type (primary, secondary and tail), in reference to the ‘fault bar allocation’ hypothesis (Jovani & Blas, 2004). In this, fault bars occur on feathers that are least important for flight, resulting in the majority of fault bars being located on the tail feathers, with the lowest numbers in the primary feathers. A variety of feather quality measures were assessed here, including the number of fault bars, average width of fault bars, feather iridescence and strength, in addition to the number of snapped and white feathers. A key finding in this study was the relationship between the average width of fault bars and average feather iridescence across all feather types. This information strengthens our knowledge of how dull feather portray honest communication signals of low fitness. Moreover, average feather strength was found to be an independent measure of quality, with generally no relationship found with other measures of quality.

Average fault bar width measurements were used to investigate the causes of fault bar production in relation to chemical profile of feathers, parasite burden, sex and age (study aim 3). This made a valuable and novel discovery, identifying a possible link between calcium deficiency and fault bar occurrence. Calcium an essential element in skeletal mineralisation and eggshell formation. Therefore, the results of this study add to the knowledge of calcium and its role in fitness, expanding to feather quality. This study also found a potential trade-off between costly immune defences facilitated at the cost of feather quality, where low numbers of endoparasite species associated with wide fault bars in the wing feathers. Poor feather quality was not found to vary between sexes, as carrion crows are monomorphic and non-migratory. In regard to age differences, the tail feathers of younger individuals were found to have the widest fault bars. This supports many other studies in highlighting the vulnerability of juveniles during the feather growth period.

Lastly, average feather strength measurements were used to investigate the consequences of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, sex and age (study aim 4). A key finding here was that stress resistant bases were associated with a high proportion of chlorine in primary flight feathers. Links to parasite burden and sex were not identified; however, in line with the above findings, younger individuals were found to have low stress tolerance in the primary feathers compared to adults. Differences in feather strength in relation to fault bar occurrence was also reviewed. Contrary to predictions, no differences in strength were found between feather regions with fault bar occurrence and those with fault bar absence.

Future research in this field could be extended to nestlings, an age group that was unfortunately excluded from this study due to the presentation of pin feathers. Moreover, research could also be broadened to additional species, as fault bars are found to impact a wide variety of passerine and non-passerine individuals. This may then open opportunities in understanding stressors faced by vulnerable species, aiding future conservation efforts.

Chapter 1: Poor feather condition in UK *Corvidae* species – general introduction

1.1 Introduction to feathers

Over tens of millions of years, birds have evolved their front extremities to accomplish flight; one of most complex modes of locomotion (Sullivan *et al*., 2017; Videler, 2007). Flight aids foraging, predator avoidance and large-scale movements in most avian populations (Videler, 2007). Wings contain two types of flight feathers; also known as remiges, which consist of primary and secondary feathers. Primary feathers are the largest and strongest flight feathers found on the outer part of the wing, providing thrust during flapping flight (Ginn & Melville, 1983; Videler, 2007; Fig.1.1). In the majority of bird species, there are 10 primary feathers, with the 10th smallest and most distal primary being classed as the 'remicle' (Ginn & Melville, 1983; del Hoyo, Elliott & Sargatal, 1992; Fig.1.1). Primary feathers are rigidly attached to the wing bone, allowing them no freedom of movement (Sullivan *et al*., 2017). Consequently, the feathers spread out when the elbow and wrist joint are fully extended (Sullivan *et al*., 2017). Secondary feathers are located between the carpal joint and the body and provide lift throughout flight (Ginn & Melville, 1983; Videler, 2007; Fig.1.1). Unlike primaries, secondary feathers have a flexible attachment to the ulna, having the ability to hinge up and down (Sullivan *et al*., 2017; Pennycuick, 2008). In order to increase the camber of the wing, these feathers are pulled downward by the tightened patagial tendon (Sullivan *et al*., 2017; Pennycuick, 2008). The tail has a generally directional purpose, providing lift and drag forces during flight (Balmford, Jones & Thomas, 1994; Norberg, 1994; Thomas, 1997; Tubaro, 2003).

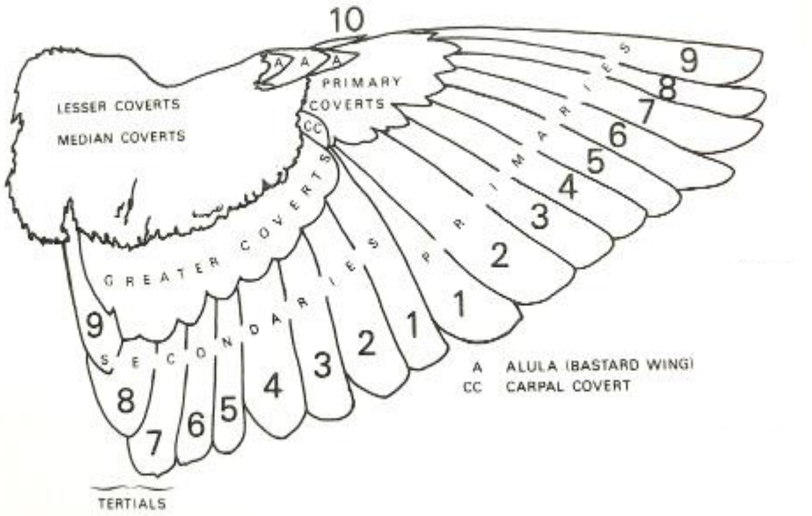


Fig.1.1 The topography of a bird's wing. Reprinted from "Moult in Birds: BTO Guide 19", by Ginn & Melville (1983), British Trust for Ornithology.

In some species, tails are also an indicator of reproductive potential, as they are often used as ornamental traits in sexual selection (Fitzpatrick & Price, 1997; Videler, 2007). Although tail feathers may be less important for flight, they are strong and robust in order to resist lift forces and to also avoid damage from contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Fig.1.1 also illustrates the coverts, a feather group which are thought to contribute to aerofoil properties (Lucas & Stettenheim, 1972; Videler, 2005). Yet, recent studies have found this feather group to serve other functions such as lift enhancement and provision of sensory information (Brown & Fedde, 1993; Carruthers *et al*., 2007; Wang & Clark, 2015). Moreover, covert function has been found to vary between species and warrants further research (Wang & Clark, 2015).

The shape and structure of the wings makes flight possible (Videler, 2007). The feathers in a wing slightly overlap and create a horizontal plane, catching the air below (Videler, 2007). The wing is also cambered like an aerofoil, capable of handling intense loads in flight (Müller & Patone, 1998; Sullivan *et al*., 2017; Videler, 2007). The camber of the wing is an important aspect in flight, outlined in Bernoulli's Principle (1738; Fig.1.2). Fig.1.2 illustrates this principle, showing the air splitting at the wing's leading edge, passing above and below the wing at different speeds, meeting again at the trailing edge (Bernoulli, 1738; NASA, 2021). The curved upper edge of the wing results in a rush of air over the top of the wing, which increases in velocity (Bernoulli, 1738; NASA, 2021). In contrast, the air below the wing moves in a straight line, maintaining constant speed and pressure (Bernoulli, 1738; NASA, 2021). Lift is then created due to high pressure moving towards the area of low pressure above the wing (Bernoulli, 1738; NASA, 2021). Due to this, the faster the bird flies, the greater the lift generated (Bernoulli, 1738; NASA, 2021). Forwards motion is then made possible by thrust (Bernoulli, 1738; NASA, 2021).

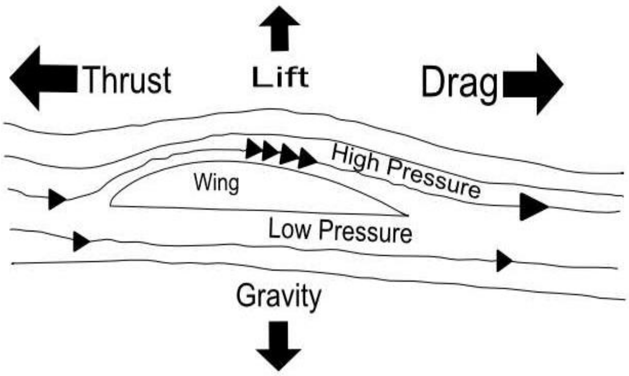


Fig.1.2 Four forces of flight (Kett, 2022).

Flight is facilitated by hollow pneumatic bones, which increase oxygen flow around the body by connecting to the respiratory system and also reduces weight (Proctor & Lynch, 1998; Sullivan *et al*., 2017). In addition, the lightweight structure of feathers aids in providing efficient flight. For example, the main feather shaft is made up of the rachis and calamus, which consist of a keratinous tube filled with lightweight medullary foam (Tubaro, 2003; Fig.1.3). Branching out from the rachis is the feather vane, which is made up of interlocking barbs (Corning & Biewener, 1998; Sullivan *et al*., 2017; Videler, 2007; Fig.1.3). Barbules branch out from the barbs and interlock with one another using minute hooks (Corning & Biewener, 1998; Sullivan *et al*., 2017; Videler, 2007; Fig.1.3). This complex interlocking system is crucial for flight, by limiting the amount of air flow through the feathers, creating more efficient lift (Corning & Biewener, 1998; Videler, 2007).

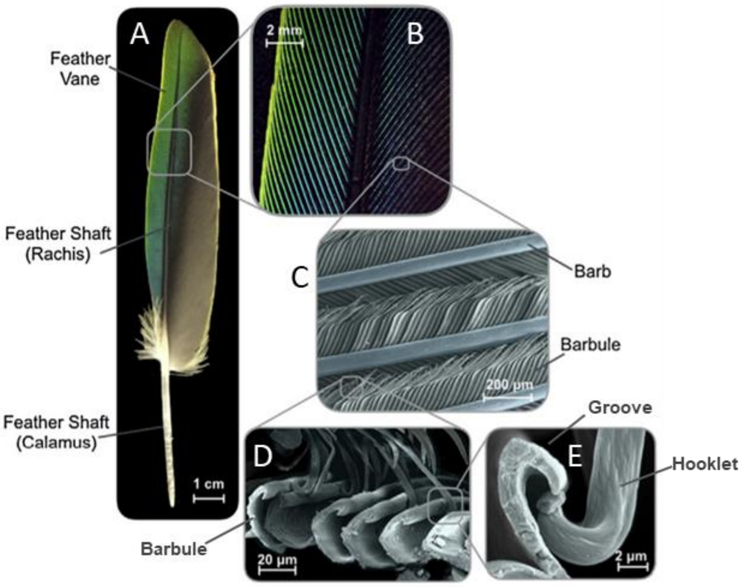


Fig.1.3 Feather anatomy diagram reprinted from Sullivan *et al.* (2016, 2017) with additional annotations. (A) Feather shaft and vane (B) Magnified section of feather vane showing barbs (C) overlapping barbules extending from barbs (D) Cross section illustrating barbule connection (E) hooklet sliding into the grooved proximal barbule.

Feathers must withstand large amounts of stress from air pressure during flight, requiring a crucial balance between stiffness and flexibility to avoid breakage (Bachmann et al., 2012; DesRochers *et al.,* 2009). A high tolerance to stress is associated with higher flexibility. In this, flexible feathers generate less lift and bend when pressure becomes too strong (Sullivan *et* *al.*, 2017). In contrast, materials that have low tolerance to stress are stiffer. Stiffer feathers are important to generate lift; however, when pressure becomes too strong, they break (Zhao *et al.,* 2020). Feather strength can be determined by flexural stiffness, described using the term 'Young's Modulus' (Zee Ma & Holditch, 2016; Worcester, 1996). Young's modulus is defined as the ratio of stress to strain in a material, where stress is the amount of force applied per unit area (compression) and strain is the extension per unit length (distortion of the material) (Jastrzebski, 1959; Zee Ma & Holditch, 2016; Worcester, 1996). Fig.1.4 shows different air pressures during flight (Corning & Biewener, 1998), using the four wing positions adapted from Brown (1948; Tab.1.1). In this study, metal foil strain gauges were attached to the wing, to calculate functional stresses based on published values for the young's modulus of feather keratin (Corning & Biewener, 1998). In this, lift forces produced negative (compressive) strains; therefore, negative peaks correspond to the downstroke and positive (tensile) peaks correspond to the upstroke (Corning & Biewener, 1998; Tab.1.1; Fig.1.4). This is because decreasing lift is linked to decreasing pressure on the wing (Corning & Biewener, 1998).

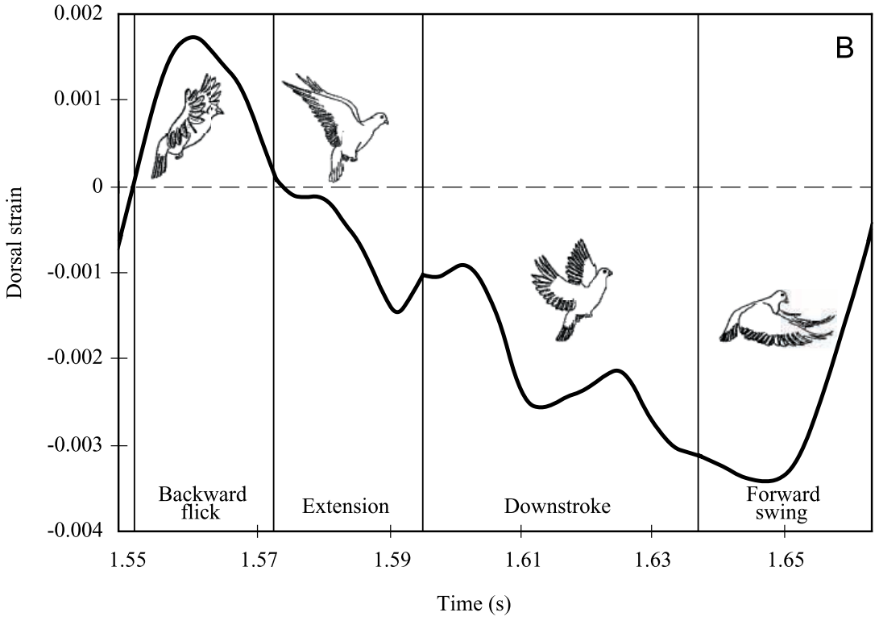


Fig.1.4 Representative strains recorded for an entire flight sequence, from the dorsal surface of a ninth primary feather shaft. Line drawings (adapted from Brown, 1948) show the position of the wings during each part of the wingbeat cycle. Reprinted from Corning & Biewener (1998).

Tab.1.1 Descriptions of the four different wing positions during flight (Brown, 1948).

Wing position	Description
(1) Downstroke:	Begins with the wings vertical, ends with them just below the horizontal and still fully extended.
(2) Forward swing:	Begins at the end of (1), includes the retraction and rotation of the manus, and ends with the wing tips facing forward and with their surfaces parallel.
(3) Changeover point:	This is the small time interval where the forward swing has finished and the next stage is about to begin.
(4) Backward flick:	The flexed wing is moved backward and upward and at the same time extended.
(5) Extension:	Here the wing, having completed (4), is extended fully prior to the repetition of (1).

Variations in strength across the different flight feathers account for differences in lift distribution across the wing, decreasing from maximum pressure at the proximal edge to a minimum at the distal edge (Müller & Patone, 1998). Consequently, lift decreases from proximal to distal, which is consistent with secondary feathers being responsible for lift (Müller & Patone, 1998). Moreover, feathers also vary in strength across the length of the structure (Butler & Johnson, 2004; Sullivan *et al.*, 2017). Feathers are stiffer and less tolerant of stress at the base and become gradually more flexible and resistant to stress towards the distal end of the feather (Butler & Johnson, 2004; Sullivan *et al.*, 2017). Therefore, it is important to consider that the more flexible end may protect the stiffer end from breaking. For example, a stiffer feather would act as a lever on the basal part of the feather, resulting in a higher risk of breakage. However, as the tip is more flexible, it reduces pressure by bending and prevents breakage of the feather closer to the base. Interestingly, a study on 13 different bird species found the distal end of flight feathers to be more flexible in larger birds (Worcester, 1996). A benefit of flexible feathers in larger birds is the potential for higher lift generation, as more flexible wings result in greater lift production in flapping flight (Gopalakrishnan & Tafti, 2010) as well as in the flight of insects (Mountcastle & Combes, 2013).

1.1.1 Feather pigments and minerals

Feather colouration provides honest communication signals of quality and fitness to conspecifics and is also important for species recognition (Griffith, Parker & Olson, 2006; Pacyna *et al.*, 2018). Plumage colourations come in two different forms; pigment-based colours and structural colours (Maia, D'Alba & Shawkey, 2011). Pigment based colours consist of carotenoids and melanins, which result from a wavelength-dependant absorbance of light (Maia *et al*., 2011; McGraw, 2006). Carotenoid pigments are acquired from diet and provide warm colours such as yellows, oranges and reds (Goodwin, 1984; Guay, Potvin & Robinson, 2012; McGraw, 2006). In contrast, melanin pigments are synthesized, giving rise to dark colours which are made up of eumelanin (black) and phaeomelanin (brown) (Guay *et al*., 2012; McGraw, 2006). In contrast, structural colours refract light, producing coherent scattering of incident light (Maia *et al*., 2011). Depending on the angle of observation, these feathers can sometimes vary in hue, presenting an iridescent shine (Maia *et al*., 2011). However, the presence of iridescence is dependent on the layering and angle of keratin amongst the melanin pigments (Brink & Van Der Berg, 2004; Doucet *et al.*, 2006; Maia *et al*., 2009). Ornamental feathers impose a handicap for the bearer, as these feathers are more susceptible to bacterial degradation (Javůrková *et al.*, 2019; Ruiz-Rodriguez *et al*., 2015). Fitter male individuals are able to maintain good feather quality as they have a larger uropygial gland which prevents bacterial growth (Leclaire *et al*., 2014; Møller, Erritzøe, & Nielsen, 2009). Therefore, good feather quality is an honest signal of immunocompetence against bacteria (Leclaire *et al*., 2014; Møller *et al.*, 2009). Iridescent feathers have also been found to have decreased hydrophobicity, again, emphasising the fitness costs associated with this variety of feather colouration (Eliason & Shawkey, 2011). Currently, little is known about the relationship between honesty signals in terms of feather colouration and fault bar occurrence, leading to a focus on this in future chapters.

Honesty signals are costly to produce, requiring sequestration of certain minerals via diet to stimulate the production of colouration pigments (Griffith, Parker & Olson, 2006; McGraw, 2003; Pacyna *et al*., 2018). For example, Hill and Montgomerie (1994) found the brightness of carotenoid-based colouration to be positively related to nutritional status in house finches (*Carpodacus mexicanus*). Moreover, a study on white-tailed eagles (*Haliaeetus albicilla*) and barn owls (*Tyto alba*) found a high concentration of calcium and zinc in the black, melanin-pigmented feathers compared to unpigmented white feathers (Neicke, Heide & Kruger, 1999). Melanic feather keratin is more resistant to stress than non-melanic keratin, owing to its increased thickness (Bonser, 1995). Therefore, due to calcium playing a role in the aggregation of melanin, this finding supports the importance of calcium in increasing feather stability (Bonser, 1995; Niecke, Heid & Kruger, 1999; Okazaki *et al*., 1985). However, in order to fully understand the relationship between melanin and metal ions, further research is required. Moreover, the chemical composition of feathers in terms of nutritional elements has not been studied in fault bars, leading to the assessment of this in future chapters.

The uptake of micro and macro-minerals into feathers is not always beneficial, as high levels of toxic heavy metals may accumulate through the diet in areas of environmental contamination (Dauwe *et al.,* 2003; Goede & de Bruin, 1984; Janssens *et al*., 2003; McGraw, 2003). Exposure to environmental contaminants such as aluminium, cadmium, mercury and lead have been found to cause sublethal chronic effects in birds (Edmonds *et al*., 2010; Scheuhammer, 1987). For example, lead poisoning is commonly seen in waterfowl species due to fishing litter, resulting in anorexia, muscle weakness and if not treated can be fatal (Stocker, 2005). Feathers have been widely used as indicators of metal pollution, as they are deposited in the feather structures during the growth period (Pacyna *et al*., 2018). This will be discussed further in section 1.1.6.2.

1.1.2 Poor feather quality

The structural integrity of feathers is crucial for energy efficient flight; therefore, resistance to abrasion and strain is an important requirement of feathers (Corning & Biewener, 1998; Echeverry-Galvis & Hau, 2013; Jovani & Rohwer, 2016). However, if stress is experienced during the growth stage of feathers, structural imperfections are formed. Once the feather is fully grown, no further changes are made to its structure as the feather is no longer in an active state (Jaspers *et al*., 2004). Consequently, poor quality feathers impact on a bird's fitness until the next moult cycle.

Feather quality can be measured in a variety of ways. For instance, feather strength in terms of rachis stiffness has been used as a proxy for feather quality in previous studies (Dawson *et al*., 2000; DesRochers *et al*., 2009; Pap *et al*., 2013). For example, a study on European starling *Sturnus vulgaris* feathers (Dawson *et al*., 2000) investigated how the rate of moult affects feather quality. This study found faster moulting primary feathers to be less resistant to wear, as they had thinner shafts and were less rigid/hard. Another study on European starlings focused on feather quality in relation to the stress hormone corticosterone (DesRochers *et* *al*., 2009), measuring rachis tensile strength. Tensile strength is the maximum stress that a material can withstand while being stretched or pulled before breaking (Britannica, 2021). This study found weaker flight feathers with altered microstructure when grown in the presence of high circulating corticosterone (DesRochers *et al*., 2009). Moreover, the bending stiffness of house sparrow *Passer domesticus* feathers was analysed in relation to chronic coccidian infestation (Pap *et al*., 2013). Results of this study found infested, non-medicated birds to grow poorer quality flight feathers in a large number of feather traits both after the first and second moults (Pap *et al*., 2013). In this, primary feathers were shorter and lighter with a smaller vane area, had thinner rachis and decreased stiffness (Pap *et al*., 2013). From this it was suggested that parasites may have serious, long-lasting effects on feather quality, ultimately impacting the fitness of the host (Pap *et al*., 2013).

Poor feather quality is also identified when particular structural characteristics are evident. For instance, structural weakness in the form of fault bars (Fig.1.5), also known as stress marks or fret marks, may lead to feather breakage and/or ragged edges due to lack of keratin (Dawson, Bortolotti & Murza, 2001; Kose, Mänd & Møller, 1999; RSPCA, 2013). These weak areas are displayed as translucent bands that appear through the width of the feather vane and are characterised by a malformation of barbules (Fig.1.5) (Erritzøe, 2006; Jovani & Rohwer, 2016). Fault bars occur on the outer and/or inner vane of the feather (Erritzøe, 2006). One of the first scientists to describe fault bars was Riddle in 1908, which has recently received increasing attention (Erritzøe, 2006; Jovani & Rohwer, 2016; Møller *et al.*, 2009). Riddle described fault bars as the “total or partial absence” of barbules, extending across the feather-vane at approximately 90° from the shaft and always running parallel with one another (Riddle, 1908). Fig.1.6 shows a detailed illustration of this occurrence, describing a loss of barbules, peculiar cornification and massing of barbs, in addition to a loss of pigment in the shaft (Riddle, 1908). Murphy, Miller and King (1989) have since offered a similar description in the study of white-crowned sparrows (*Zonotrichia leucophrys gambelii*), describing fault bars as a “barbule-free segment of a barb” (Fig.1.7).

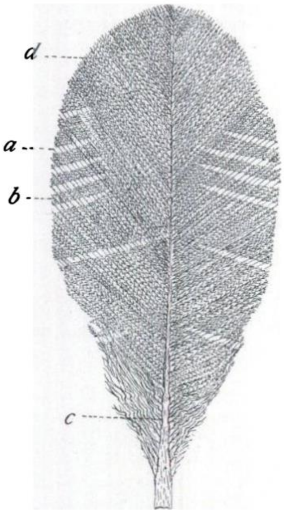


Fig.1.5 A poorly nourished chick feather showing abnormalities: a) abnormal area; b) "fundamental bar" (a day's growth); c) constrictions; d) region of defective lines shown plainly in this feather (Riddle, 1908).

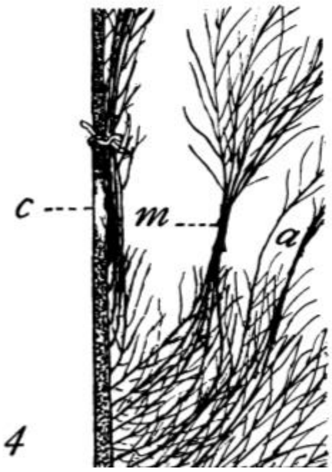


Fig.1.6 Starved chick feather showing a) loss of barbules in the fault-bar region; m) very peculiar cornification and massing of barbs; c) almost total absence of pigment in the shaft (Riddle, 1908).

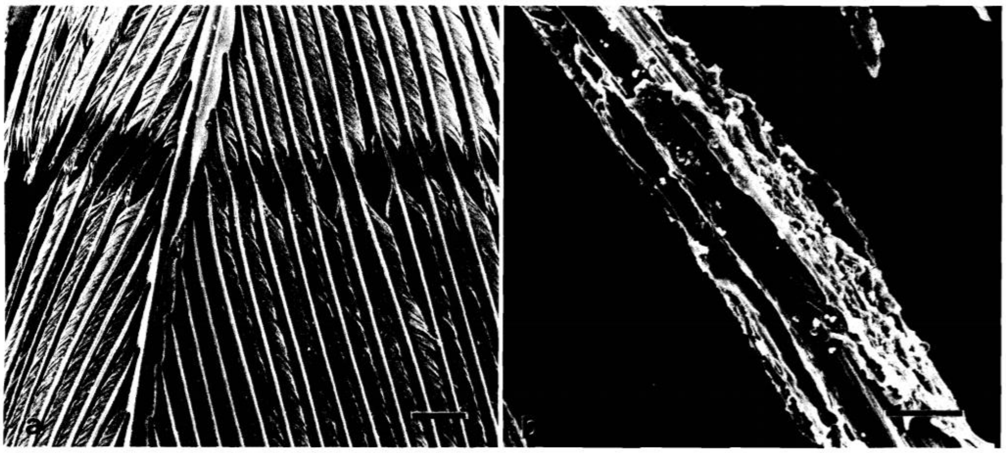


Fig.1.7 Scanning electron micrographs of (a) a fault bar (scale bar – 100um) and (b) the barbule-free segment of a barb characterising a fault bar (scale bar – 100um) in a white-crowed sparrow rectrix (Murphy, Miller & King, 1989).

Riddle (1908) stated that there are five types of feather faults, with varying severity. The first type of feather defect was described in the same way as the commonly known ‘fault bar’. He then moved on to describe the second type of feather malformation, which was the most extreme form of defect. This particular malformation consisted of an area of feather which entirely lacked barbs and barbules and can be seen in Fig.1.8 (Riddle, 1908). In contrast, the third type of defect could not be represented in a drawing due to its inconspicuous nature (Riddle, 1908). He described this feature as a “very minute depression” across the feather surface which appear in the same direction as the first type of feather defect (Riddle, 1908). He then went on to suggest that this could be a result of differences in light reflectivity (Riddle, 1908). In more recent years, the recognition of the third type of feather defect under a similar description was studied, which are now commonly known as ‘pallid bands’ (Ross *et al*., 2015; Fig.1.9). Pallid bands are weakened areas across the width of the feather vane and have reduced melanin (Fig.1.9) (Ross *et al*., 2015). This phenomenon has also been found to be a product of a diet deficient in sulphur-containing amino acids (Jovani & Rohwer, 2016; Murphy, King & Lu, 1988). Four common sulphur-containing amino acids are methionine, cysteine, homocysteine, and taurine (Brosnan & Brosnan, 2006). Methionine and cysteine are incorporated into proteins, with feather proteins containing proportionately more cystine than most other tissue and food proteins (Brosnan & Brosnan, 2006; Murphy & King, 1982; Murphy *et al*., 2007).

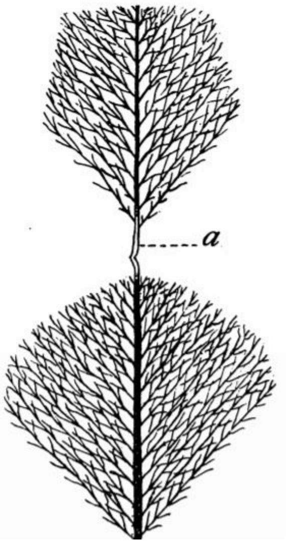


Fig.1.8 Riddle’s second type of feather defect. Abnormal region of a plume from an ostrich (*Struthio camelus*) chick. ‘*a*’ shows fault-bar in which shaft only is present. Reprinted from Riddle (1908).

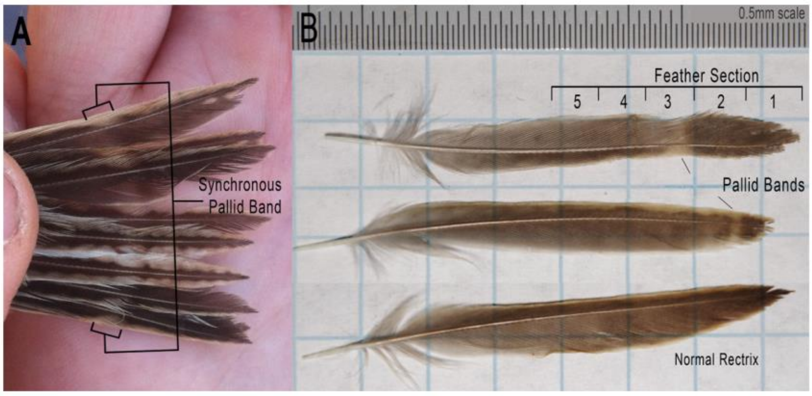


Fig.1.9 Photographs of grasshopper sparrow (*Ammodramus savannarum*) rectrices exhibiting pallid bands. Reprinted from Ross *et al.* (2015).

Riddle's fourth type of feather defect can be observed as a weakened or kinked area in the barb, where the shaft/rachis is constricted and weakened (Riddle, 1908; Fig.1.10). Fig.1.10 shows his illustration of a deeply constricted feather germ (also known as a feather pin) taken from a northern cardinal *Cardinalis virginianus* (Riddle, 1908). When this feather emerged from its sheath, it presented an extremely wavy appearance (Riddle,1908). Riddle suggested that the extreme feather constrictions were associated with its confinement and captivity in a cage (Riddle, 1908). The fifth type of feather defect is the least common and is described as a longitudinal fault bar on one side of the feather vane (Fig.1.11) (Riddle, 1908). Riddle suggested that this type of feather defect is caused by prolonged defective nutrition of a "segment of the circle of growing feather-elements" (Riddle, 1908). This is comparable to a type of fault identified in later years as a 'fault spot', where translucent patches run longitudinally across the feather (Erritzøe, 2006; Fig.1.12).

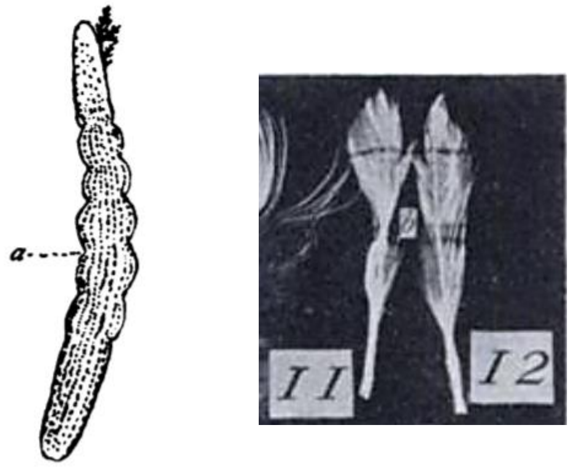


Fig.1.10. Left: Riddle's fourth type of feather defect. The longitudinal section shows at '*a*' an indentation of the pulp cavity by the epidermal layers. Right: Expanded sparrow *Passer domesticus* tail feather germs with '*b*' indicating a fault bar. Reprinted from Riddle (1908).

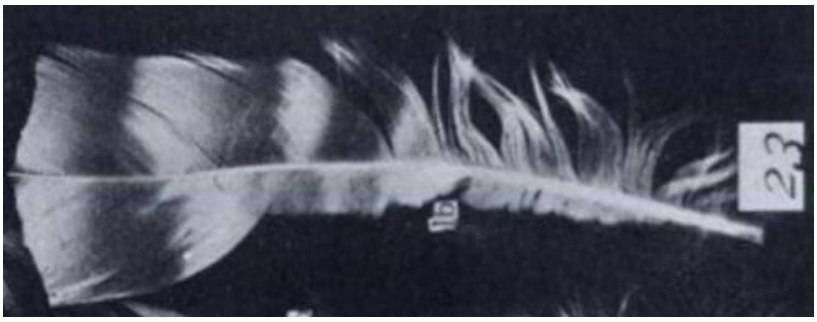


Fig.1.11 Riddle's fifth type of feather defect shown at 'lb', the 'longitudinal' fault bar. Reprinted from Riddle (1908).

Fig.1.12 The primary wing feathers from a red-billed (*Leiothrix Leiothrix lutea*) with a 'fault spot' in the fourth feather in the image. Photo taken by Wolf Dieter Busching. Reprinted from Erritzøe (2006).

In addition to Riddle's five types of feather defect, feather holes (0.5-1mm in diameter) have also been identified in connection to feather quality (Vágási, 2014; Fig.1.13). Originally, feather holes were thought to have been associated with chewing lice but have recently been found to be connected to fault bars (Jovani & Rohwer, 2016; Vágási, 2014). However, Pap *et al* (2007) found that feather holes had a more even spatial distribution, which contrasts with the 'fault bar allocation hypothesis' (Jovani & Blas, 2004). In this, fault bars occur on feathers that are least important for flight, resulting in the majority of fault bars being located on the tail feathers, with the lowest numbers in the primary feathers (Jovani & Blas, 2004). This evolutionary strategy reduces the negative consequences of fault bars on flight performance, minimising direct fitness costs (Jovani & Blas, 2004; Jovani *et al*., 2010). Pap *et al.* (2007) therefore suggested that differences seen in the distribution of feather holes and fault bars may be due to feather holes being less harmful, which has resulted in being less influenced by natural selection (Pap *et al*., 2007).

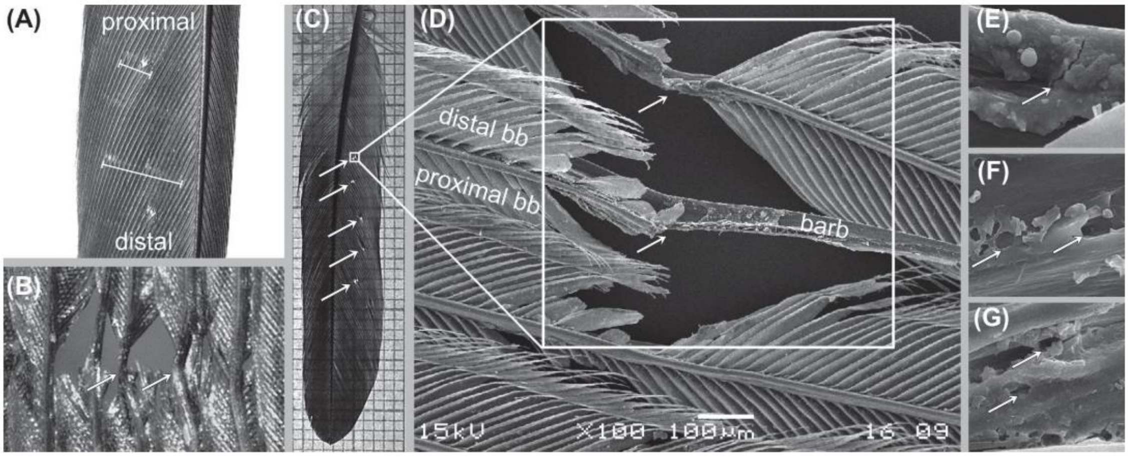


Fig.1.13 Feather holes of house martin (*Delichon urbica*) feathers. (A) Some holes are positioned along light fault bars (white section marks) and are elongated quasi horizontally; (B) the denuded portions of barbs are frequently bent (see also D); (C) feather with several holes; (D) close-up view of the damaged barbs and barbules (bb) on a scanning electron micrograph; (E) – (G) crop details of damaged barbs (marked with arrows on D) with multiple signs of fractures, micro-cracks and surface damages. Reprinted from Vágási (2014).

1.1.3 Aberrant White Feathers

Abnormal lack of melanin has also been identified as a characteristic of poor feather quality and is typically referred to as 'leucism' (van Grouw, 2013). Melanin is absent from areas in which it is normally deposited. For example, Fig 1.14 shows abnormal white colouration in carrion crow (*Corvus corone*) feathers that are expected to be entirely black. Feathers with white colouration are more susceptible to damage than those of normal melanin deposition (Bonser, 1995; RSPCA, 2013). Due to a lack of melanin, white feathers in general become more susceptible to UV damage and, therefore, deteriorate at a faster rate than dark feathers (Kaiser, 2008). Consequently, the aberrant white feathers may compromise the wing, as they are suggested to be a weak area in the overall wing structure. The cause of aberrant white feathers is generally thought to be associated with genetic factors (Sage, 1962); however, it has now been found to link more commonly with the physical condition and age of individuals and can also include environmental conditions e.g. food availability (van Grouw, 2018). Dietary imbalance results in the inability to extract the necessary nutrients required for melanin synthesis, resulting in lack of pigment. This type of abnormality can be seen in association with fault bars and frequently affects carrion crow and hooded crow (*Corvus cornix*) populations due to their requirement of a high protein diet (van Grouw, 2018). This particular phenomenon is yet to be quantified, leading to a focus on this in future chapters. An early study by Harrison (1963) stated that carrion crows were particularly susceptible to aberrant white feathers in urban or suburban areas, due to an abnormal or deficient diet. In addition, carrion crows regularly feed at rubbish tips (Harrison, 1963). On the other hand, aberrant white feathers which lack fault bars are caused by heritable disorders such as vitiligo (pigment disease) are connected to a 'progressive greying' (van Grouw, 2018). This term refers to the progressive loss of melanin with each successive moult and was recorded in 90% (N=1516) of blackbirds (*Turdus merula*) in 2011 with aberrant white feathers by the British Trust for Ornithology (BTO) (van Grouw, 2018).

Fig.1.14 Left wing of carrion crow (*Corvus corone*) exhibiting aberrant white feather colouration due to lack of melanin pigment.

1.1.4 Impacts of poor feather condition

The occurrence of fault bars is particularly detrimental to survival when present on the remiges/flight feathers, as breakage of these feathers compromise flying ability (Bortolotti, Dawson & Murza, 2002). This is due to a reduction in wing area, as this consequently increases wing loading (Navarro & González-Solís, 2007, Velando, 2002). When a single feather is broken, the cost of failure, in terms of survival, should be much less than that of a bone or limb (Corning & Biewener, 1998). However, this cost is increased by the fact that broken feathers cannot be repaired and are generally replaced only annually in the wing moult (Corning & Biewener, 1998). Feathers can break for a number of reasons, for instance, predator threats, impact damage and degradation from poor structural quality (Dawson *et* *al*., 2001; Kose *et al*., 1999). Broken feathers then impact the ability to travel to areas for foraging and increases chances of predation (Erritzøe, 2006; Møller *et al.*, 2009). A high number of fault bars also impinges on individual success during mate choice due to lack of fitness (Erritzøe, 2006; Møller *et al.*, 2009). For example, magpies *Pica pica* with snapped tails are less successful in mating than those with full tails (Fitzpatrick & Price, 1997). Moreover, American kestrels (*Falco sparverius*) with above-average numbers of fault bars have lower survival probabilities, highlighting the connection between feather quality and fitness (Bortolotti *et al*., 2002). Similarly, a study on willow tits (*Poecile montana*) and crested tits (*Lophophanes cristatus*) found a 5% reduction in survival rate for each fault bar on the rectrices/tail feathers (Eggers & Low, 2014). Furthermore, a study conducted on the feather quality of prey captured by sparrowhawks (*Accipiter nisus*) and goshawks (*Accipiter gentilis*) found that individuals with higher frequencies of fault bars were more susceptible to predation (Møller *et al.*, 2009). Moreover, the number of fault bars in the prey of goshawk was three times higher than that expected for the general population (Møller *et al.*, 2009). Little is currently known around the consequences of fault bar occurrence in relation to other feather quality measures such as feather strength, leading to a focus on this element in future chapters.

As stated previously, due to the negative consequences of fault bars on flight performance, birds have adapted in order to minimise direct fitness costs (Jovani & Blas, 2004; Jovani *et al*., 2010). This evolutionary strategy is explained by the 'fault bar allocation hypothesis' which states that fault bars occur on feathers that are least important for flight (Jovani & Blas, 2004). Consequently, this results in the majority of fault bars being located on the tail feathers, which may be due to the tail potentially playing a less important aerodynamic role in flight (Jovani & Blas, 2004). In contrast, few fault bars are found in the primary feathers, which have high strength requirements for proving thrust in flight (Jovani & Blas, 2004). This hypothesis is supported by many studies, in a range of species (Bortolotti *et al*., 2002; Jovani & Blas, 2004; Jovani *et al*., 2010; Murphy *et al*., 1989; Sarasola & Jovani, 2006; Slagsvold, 1982b). However, Serrano and Jovani (2005) discovered a high number of fault bars in the outermost, important tail streamer feathers of barn swallows (*Hirundo rustica*). They suggested that this was due to the recent nature of tail streamer evolution in hirundines, stating that flight performance is much less sensitive to shape changes in the tail than in the wings (Serrano & Jovani, 2005).

This could explain why evolutionary forces have not counteracted the increase of fault bars associated with feather elongation of tail streamers (Serrano & Jovani, 2005).

1.1.5 Fault bar formation theories

Fault bars are produced as a result of stressful or adverse environmental conditions during ontogeny or annual moult (Erritzøe, 2006; Jovani & Blas, 2004; Michener & Michener, 1938; Møller *et al.*, 2009). This concept was verified by Bortolotti *et al.* (2008) in which fault bars were found at points of high corticosterone levels in the feather. Growth abnormalities caused by stressors can also be seen in other keratin structures such beaks, nails, horns, hair, claws and hoofs (Jovani & Rohwer, 2016). For example, the growth abnormalities in human nails are regarded as an indicator of disease or nutritional deficiency (Cashman & Sloan, 2010). It has been suggested that the main cause of fault bar production is stress caused by nutritional deficiency (Stocker, 2005). However, a variety of other possible causes have also been identified, including age and sex, heredity traits, human handling, environmental factors, parasite burden and disease (Erritzøe, 2006).

There are different hypotheses about the formation of fault bars. The earliest describes a lack of nutrient supply to the growing feather at specific points of formation, with reference to alterations in blood pressure (Riddle, 1908). In diurnal birds, the majority of fault bars are produced in the first few hours of the night, which may be connected to a decrease in blood pressure during this time (Jovani & Diaz-Real, 2012; Riddle, 1908). It was suggested by Riddle (1908) that fault bars were the result of the follicle structure collapsing at this point. Soon after, Duerden (1909) explored Riddle's theory, concluding that barbule malformation could also result from mechanical damage rather than just a failure of nutrient delivery. More recently, Murphy *et al*. (1989) stated that night-time blood pressure reduction was not sufficient to collapse the feather follicle structure; therefore, unlikely to induce a fault bar. They suggested that unusual muscle contractions around the soft feather shaft during feather growth result in barbule damage (Murphy *et al*., 1989). Moreover, the concept of mechanical damage was discussed further, explaining that many types of stress may lead to an "autonomic reaction powerful enough to override a regional inhibition of feather muscles". In addition, these sudden muscle contractions have also been discussed in relation to fright moults - a response to stress such as a predatory event, resulting in the immediate shedding of feathers (Jovani & Rohwer, 2016). It was suggested that the production of fault bars may be a mild form of response to shock (Murphy *et al*., 1988; Murphy *et al*., 1989). This then leads to questions around the stress events, in terms of severity and length of time. Jovani & Diaz-Real (2012) found that fault bars were not the result of a chronic (long-term) stress, but that of an acute (short-term) stressor. From this, they suggested that fault bar strength/width is not related to the duration of the stress, but to the intensity of the stress (Jovani & Diaz-Real, 2012).

1.1.6 Factors associated with poor feather quality

1.1.6.1 Malnutrition

Malnutrition in avian species is caused by factors such as lack of food availability, adverse weather conditions and parental neglect in nestlings (Erritzøe, 2006; Murphy *et al*., 1988). It can result in metabolic bone disease (MBD) or osteodystrophy that is caused by an imbalance of calcium, phosphorus and vitamin C, resulting in brittle bones, deformed limbs and soft beaks in birds (Forbes & Zsivanovits, 2002; Stocker, 2005). Due to the keratinous structure of feathers, malnutrition is thought to be associated with fault bar formation, resulting in commonly used alternative names of 'hunger streaks' and 'hunger bars' (Jovani & Rohwer, 2016). Riddle (1908) stated that the production of fault bars is a result of "poor nutritive conditions in the feather-germs". This hypothesis has since been supported by researchers such as Slagsvold (1982a) and Newton (1986). In this, Slagsvold (1982a) found that juvenile hooded crows (*Corvus corone cornix*) displayed more feathers with fault bars and white colouration when undernourished. Similarly, Newton (1986) found a greater number of fault bars in juvenile sparrow hawks (*Accipiter nisus*) when they had received less food during rainy days. However, Jovani and Rowher (2016) have since examined 74 scientific papers in order to determine the reliability of Riddle's hypothesis. From this, an overarching conclusion was given that malnutrition does not strongly impact the production of fault bars (Jovani & Rohwer, 2016). Further studies have also come to this conclusion. For example, a study conducted on white-crowned sparrows (*Zonotrichia leucophrys gambelii*) found that fault bars are an unreliable index of a nutritional status due to a lack of correlation with starvation days (Murphy *et al*., 1989). Furthermore, a study also found a correlation with human handling and fault bars rather than malnutrition (Negro, Bildstein & Bird, 1994).

1.1.6.2 Environmental Contamination

It is well known that environmental factors impact the health of organisms (Jaspers *et al.*, 2004). Dumonceaux & Harrison (1994) stated that birds are commonly poisoned through environmental contamination. Toxins enter birds through direct ingestion of contaminated water, air or food, or through indirect contamination through the food chain (Dumonceaux & Harrison, 1994). During feather growth, heavy metals bind to the protein molecules in the feather, reflecting levels of contamination during this short time frame (Malik & Zeb, 2009). Consequently, feathers are commonly analysed to identify environmental contamination. For example, rusty blackbird (*Euphagus carolinus*) feathers have been used as a bio-monitor of heavy metal contamination in five regions across North America (Edmonds *et al.,* 2010). This study found high concentrations of mercury, with some regions exceeding published minimum levels for adverse effects on birds (Edmonds *et al*., 2010). In addition, a study on various North American songbird species found significantly lower levels of mercury in historic samples dated prior to 1900 compared to those post-2000, showing an increase in exposure to this particular environmental contaminant over time (Perkins *et al*., 2019).

Environmental contamination in the form of acid rain is formed by emissions of sulfur dioxide and nitrogen oxide, which produce acids when they react with atmospheric water molecules (Graveland, 1998). This impacts bird species in several ways. A study by Scheuhammer (1991) found that acidification impacts the availability of calcium-rich prey e.g. snails and increases dietary exposure to toxic metals such as lead and aluminium. This is significant as calcium is required for egg shell formation and skeletal growth (Dawson & Bidwell, 2005; Graveland, 1998). A study by Dawson and Bidwell (2005) supported this in their study of tree swallows (*Tachycineta bicolor*), finding improved nestling growth and fitness in individuals that were given calcium supplements. They also found that these individuals had higher survival rates after leaving the nest, suggesting that calcium availability impacts fitness (Dawson & Bidwell, 2005). In addition to the limitation of dietary nutrients, in some circumstances, acid rain also impacts bird populations by limiting habitat availability in the form of forest dieback (Graveland, 1998).

1.1.6.3 Life Stage

Young birds are more vulnerable to fault bar production, as the feather growth period is very sensitive and is also an important time for general body growth (Hawfield, 1986; Serrano & Jovani, 2005). Due to this, nestlings are vulnerable to fault bar production, even under low levels of stress (Erritzøe, 2006). Many studies have found a lower number of fault bars in adult birds in comparison to young birds (Hawfield, 1986; Jovani & Blas, 2004; Serrano & Jovani, 2005; Slagsvold, 1982a). In relation to young individuals, a study by Machmer *et al.* (1992) found that the smallest osprey (*Pandion haliaetus*) nestling had the highest number of fault bars. Interestingly, this study also found that fault bar production occurred less as the nestlings became older (Machmer *et al.*, 1992). This is also supported by Jovani and Tella (2004), who found a significant decrease in fault bar production throughout the growth period of nestlings. They suggested that this could also be due to an increase in resilience to adverse weather conditions (Jovani & Tella, 2004). In addition, a study conducted on the upland buzzard (*Buteo hemilasius*), found that sibling competition was a greater source of stress than variations in relative nutritional condition (Yosef, Gombobaatar & Bortolotti, 2013). This suggests that the pressures of nestling conflicts are also a factor in their vulnerability to fault bar production.

1.1.6.4 Sex

A study on the hooded crow (*Corvus cornix*) found that the mortality of juveniles was reflected in the sex ratio of broods, finding a greater number of fault bars in the feathers of juvenile males than females (Slagsvold, 1982b). This can be explained by the fact that male hooded crows require a higher parental investment than rearing a female due to their larger body mass (Slagsvold, 1982b). Similarly, female American kestrels (*Falco sparverius*) were found to have higher numbers of fault bars than males (Dawson *et al.*, 2001). Although females are larger than males in this species, body size was not related to feather condition (Dawson *et al.*, 2001). In this case, it was suggested that the difference was due to females experiencing higher levels of stress during migration, as they tend to travel further than males and moult during this time (Balgooyen, 1976; Dawson *et al.*, 2001). This shows that sex can play an important role in the propensity to producing fault bars, depending on the species of bird.

1.1.6.6 Parasite Burden

Parasite burden has a significant impact on individual performance, as parasites compete with the host for nutrients, triggering costly immune responses (Hudson, Dobson & Newborn, 1998; Møller, 1997; Reed *et al*., 2012; Sheldon & Verhulst, 1996). This is particularly detrimental to young individuals, as they have a less efficient immune system than adults and are more exposed to nest-dwelling parasites (Reed *et al*., 2012; Ros *et al.,* 1997; Szép & Møller, 1999). Research in regard to parasites and their impact on feather quality are generally limited to ectoparasites due to their direct contact with the feathers (Blanco, Tella & Potti, 1997; Harper, 1999). For example, Harper (1999) found that house finches (*Carpodacus mexicanus*) and yellowhammers (*Emberiza citronella*) with high numbers of feather mites grew duller plumage, relatively shorter wings and had lower protein reserves. On the other hand, Blanco *et al.* (1997) found a mutualistic relationship between red-billed choughs (*Pyrrhocorax pyrrhocorax*) and feather mites, suggesting a species-specific interaction with parasites. More recently, endoparasites have also been found to impact feather quality, due to their complex relationship with the host. Endoparasite species live inside the host and exist in the form of helminths (parasitic worms), protozoa (one-celled organisms) and some arthropods (e.g. ticks and mites) (Krone, 2007). For example, low breeding plumage quality was found to be associated with the presence of cestode helminths in female godwits (*Limosa lapponica taymyrensis*) (Piersma *et al*., 2001). Moreover, in young male wild turkeys *Meleagris gallopavo*, coccidian protozoa parasites were found to negatively impact the expression of iridescent structural coloration (Hill, Doucet & Buchholz, 2005). In this, iridescent coloration may serve as a condition-dependent signal of male health; therefore, this provides evidence of a link between parasite burden and feather quality (Hill *et al*., 2005). Further studies have supported these findings; for instance, chronic coccidian infestation in house sparrows *Passer domesticus* was analysed in relation to feather bending stiffness (Pap *et al.,* 2013). Results of this study found infested, non-medicated birds to grow poorer quality flight feathers in a large number of feather traits both after the first and second moults (Pap *et al.,* 2013). In this, primary feathers were shorter and lighter with a smaller vane area, had thinner rachis and decreased stiffness (Pap *et al.,* 2013). From this it was suggested that parasites may have serious, long-lasting effects on feather quality, ultimately impacting the fitness of the host (Pap *et al.,* 2013). Endoparasites and the immune system have also been found to play an important role in fault bar formation and feather quality

(Freed *et al*., 2005; Jovani, Montalvo & Sabaté, 2014; Møller, Kimball & Erritzøe, 1996). For example, in house sparrows (*Passer domesticus*) there is a positive relationship between immune response and the number of fault bars on feathers (Møller *et al*., 1996). In this study, immune response was measure in the form of bursa Fabricius volume, an organ involved in antibody synthesis (Møller *et al*., 1996). This implies that a trade-off between immunity and feather quality may occur in some instances.

1.2 The Corvid Family

The propensity to develop fault bars is more prominent in certain species and also between individuals (Jovani & Rohwer, 2016). Corvidae species commonly suffer from poor feather condition (van Grouw, 2018; RSPCA, 2013), resulting in a focus on this family. The corvidae family are commonly known as the corvid or crow family, consisting of 130 different species worldwide (Droege & Topfer, 2016). The corvid family are renowned for being among the most intelligent group of birds, being considered to have equal intelligence to non-human primates (Clayton & Emery, 2007; Prior, Schwarz & Güntürkün, 2008).

1.2.1 Ecosystem services provided by corvids

Corvids play a vital role in our ecosystem, providing important ecosystem services such as carcass removal and seed dispersal (Inger *et al.*, 2016; Pesendorfer *et al.*, 2016). The removal of carcasses via scavenging is very important in maintaining overall hygiene in the natural environment (Whelan, Wenny & Marquis, 2008). Moreover, scavengers assist in holding more energy in higher trophic levels and encourage connections between heterotrophic and detrital food webs (Inger *et al*., 2016; Rooney *et al.*, 2006; Wilson & Wolkovich, 2011). This redistribution of energy is important for nutrient cycling within ecosystems, as a large amount of energy is lost between trophic levels (Inger *et al*., 2016; Rooney *et al.*, 2006; Wilson & Wolkovich, 2011). In addition to this, seed dispersal is a key component in maintaining community structure and biodiversity (Bascompte & Jordano, 2007; Whelan *et al.*, 2008). Corvids disperse seeds through ‘food-caching’ behaviour, which is also known as ‘scatter-hoarding’, where individuals hide food for when resources are low (Pesendorfer *et al*., 2016). When the birds do not return to some of the stored food, the seeds then sprout and help plants to colonise new areas (Cain, Milligan & Strand, 2000; Pesendorfer *et al*., 2016). This is particularly important in eliminating habitat fragmentation, as long-distance seed dispersal aids in maintaining habitat connectivity (Pannell, 2007). The mutualistic relationship between corvids and plant species can also be considered as a tool in conservation, as suitable climates for plants are likely to shift rapidly in the near future (Pesendorfer *et al*., 2016). Not only do corvids expand the range of important plant species, but also support genetic diversity and population health (Betancourt *et al*., 1991; Pesendorfer *et al*., 2016). For example, pinyon jays (*Gymnorhinus cyanocephalus*) contribute to high levels of heterozygosity in the pinyon pine *Pinus edulis* population in western U.S. (Betancourt *et al*., 1991).

1.2.2 Corvids and human conflict

Corvids are vulnerable to persecution due to a long-standing negative cultural perception. For example, farmers and gamekeepers have persecuted corvids in order to protect their crops and livestock (Holden & Cleeves, 2010). However, populations have now stabilized after a recent reduction in game keeping and colonization of towns (Holden & Cleeves, 2010). Carrion crows and magpies (*Pica pica*) are also well known for taking songbird eggs and chicks from nests (Holden & Cleeves, 2010). However, research shows that this has “little overall effect” on small bird populations (Gibbons *et al*., 2007; RSPB, 2015). A recent study has shown that other limiting factors are a greater concern for small bird populations than nest predation, with habitat fragmentation proving to be more detrimental (Madden, Arroyo & Amar, 2015). Habitat fragmentation, in turn, is also responsible for an increased density of corvids in small forests, as agricultural lands mix with and divide popular habitats (Andrén, 1992). Further research is necessary in order to understand the relationship between levels of predation and habitat management, resulting in the implementation of non-lethal population management of corvids (Gibbons *et al*., 2007).

1.2.3 Study Species

This study focuses on the three most common Corvid species that are found in the United Kingdom. These species are carrion crow (*Corvus corone*), magpie (*Pica pica*), and jackdaw (*Corvus mondula*) (Fig.1.15).

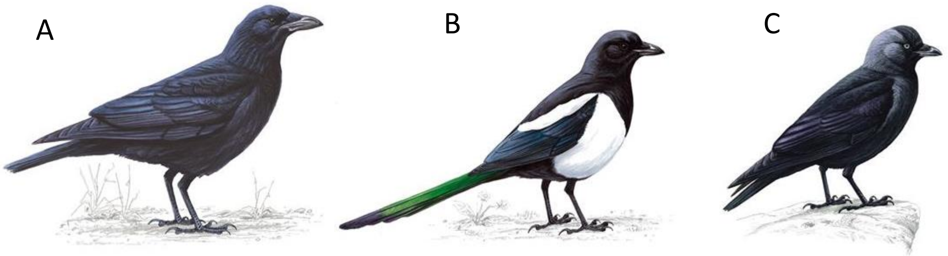


Fig.1.15 (A) Carrion crow *Corvus corone* (B) magpie *Pica pica* (C) jackdaw *Corvus mondula*. Reprinted from online source "Crows", by The Royal Society for the Protection of Birds (RSPB) https://www.rspb.org.uk/birds-and-wildlife/wildlife-guides/bird-a-z/crow-family/

The carrion crow population has increased over time, owing to their omnivorous diet and ability to adapt to changing habitats (Miller *et al*., 2015). More specifically, the British Trust for Ornithology (BTO) stated that between 1995 and 2016, the carrion crow population increased by 29% in England (BTO, 2017; Fig.1.16). In addition, the jackdaw population increased in England by 68% during this time (BTO, 2017; Fig.1.16). In contrast, after some fluctuation, the magpie population in 2016 returned to that seen in 1995 (BTO, 2017; Fig.1.16). It is suggested that this stabilisation could be due to a reduction in control by gamekeepers after the First World War (BTO, 2017; Tapper 1992). The three focus species are found in a wide variety of habitats, having a preference for open land away from dense forests (BirdLife International, 2017). Moreover, due to being highly adaptable they have also been able to colonise new urban and suburban habitats, regularly visiting rubbish tips (Holden & Cleeves, 2010). It has been suggested that landscape changes have benefitted these species, with increased occurrence in agricultural habitats (BTO, 2017; Gregory & Marchant, 1996). Population increases of carrion crow and magpie within woodlands were found between 1964 and 1993; however, woodland jackdaw populations declined during this time (Gregory & Marchant, 1996).

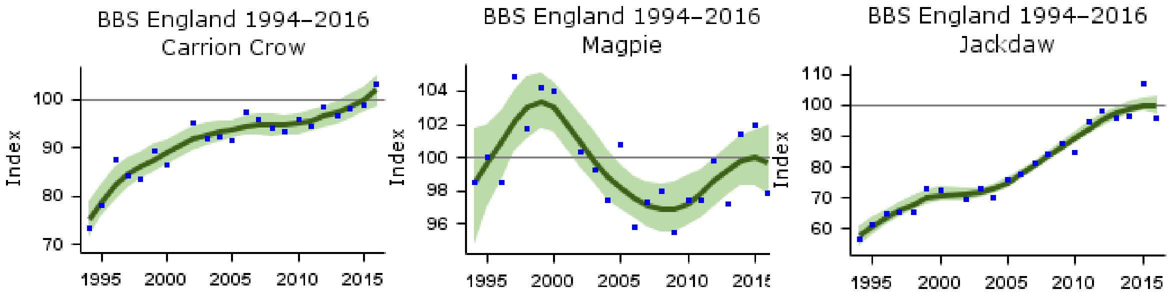


Fig.1.16 Breeding bird survey (BBS) population data for carrion crow *Corvus corone*, magpie *Pica pica* and jackdaw *Corvus mondula* in England between 1994-2016. Smoothed population index of relative to an arbitrary 100 in the year given, with 85% confidence limits in green. Reprinted from British Trust for Ornithology (BTO) /Joint Nature Conservation Committee (JNCC) BirdTrends research report 708 by Woodward *et al.* (2018).

Their diet consists of a variety of food types, ranging from carrion, eggs, insects and other invertebrates such as worms and beetles (Holden & Cleeves, 2010). They also consume a variety of vegetable matter such as grain, fruit and seeds (Holden & Cleeves, 2010). Due to their opportunist feeding habits, carrion crows living in coastal regions also eat shellfish, which they open by dropping onto rocks (Holden & Cleeves, 2010). A study on the gizzard contents of these species found that Jackdaws consume the highest level of plant-based food sources amongst the three species, making up the majority of its diet (Holyoak, 1968) (Fig.1.17). Magpies were found to consume the highest level of invertebrates, forming the majority of its diet (Holyoak, 1968) (Fig.1.17). Carrion crows and magpies were found to consume higher levels of vertebrate food sources than jackdaws, with magpies consuming the highest level (Holyoak, 1968) (Fig.1.17). However, eggs were included in the vertebrate food type, which explains this result as magpies consume more eggs than carrion crows (Holyoak, 1968). Moreover, carrion crows consume higher levels of carrion than magpies (Holyoak, 1968).

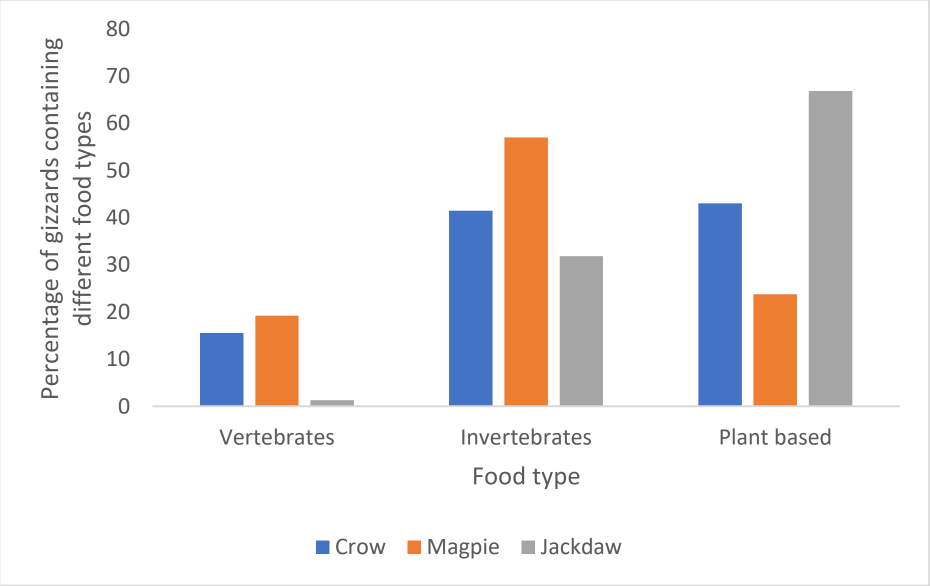


Fig.1.10 Percentage of gizzards containing different food types in carrion crow (N=234), magpie (N=77) and jackdaw (N=222). Data obtained from Holyoak (1968). Please note that eggs were included in the vertebrate food type.

The carrion crow is a solitary species, practicing serial monogamy in its mating system and maintaining large territory areas (BirdLife International, 2017; Clayton & Emery, 2007). In contrast, magpies are largely gregarious, practice serial monogamy and are sedentary over most of its range (BirdLife International, 2017). Jackdaws are also gregarious but tend to move further, with their population including some migrant individuals from Europe (BirdLife International, 2017). In relation to their plumage, adults of these species generally have freshly moulted feathers by the start of autumn, with non-breeding second year birds starting their moult c.1-2 weeks before breeding birds (Svensson, 1992).

1.2.4 Selection of focal subjects

In order to select the species most commonly affected by poor feather condition for this study, RSPCA Stapeley Grange admissions data from 2013 to 2016 were analysed. This focused on the three most common corvid species that are found in the United Kingdom. These species are carrion crow, magpie and jackdaw (Fig.1.15).

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) have four wildlife rehabilitation centres spread across the UK - East Winch in Norfolk, Mallydams Wood in East Sussex, Stapeley Grange in Cheshire and West Hatch in Somerset (RSPCA, 2015). The study site for this research project was RSPCA Stapeley Grange Wildlife Centre, which was originally the home of Mrs Cynthia Zur Nedden (RSPCA, 2019). After she passed away in 1990, the RSPCA received the building as a donation in order to 'establish an animal home and refuge with a clinic' (RSPCA, 2019). The centre then opened in 1993 and has been receiving ever-increasing numbers of admissions, making it the busiest RSPCA wildlife centre in the UK (RSPCA, 2019). Wildlife rehabilitation entails the temporary care and treatment of "injured, diseased, and displaced indigenous animals" and the resulting release of healthy individuals to appropriate natural habitats (Miller, 2012).

During admission, key information is documented in relation to each individual. This includes date, time, location found, admission reason, stage of life, finder details and inspector reference numbers. At the end of each rehabilitation case, the end result and date are then recorded, following with the number of days spent in care. Poor feather condition is documented in hand-written progress notes made on each individual during their time in rehabilitation. This information is not available on the electronic database unless poor feather condition is specified as a justification for euthanasia. The reasons given for euthanasia often differs from the reason for admission, as these comments are made after triage assessment. Good feather condition is not often documented; therefore, no mention of feather condition results in the assumption that the feather condition is of a good quality. It is important to acknowledge that poor feather condition cases were underestimated using this method, as poor feather quality is often overlooked during triage if there is a more prominent reason for euthanasia. For example, the individual may be clinically blind and also have poor feather condition. However, the reason for euthanasia will be documented to be the former of the two reasons, as this is adequate for justifying the decision to euthanise the individual. Likewise, feather condition is sometimes undocumented if a more prominent treatable ailment is recognised.

Tab.1.2 shows the number of admissions for each year across the three species, with the number of individuals documented to have poor feather condition and those of which euthanised/put to sleep (PTS) due to this. Poor feather condition was most prominent in carrion crows; therefore, this species was selected as the focal subject of this research.

Tab.1.2 Number of carrion crow *Corvus corone*, magpie *Pica pica*, jackdaw *Corvus mondula* admissions documented to have poor feather condition in addition to those euthanised/put to sleep (PTS) as a result of this between 2013 and 2016.

Year	Number of admissions	Admissions with poor feathers		PTS due to poor feathers		
		Number	% of total	Number	% of total	% of poor
Carrion crow						
2013	98	41	41.8	38	38.7	92.6
2014	111	70	63.1	59	53.2	84.3
2015	106	59	55.7	50	47.2	84.7
2016	132	79	59.8	72	54.6	91.1
Magpie						
2013	92	15	16.3	10	10.9	66.7
2014	132	30	22.7	23	17.4	76.7
2015	95	20	21.1	12	12.6	60.0
2016	110	16	14.5	11	10.0	68.8
Jackdaw						
2013	51	7	13.7	5	9.8	71.4
2014	93	17	18.3	11	11.8	64.7
2015	92	12	13.0	9	9.8	75.0
2016	99	16	16.2	11	11.1	68.8

1.2.4.1 Admission Reason

Within the admissions documented above, 18 different reasons for admission occurred - the descriptions of which can be found in Tab.1.3. Although casualties may display more than one reason for admission, the reason that best describes the problem is selected during admission. Individuals with poor feather condition that were damaged by reasons marked with an asterisk were not assessed due to direct impact on feather condition.

Tab.1.3 Descriptions of avian admission reasons. Reasons marked with an asterisk were excluded from data review due to having a direct impact on feather condition.

Admission Reason	Definition
Grounded	Individual capable of flight found unable to fly
Injury (cause uncertain)	Damage to the body from unknown incident
*Collision	Damage gained from striking a solid surface in flight
Legal case animal	Individual connected with a dispute resolved by court
Abnormal behaviour	Individual displaying behaviour that is not of a normal nature
*Attacked by another animal	Damage caused by an attack from another animal
Weakness	Individual lacks strength required to perform normal bodily functions
Disease	Individual suffering from a pathological condition
Orphan	Nestling which lacks parental care and requires handfeeding
Inexperienced juvenile	Fledgling which lacks parental care and requires supportive care
Dead on arrival	Individual deceased at the time of admission
*Caught/entangled	Damage caused to individual due to the restriction of bodily movements by foreign object
Starvation	Individual requires rehabilitation due to lack of access or ability to consume food
Fishing litter	Damage caused by fishing equipment
Shot	Damage caused by firearm
*Oiling	Damage caused by oil spill
Unknown	Admission reason not known
Other	Admission reason does not fall into the above categories. This should be written within the admission comments section.

Tab.1.4 shows that a large proportion of admissions with poor feather condition were orphaned, which highlights that juvenile individuals are strongly affected by fault bar production. This has been reported in other studies, where a lower number of fault bars in adult birds was found in comparison to young birds (Jovani & Blas, 2004; Serrano & Jovani, 2005; Slagsvold, 1982a & Hawfield, 1986). It is important to acknowledge that juveniles are dependent on their parents for food, whereas adults are able to move away from the nest and forage in more distant areas when they require additional nutrients for feather growth during moulting periods. In addition, juveniles also face energy reserve challenges during growth, with trade-offs between body and feather growth (Machmer *et al*., 1992). Results also found that a large number of individuals with poor feather condition were grounded. Tab.1.3 states that grounded admissions are unable to fly in cases where the individual is normally capable of flight, which links directly to the manifestation of poor feather condition. Low feather quality often leads to feathers becoming snapped and unusable for flight (Kose *et al*., 1999; Dawson *et al*., 2001; RSPCA, 2013); therefore, this highlights the severity of some poor feather condition cases seen as Stapeley Grange.

Tab.1.4 Carrion crow admission reasons between 2013 and 2016 in relation to poor feather condition. Reasons with less than 5 admissions were excluded from displayed results (N=447). PTS = put to sleep due to poor feather condition.

Admission reason	Total no. admissions	No. poor	% Total poor	No. PTS	% total PTS	% poor PTS
Grounded	134	75	56.0	66	49.3	88.0
Injured	98	25	25.5	17	17.3	68.0
Other	47	33	70.2	28	59.6	84.8
Weakness	9	6	66.7	6	66.7	100.0
Orphan	136	93	68.4	84	61.8	90.3
Inexperienced juv.	11	7	63.6	7	63.6	100.0

1.2.4.2 Rehabilitation Outcome

The rehabilitation outcome of individuals with poor feather condition were assessed to outline the chances of rehabilitation success. Rehabilitation outcomes were as follows – put to sleep/euthanised (PTS) on admission (O/A), PTS before 48 hours, PTS after 48 hours, dead O/A, died before 48 hours, died after 48 hours and released. If an individual was PTS or died after 48 hours, this includes any period of time after the stated 48 hours. This could be 3 days or 3 months etc. Tab.1.5 shows that the majority of poor feather admissions were PTS on arrival. This is due to the fact that RSPCA protocol states that euthanasia is recommended for individuals with poor quality feathers (RSPCA, 2013). This highlights the severity of this issue and shows that poor feather condition is not treated in rehabilitation. Moreover, this also shows that admissions had poor feather condition before arriving at the rehabilitation centre, proving that poor feather condition is not a result of captivity.

Tab.1.5 Carrion crow rehabilitation outcomes between 2013 and 2016 in relation to poor feather condition (N=447). PTS = put to sleep due to poor feather condition. O/A = on admission.

Admission reason	Total no. admissions	No. poor	% Total poor	No. PTS	% total PTS	% poor PTS
PTS O/A	256	171	66.8	167	65.2	97.7
PTS before 48hrs	45	31	68.9	26	57.8	83.9
PTS after 48hrs	57	29	50.9	26	45.6	89.7
Dead O/A	6	0	0	0	0	0
Died with 48hrs	6	1	16.7	0	0	0
Died after 48hrs	17	4	23.5	0	0	0
Released	60	13	21.7	0	0	0

1.2.4.3 Location

The location of admissions generally consisted of the full address of where an individual was found. However, a full address is not always available, resulting in general county records. The counties recorded during 2013-2016 can be found in Tab.1.6, which shows that the large majority of admissions were received from Cheshire, which is the location of Stapeley Grange. This is due to a large number of admissions being received from locations nearby to RSPCA Stapeley Grange from members of public. A substantially lower number of admissions were received from counties further afield. However, high numbers of admissions were received from some locations, even though they were further away. This could be due to the transfer of animals from veterinarian practices and other rehabilitation centres. This naturally creates bias in the data due to uneven sample sizes. Moreover, specific locations that provide information on habitat are often missing. Therefore, this factor was not followed up.

Tab.1.6 Number of carrion crow admissions received between 2013 and 2016 different counties in relation to poor feather condition. Location is arranged in order of closest to furthest distance from Stapeley Grange. PTS = put to sleep due to poor feather condition. O/A = on admission.

Location	Total no. admissions	No. poor	% Total poor	No. PTS	% total PTS	% poor PTS
Cheshire	141	70	49.6	65	46.1	92.9
Staffordshire	67	39	58.2	33	49.3	84.6
Shropshire	30	14	46.7	12	40.0	85.7
Greater Manchester	21	13	61.9	9	42.9	69.2
Merseyside	45	31	68.9	25	55.6	80.6
Derbyshire	5	2	40.0	2	40.0	100.0
Clwyd	19	15	78.9	11	57.9	73.3
Powys	1	1	100.0	1	100.0	100.0
West Midlands	38	18	47.4	16	42.1	88.9
Lancashire	44	31	70.5	30	68.2	96.8
Gwynedd	4	2	50.0	1	25.0	50.0
West Yorkshire	25	12	48.0	10	40.0	83.3
South Yorkshire	7	4	57.1	4	57.1	100.0
North Yorkshire	3	1	33.3	1	33.3	100.0
Glamorgan	1	1	100.0	0	0.0	0.0

1.3 Research Programme

This study focused on the most commonly affected corvid species, the carrion crow, assessing feather quality measurements along with the identification of possible causes and consequences of this issue. Additional details are outlined below in the form of study aims:

- **Aim 1 (Chapter 3):** To investigate the macroscopic and microscopic characteristics of fault bars. This aimed to review existing fault bar formation theories and imagery with the use of advanced technology.
- **Aim 2 (Chapter 4):** To assess relationships between different feather quality measures. This consisted of the number and severity of fault bars, feather iridescence, feather strength and the occurrence of snapped and aberrant white feathers.
- **Aim 3 (Chapter 5):** To investigate the causes of fault bar production in relation to the chemical profile of feathers, endoparasite burden, sex and age.
- **Aim 4 (Chapter 6):** To investigate the consequences of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, sex and age.

Chapter 2: General methodology

2.1 Study subjects

Ninety deceased carrion crows were collected after admission to RSPCA Stapeley Grange Wildlife Centre during 2017 and 2018. Carrion crow cadavers were collected after euthanasia and stored in individual bags labelled with corresponding identification information. Cadavers were then stored in a freezer at -18°C until post-mortem. These cadavers were collected year-round, with the majority being collected in the peak season of spring and summer. Out of the ninety birds that were collected, forty-seven individuals were used for analysis due to the exclusion of nestlings. This was due to the developing feathers being in pin formation, which could not be used for feather quality measurements (Fig.2.1). In addition, nestlings had undeveloped sex organs, which were necessary for sex identification.

Fig.2.1 Image of nestling carrion crow displaying pin feathers.

In 2017, twenty-three carrion crows were collected, with the majority of admissions being found in Cheshire. Individuals were admitted due to a range of reasons, with half of admissions being grounded. This admission reason is documented when an individual that is normally capable of flight is found unable to fly. Regarding rehabilitation outcome, the large majority of individuals were put to sleep (PTS) on admission (O/A). Nine of these admissions were euthanised due to poor feather condition. In 2018, twenty-four carrion crows were collected. Admissions data is not available for these individuals, as identification information was not recorded by staff during this year. This occurred in order to speed up the process of body collection and storage.

2.2 Methodologies for each study aim

2.2.1 Aim 1 (Chapter 3): To investigate the macroscopic and microscopic characteristics of fault bars

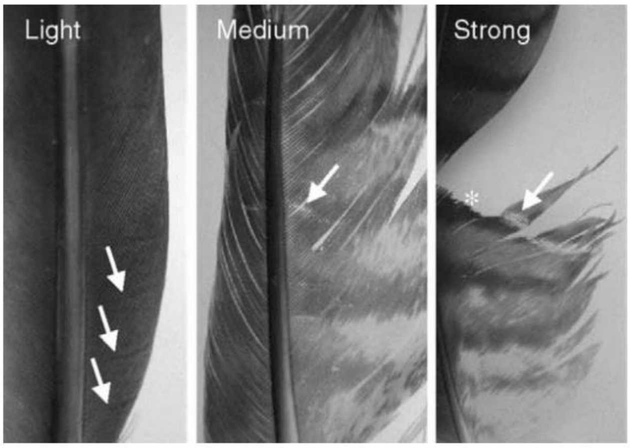


Fig.2.2 A gradient of fault bar strengths in swainson's hawk Buteo swainsoni feathers. The asterisk indicates break of feather barbules due to a heavy fault bar (Sarasola & Jovani, 2006).

Ten feathers were selected from 10 individuals, containing examples of different fault bar severities on a single feather. In this, wing feathers were selected based on the occurrence of a range of different fault bar severities presented together on the same feather. Fault bar samples were selected using categories outlined by Sarasola & Jovani (2006; Fig.2.2). These categories acknowledge the variation of widths in fault bars, classifying them as light, medium and strong (Fig.2.2). They described these categories in the following way: Light fault bar – the "absence of some barbules producing a visible discontinuity on the structure of the feather"; medium fault bars - "narrow, <1 mm, translucent line across the feather"; heavy fault bars were >/=1 mm, "translucent line across the feather". This study categorised fault bars in a slightly different way in accordance to how they occurred in carrion crow feathers. This was due to the rare occurrence of fault bars measuring over 1mm in width. Usually, two fault bars were positioned in a very close proximity, appearing as single very wide fault bar. Light fault bars were described as <0.1mm in accordance with the above category. Medium fault bars were described as 0.1mm-0.59mm wide, with heavy fault bars classified as $\geq$0.6mm. Each fault bar was measured to the nearest 0.05mm with a digital calliper.

Feathers were first washed alternately with acetone and distilled water to remove external contaminants (Dauwe *et al.*, 2003). Four 1cm^2 samples were then cut from each feather representing the following: feather vane with no fault bars present, a light fault bar, medium fault bar and heavy fault bar. Samples were attached to aluminium specimen stubs using conductive carbon adhesive discs and coated with gold to create a conducting surface (Leslie & Mitchell, 2007). Samples were placed into the Quanta 200 Scanning Electron Microscope (SEM) and photographed at 150X magnification.

Each barbule width was measured using the scale bar. Fig.2.3 shows the measurement points of each barbule. The mid-point of each 'squeezed' section was used for this measurement. The width of each barbule in an image was measured and the mean barbule width calculated for each image. The number of barbules measured per sample varied slightly due to the movement of each sample around the rotating specimen holder stage. SEM and photographic imagery of other samples of interest were also assessed, to be discussed further in the data chapter.

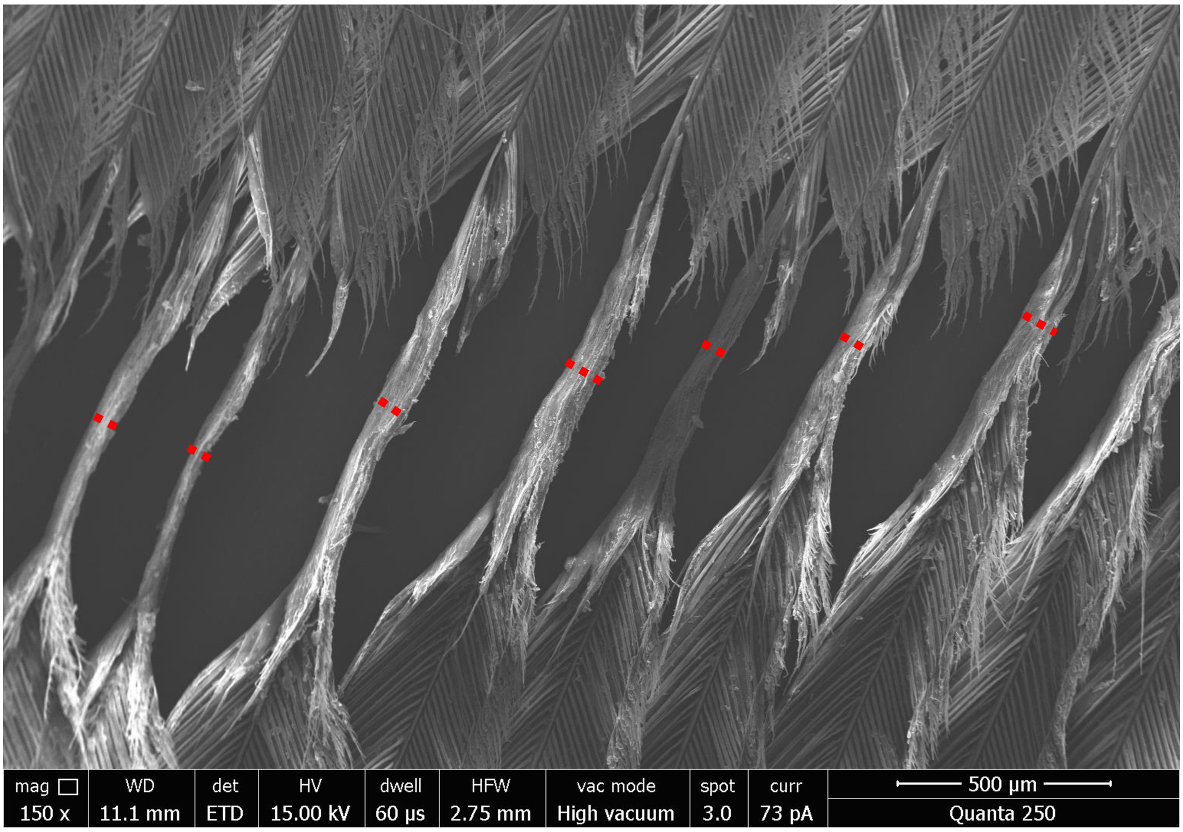


Fig.2.3 Scanning electron microscope (SEM) image of constricted barbules at a fault bar site of a flight feather of a carrion crow. The red dashed lined indicate points of measurement to calculate the average width of barbules (mm).

In addition to assessing the characteristics of fault bars, this aim also identified further evidence of structural damage caused by fault bars. In this, the feather shaft and growing pin feathers were assessed. It should be noted here that later images of feather shaft damage were collected using SEM equipment at Staffordshire University. This particular instrument was used under low vacuum and no gold coating was used (JOEL JSM-660LV). These images can be identified using labelling of 'low vacuum'.

2.2.2 Aim 2 (Chapter 4): To assess relationships between feather quality measures

2.2.2.1 Feather samples

Forty-seven individuals were used in this chapter, outlined in section 2.1. Six feathers were collected from the right-hand wing of each individual. This consisted of three primary and three secondary remiges/flight feathers; see Fig.2.4 for exact feather locations. These particular feathers were chosen in order to represent different parts of the wing, covering the entire wing area. It was important to measure primary and secondary flight feather separately due to their different functions in flight (Ginn & Melville, 1983; Videler, 2007). Feathers 1, 3 and 5 were selected within the flight feathers in order to provide standardised measurements whilst accounting for each feather differing slightly in size according to its position on the wing flight (Ginn & Melville, 1983; Videler, 2007). In addition, three rectrices/tail feathers were collected. The tail feathers were selected at random due to the difficulty in identifying specific feather positions. This was due to many individuals suffering from injuries or trauma which led to the loss of various tail feathers. The random nature of tail feather collection does not hinder the quality of data collection due to rectrices being equal in length and function in species with non-forked tails (Tubaro, 2003). All tail feathers were stored in an envelope, with three being removed for analysis unsystematically.

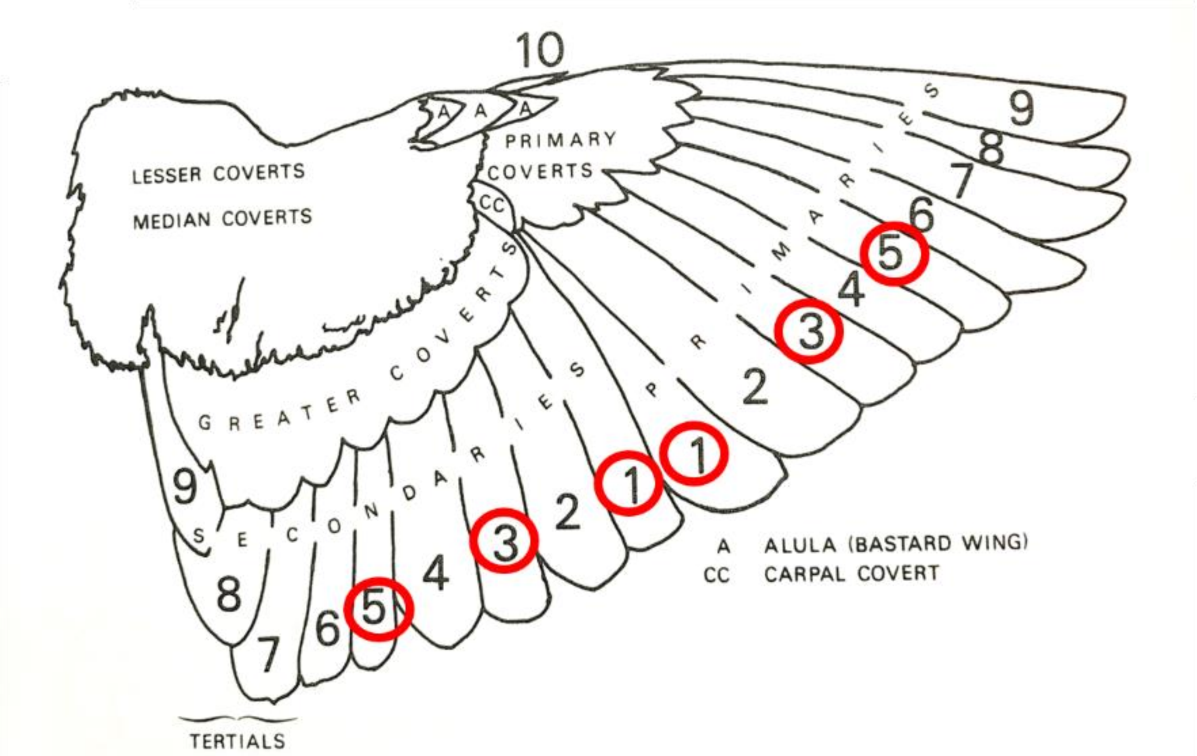


Fig.2.4 Topography of a passerine wing. Red circles indicate feathers selected for analysis. Reprinted from Ginn & Melville (1983).

2.2.2.2 Study measures

Various measures were used in this aim, gaining averages for each feather type using the selected feathers outlined in Fig.2.4. These measures consisted of the average number of fault bars, the average width of fault bars, the number of snapped feathers, the number of white feathers, average feather iridescence and average feather base strength. The first four measures are potential indicators of poor feather quality, investigating relationships between which (Jovani & Rohwer, 2016; Bortolotti *et al*., 2002; van Grouw, 2013). The latter two variables served as potential measures of high-quality feathers/condition (Dawson *et al*., 2000; Harper, 1999). This allowed the assessment of possible correlations between fault bar measures and general measures of feather quality.

2.2.2.2.1 Average number and width of fault bars

Using the selected feathers, the number of fault bars was counted in each individual feather. Only fault bars that were clearly visible were counted (Møller *et al.*, 2009). Within the 47 individuals, feather samples with fault bar presence were as follows for each of the feather types: primary (31), secondary (36), tail (43). Figures 2.5-2.7 display frequency histograms for the number of fault bars within each of the feather types. Please note that three feathers are measured per feather type as stated above. Each recorded fault bar was measured to the nearest 0.01mm with digital callipers. The average (mean) number of fault bars was then calculated across the three selected feathers for each feather type (primary, secondary and tail). The average fault bar width (mean; mm) was then calculated in the same way as stated above for the number of fault bars (across the three feathers per feather type). Mean values were then used for further analysis.

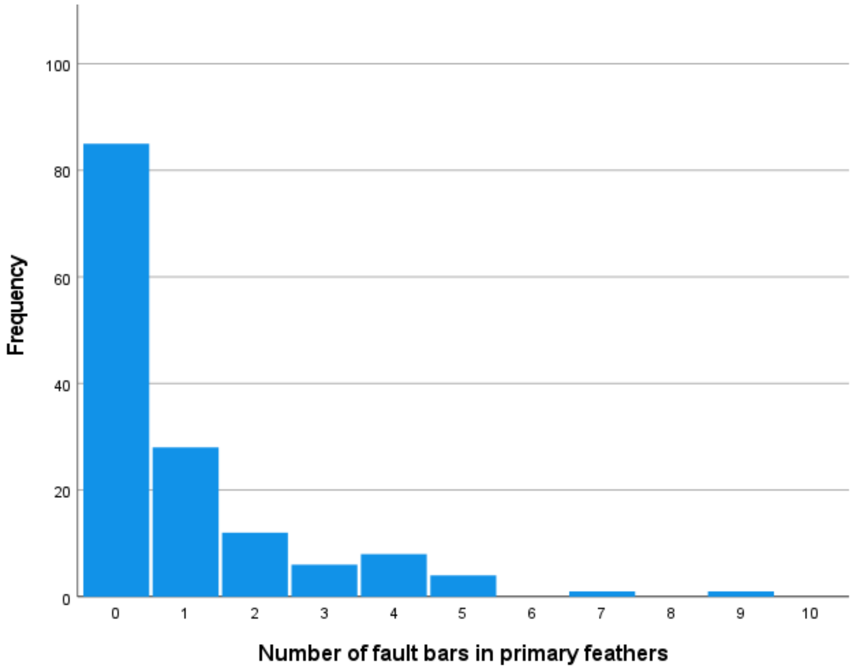


Fig.2.5 Frequency of primary feathers displaying fault bars (n=141 feathers from 47 individuals)

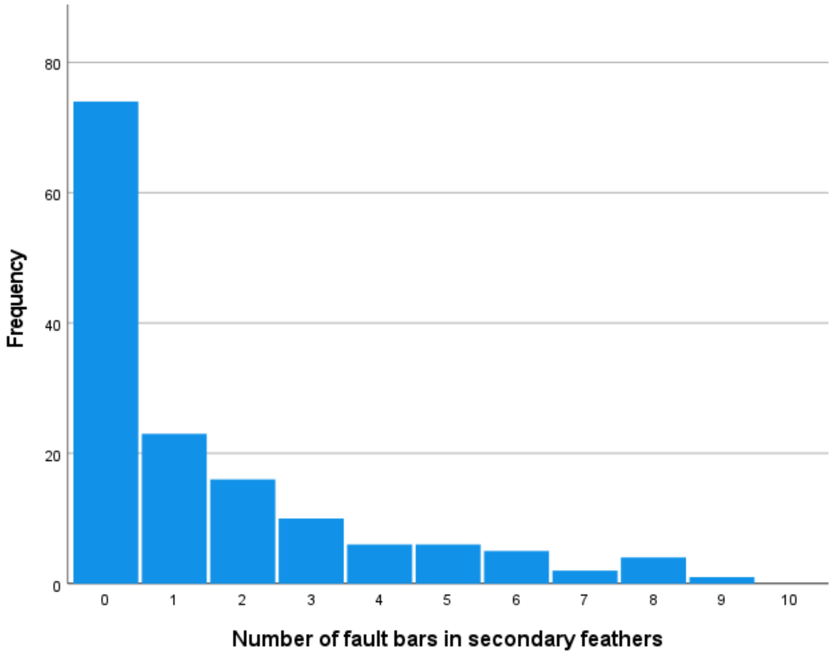


Fig.2.6 Frequency of secondary feathers displaying fault bars (n=141 feathers from 47 individuals)

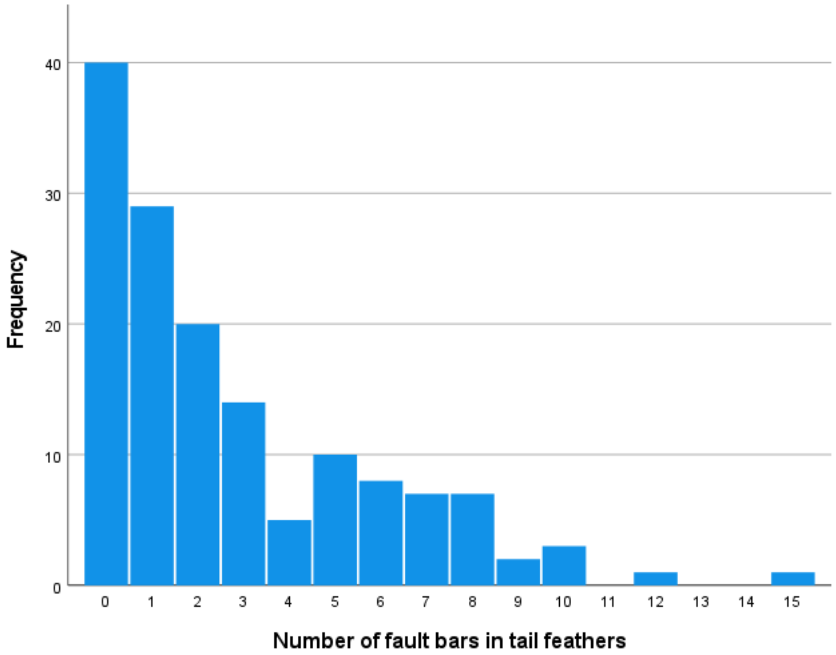


Fig.2.7 Frequency of tail feathers displaying fault bars (n=141 feathers from 47 individuals)

2.2.2.2.2 Number of snapped and/or white feathers

Using the selected feathers stated above (section 2.2.2.1), the number of snapped feathers (see Tab.2.1 for frequencies) and the number of white feathers (see Tab.2.2 for frequencies) was recorded. For each feather type, presence of snapped and/or white feathers was recorded within the three selected feathers. For example, data consisted of 0, 1, 2 or 3 for each feather type.

Tab.2.1 Number of individuals recorded to have 0, 1, 2 or 3 snapped feathers within each feather type: primary, secondary and tail (n=47).

No. snapped feathers	Primary	Secondary	Tail
0	34	39	37
1	6	3	3
2	3	3	3
3	4	2	4

Tab.2.2 Number of individuals recorded to have 0, 1, 2 or 3 white feathers within each feather type: primary, secondary and tail (n=47).

No. white feathers	Primary	Secondary	Tail
0	27	28	43
1	1	1	0
2	5	3	1
3	14	15	3

2.2.2.2.3 Average feather iridescence (T/%)

The percentage of light reflectance from remiges and rectrices was measured with Ocean Optics USB2000+UV-VIS-ES. For each feather category, the three selected feathers were taped in an overlaid manner to a white sheet of paper to simulate feather arrangement on the body (Maia *et al.*, 2009). This particular measurement was chosen, as other studies have found this to provide a reliable interpretation (Maia *et al.*, 2009). Three measurements were taken at 3 specified measurement points across the overlaid feathers to calculate the mean iridescence (Fig.2.8). To determine the three points of measurement, the overall feather length was measured between the base of the feather vane (also known as the superior umbilicus) and the feather tip. From this, the central and quarterly points of the feather length were identified; which formulated the three measurement locations (Fig.2.8). A premium reflection fibre optic light probe (400um, SR, 2m, BX Jacket KB) was positioned at a 45° angle of incidence in order to gain diffuse reflection measurements (Maia *et al.*, 2009). A pulsed xenon lamp provided the light source between 220-750nm (220Hz, ~5500hrs at 50Hz). Reflectance values were measured in percentage of light transmission across a restricted range of light wavelengths - from 300 to 700 nm (Pérez-Rodríguez, Mougeot & Bortolotti, 2011). Iridescence measurements were calculated relative to an Ocean Optics PN:WS-1 diffuse reflectance standard and to a dark reflectance standard which was computed using the program Overture Spectroscopy software version 1.0.1.

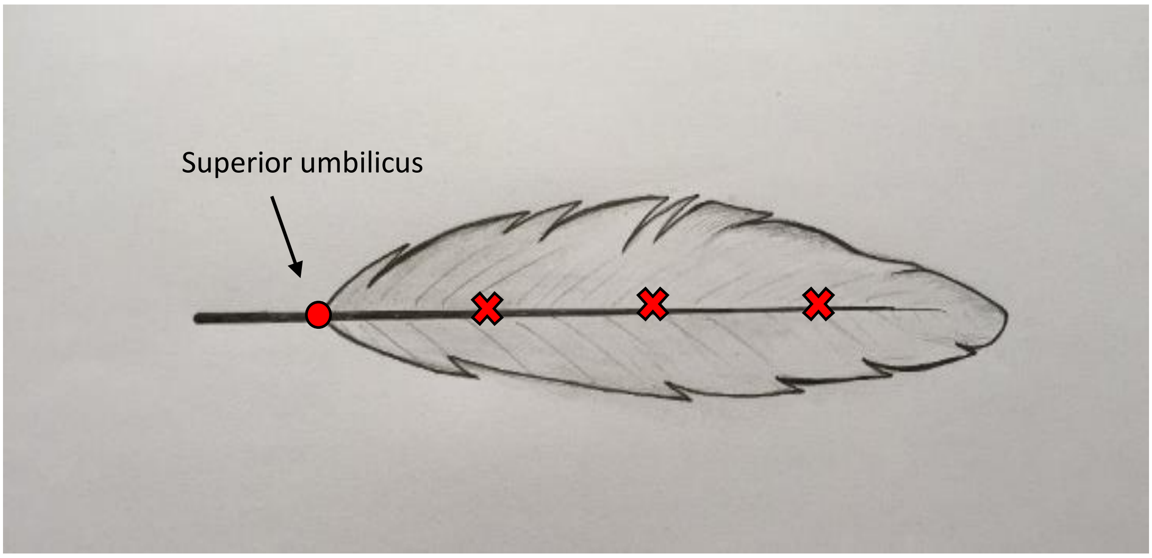


Fig.2.8 Diagram of feather with labelled superior umbilicus and measurement points at quarterly intervals.

2.2.2.5 Average feather base strength

Measurements were gained using a three-point flexural test on a Tinnius Olsen H50KS test machine which was fitted with a 100N load cell (Fig.2.9). This test was performed on samples to gain information on their ultimate flexural load, which is the maximum stress that the sample can tolerate without permanent damage (Jayakrishna, Rajiyalakshmi & Deepa, 2018). The feather base (superior umbilicus seen in Fig.2.8) was selected as the loading point for each feather. The loading point describes the location on the feather in which pressure is applied to induce buckling (Fig.2.9). This method was utilised in order to take advantage of both full and snapped feathers. The number of snapped feathers used for each feather type are as follows out of the 117 sampled feathers (primary n=24, secondary n=15, tail n=20). Morphological measurements of each feather base were taken to the nearest 1 x 10^{-5}m with digital callipers, in order to calculate the area the loading point in correspondence with the oval shape of the shaft (Lees *et al*., 2017). These measurements were taken in order to accurately determine the strength of the loading point depending on the thickness of the superior umbilicus in different feathers. For each feather base, the amount of pressure required to buckle each feather was measured until the feathers bent to approximately 90 degrees (Fig.2.9). Tinnius Olsen Horizon software was then used to identify the buckling point of each feather. This was determined by identifying the highest value on the force displacement curve (Fig.2.9).

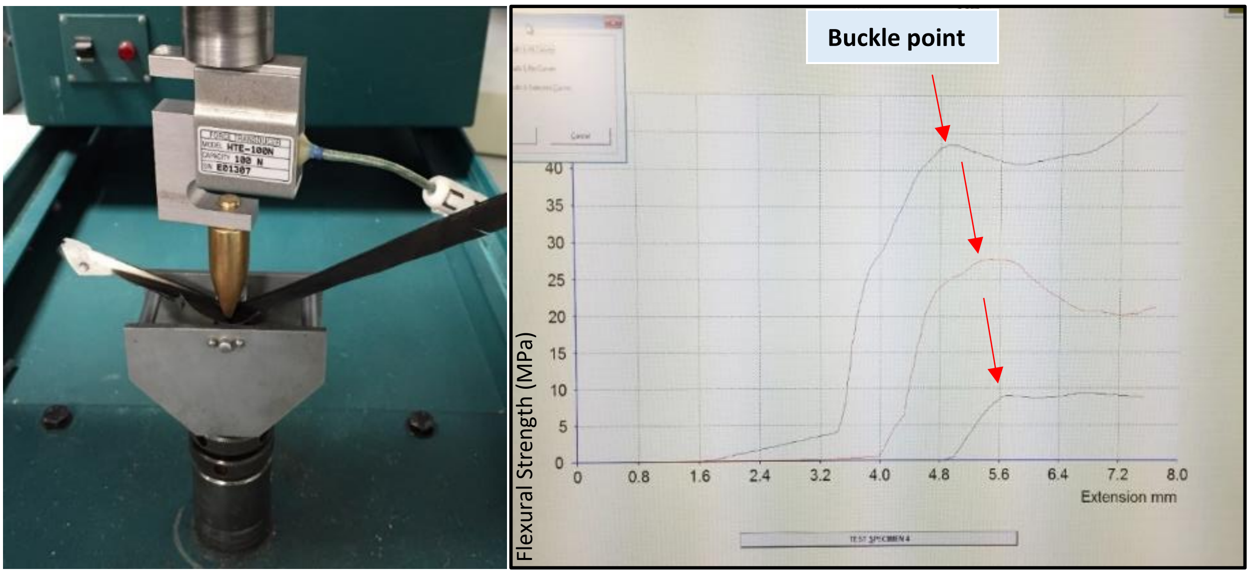


Fig.2.9 Image on left shows feather subjected to three point bending to the point of buckling at approximately 90 degrees. Image on right shows the buckle point (force bending moment) on force displacement curve.

2.2.3 Aim 3 (Chapter 5): To investigate the causes of fault bar production in relation to the chemical profile of feathers, endoparasite burden, sex and age

Chapter 5 was structured around two studies, using different feathers in each. Study 1 was conducted on a smaller sample size than study 2, due to the intensive nature of data collection for this study.

2.2.3.1. Study 1: In-depth analysis of relationships between chemical composition and fault bars

Study 1 was a small in-depth analysis (n=10), using the fault bar samples outlined in the previous chapter (section 2.2.1): feather vane with no fault bars present, a light fault bar, medium fault bar and heavy fault bar. This study was conducted to investigate differences in feather chemical composition at fault bar sections of different severities in relation to fault bar free sections. Four categories were used: 1. No fault bar present 2. Light fault bar 3. Medium fault bar 4. Heavy fault bar (see section 2.2.1 for category guidance). The distance from feather base was measured for each sample to determine the position of each sample. This is an important measure, as studies have shown that the chemical composition of feathers is non-uniformly distributed throughout the length of the feather (Howell *et al.*, 2017). Samples were analysed using SEM, viewed at 150X magnification. The average barbule width in each feather was assessed by measuring the width of each barbule, calculating the mean across all barbules for each measuring point (1 – 4). Fig.2.3 shows the measurement points of each barbule. Energy-dispersive X-ray spectroscopy (EDS) was then used to identify elements that were selected automatically by the INCAEnergy software provided by Oxford Instruments. The automatic selection of elements was achieved through labelled peaks that were auto scaled against one another (Oxford Instruments Analytical, 2006). These peaks were created by count rates of X-rays, using the K alpha number of each element across the energy scale (Oxford Instruments Analytical, 2006). This automatic selection allowed for a non-bias recognition of chemical elements. The elemental weight of chemicals within feathers was recorded as a percentage of the whole sample composition. It is important to note that elements may not be necessarily absent, but the value may be below the limit of detection (CDL). The CDL of this method is typically in the range of 0.001-0.003 mass fraction (Newbury & Ritchie, 2013).

2.2.3.2. Study 2: Main analysis for investigating the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age

Study 2 used 38 individuals (see section 2.3 for further information), investigating the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age. This study used the measure of average (mean) width of fault bars on the following feathers - the third primary feather along with the third secondary and tail feather 3. Fewer feathers were used for this study due to the intensive nature of chemical analysis. These feathers were chosen in order to represent the central area of each feather type on the wing (see Fig.2.4 for locations).

2.2.3.2.1. Feather chemical composition methodology

A 1cm^2 fragment was cut from the base of each feather vane (Fig.2.10). The location of this fragment was chosen to maintain consistency due to the use of snapped feathers. Samples were analysed using SEM-EDS at 100X magnification, analysing of chemical elements within feathers aimed to identify potential environmental pollutants and/or nutritional deficiencies. Environmental contamination was assessed using the identification of high levels of heavy metals such as silver, aluminium, arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead and zinc (Dauwe *et al.*, 2003). Moreover, nutritional deficiencies were assessed using the identification of calcium, zinc and iron (McGraw, 2007). Due to the absence of heavy metals relating to environmental contamination, this factor was no longer explored. Therefore, this 'possible cause' of poor feather condition was ruled out and the chemical element analysis was solely focussed on nutritional deficiencies.

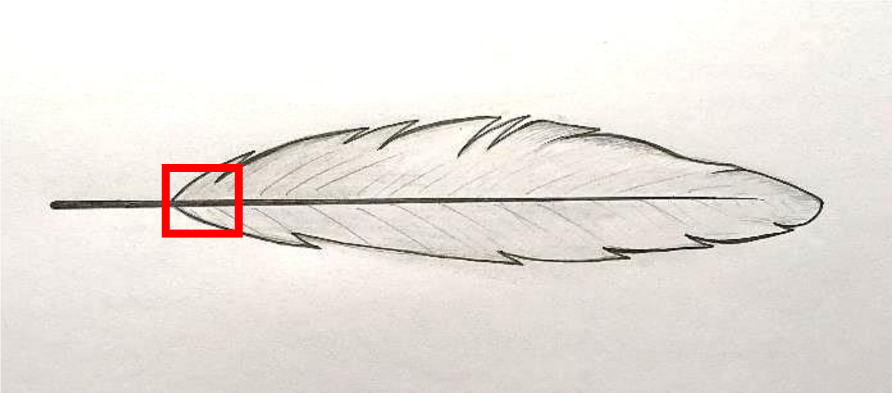


Fig.2.10 Diagram of feather with sample location identified with red box at superior umbilicus.

2.2.3.2.2 Endoparasite burden methodology

Studying endoparasite burden aimed to provide information on how internal helminths may impact feather quality, as they are known to impact immunocompetence and development due to the withdrawal of nutrients from the host (Hill & Montgomerie, 1994; Piersma *et al.*, 2001; Jovani *et al*., 2014). During post-mortem, the intestinal tract was removed from below the ventriculus/gizzard and above the cloaca, as per standard veterinary practice. This incorporated both the small and large intestine. The intestinal tract was then weighed in grams and the length was recorded in centimetres. The intestinal tract was flushed with water using a laboratory wash bottle. The contents were collected in a 50ml falcon tube and labelled with each individual's identification number. In addition, a cut was made down length of intestinal tract in order to detect any remaining parasites. Pinched fingers were passed across the open length of the intestinal tract in order to remove any remaining parasites. These were then added to the falcon tube. Intestinal contents samples were left to settle for 2 hours in order to remove the excess water from above the sample. Isopropyl alcohol (70%) was then added to preserve the samples. Intestinal contents samples were observed using light microscopy to identify helminth presence. The number of helminth species were recorded along with the abundance of each species using identification guidance from Sepulveda and Kinsella (2013). These consisted of a thorny-headed worm species (*Acanthocephalans*) (Fig.2.11A1&A2) and three types of flatworm (*Platyhelminths)* – two tapeworm (*Cestode*) species (Fig.2.11B) and a trematode species (Fig.2.11C). The two tapeworm species were labelled as 'small-headed' species and 'large-headed' species. Identification to the species level was not possible due to rapid degradation of DNA, which was required for DNA analysis. This method was attempted but did not succeed. While not identifiable to the species level, the two tapeworm species could be distinguished by the size of the head (small/large).

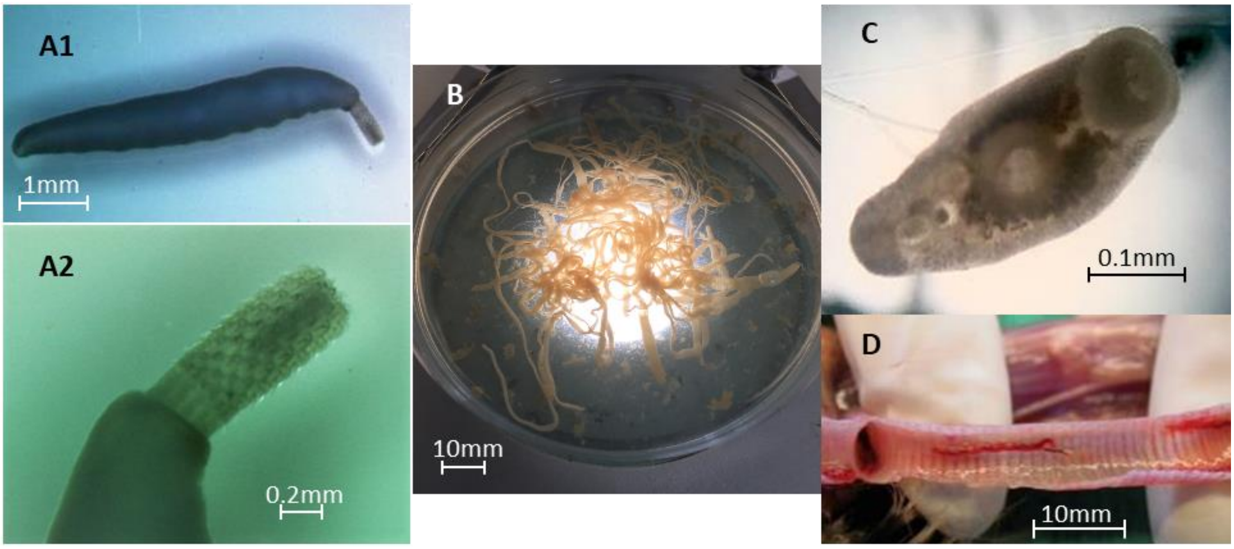


Fig.2.11 Helminth species found in carrion crows.
(A1) Thorny-headed worm/acanthocephalan
(A2) Fixatory organ/proboscis of the thorny-headed worm/acanthocephalan
(B) Tapeworms/cestodes in petri dish including both small and large-headed species
(C) fluke/trematode
(D) Gapeworm/*Syngamus trachea* nematode in the dissected trachea of a gray crowned crane *Balearica regulorum* reprinted from "Infectious Diseases" (Wernery, 2016).

Helminth infections of carrion crows are usually the result of oral consumption, often via an intermediate host such as snails (Demis, Anteneh & Basith, 2015; Tain, Perrot-Minnot & Cézilly, 2006; Wernery, 2016). Thorny-headed worms are found in the alimentary tract of avian hosts, using their fixatory organ (proboscis) to deeply insert into the intestinal wall (Wernery, 2016) (Fig.2.11A1&2). Enteritis and weight loss can be seen in avian hosts of this parasite and have been found to alter the behaviour of other taxa e.g. the amphipod (*Gammarus pulex*) (Tain *et al.*, 2006; Tarello, 2009). Tapeworms are found in the intestinal tract and can cause debilitating impactions when present in large numbers (Demis *et al.*, 2015) (Fig.2.11B). Trematodes are also found in intestinal tract; however, some species have also adapted to inhabit other organs/cavities (Wernery, 2016) (Fig.2.11C). Fluke infections have been found to result in locomotor difficulty and inappetence in avian hosts (Hess, 2019).

As per standard veterinary practice, the trachea was also removed during post-mortem, located below the tongue and above the syrinx. The trachea length was then measured in centimetres. A cut was then made down the length of the trachea with scissors in order to reveal the contents. Only one helminth was present, which was the gapeworm (*Syngamus* *trachea*) (Fig.2.11D). *Syngamus trachea* is a strongylid nematode which affects the respiratory tract of avian hosts (Wernery, 2016). The number of gapeworms was recorded.

In order to select a variable to represent endoparasite burden, it was first important to identify whether a large number of parasites was associated with a larger intestine. In order to test this, a suitable measure for the intestine was determined (weight or length). A spearman's rank correlation was used to test the relationship between intestinal weight and length, finding a strong positive correlation ($r_s(73)=0.521$, $p<0.001$). This shows that both measures are equal in representing intestine size. Therefore, intestinal weight was selected for further interpretation. No association between number of parasite species and intestinal weight was found (rs(69)=0.038, p=0.755). In order to account for tracheal parasites, a test was conducted to identify whether large number of gapeworms were associated with a longer trachea. Trachea length was not found to correlate with the number of gapeworm ($r_s(73)=0.184$, $p=0.130$). This shows that parasite burden was not impacted by tracheal length.

A variable was then chosen to represent parasite burden (parasite presence or the number of parasite species). In this, parasite presence was categorised as 'present' or 'absent' across all species. A strong positive correlation was found between parasite presence and the number of parasite species ($r_s(73)=0.776$, $p<0.001$). This shows that both variables are equal in their representation of parasite burden. Therefore, the number of parasite species was selected for further interpretation, as it also illustrates species diversity. In order to validate the use of this variable for future analysis, the relationship between the number of parasite species and the frequency of each parasite species was determined. Tab.2.1 shows that the number of parasite species was a good representation of parasite abundance. Therefore, this variable will be used for future analyses. Interestingly, Tab.2.1 shows a positive relationship between the frequency of small-headed tapeworm, large-headed tapeworm and flukes. No other relationship between the frequency of other parasite species was found.

Tab.2.1 Spearman's rank correlation output testing relationships between the number of parasite species and the frequency of each species (n=73). Emboldened values indicate p value. Non-emboldened values indicate r value. Yellow highlighter represents significant values.

	No. parasite species	**Freq. Gapeworm *Syngamus trachea***	**Freq. Small - headed tapeworm**	**Freq. Large-headed tapeworm**	**Freq. Thorny-headed worm**	**Freq. Fluke**
No. parasite species	1.000	0.405	0.466	0.705	0.388	0.359
	.	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.002**
Gapeworm	0.405	1.000	-0.105	0.188	-0.062	-0.101
	<0.001	.	**0.377**	**0.111**	**0.603**	**0.396**
Small-headed tapeworm	0.466	-0.105	1.000	0.282	-0.002	0.424
	<0.001	**0.377**	.	**0.016**	**0.987**	**<0.001**
Large-headed tapeworm	0.705	0.188	0.282	1.000	0.229	0.283
	<0.001	**0.111**	**0.016**	.	**0.052**	**0.015**
Thorny-headed worm	0.388	-0.062	-0.002	0.229	1.000	0.168
	0.001	**0.603**	**0.987**	**0.052**	.	**0.157**
Fluke	0.359	-0.101	0.424	0.283	0.168	1.000
	0.002	**0.396**	**<0.001**	**0.015**	**0.157**	.

2.2.3.2.3 Identification of age and sex methodology

The age variable was included, as it is a contributing factor to fault bar production, with young individuals being the most susceptible (Erritzøe, 2006; Hawfield, 1986; Jovani & Blas, 2004; Serrano & Jovani, 2005; Slagsvold, 1982a;). Four categories were formed following Svensson (1992) (Tab.2.2; Fig.2.12). The four age categories consist of nestling, first year juvenile, second year juvenile and adult. Tab.2.2 gives information on each of the age categories with reference to inner mouth colouration and descriptions of feather characteristics with details on seasonal differences. Fig.2.12 gives a visual representation of the variations of inner mouth colourations found within each age category.

Tab.2.2 Carrion crow ageing descriptions taken from Svensson (1992).

Category name	Inner mouth colour	Feather characteristics
Nestling	Fully pink	- Feathers fully in pin formation
First year juvenile	Fully pink	- **Autumn**: Flight feathers are blackish-brown, with some gloss when fresh in Jul-Aug, but with less gloss in Oct, and tips of tail feathers considerably worn by then.
Second year juvenile	Partially pink	- **Autumn**: Plumage as in adults. - **Spring**: Plumage as in first year juveniles in autumn, but tips of tail and primary feathers heavily worn.
Adult (exact year unknown)	Fully black	- **Autumn:** Fresh plumage from Sep. Flight feathers blackish with bluish and green gloss. Tips of tail feathers fresh at least through Dec. - **Spring:** As in autumn but tips of tail and primary feathers slightly worn.

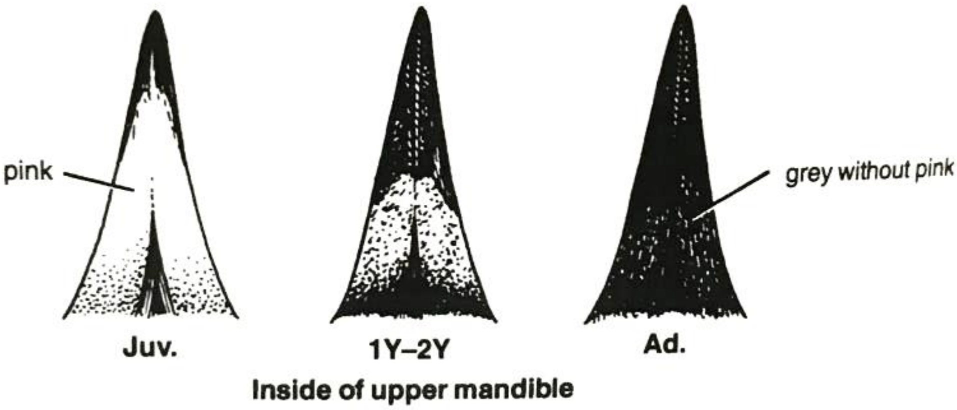


Fig.2.12 Inside of upper mandible character simplified after Kalchreuter (1971), modified according to research by J.Pettersson (*pers. comm.*) in Svensson (1992).

Some studies have found a difference in feather quality between sexes in other bird species, therefore, this factor was also assessed (Balgooyen, 1976; Dawson *et al.*, 2001; Slagsvold, 1982b). A post-mortem procedure was carried out on cadavers with practical training from Sara Abreu, a veterinarian intern at Stapeley Grange Wildlife Centre. In a female individual, only the left ovary and oviduct develop (Panto, 2017). However, in male individuals, both testes develop (Panto, 2017). The texture and appearance of the genitals also differs between sexes (Panto, 2017). The ovary consists of a cluster of small spheres, whereas the testes are smooth singular spheres (Panto, 2017) (Fig.2.13).

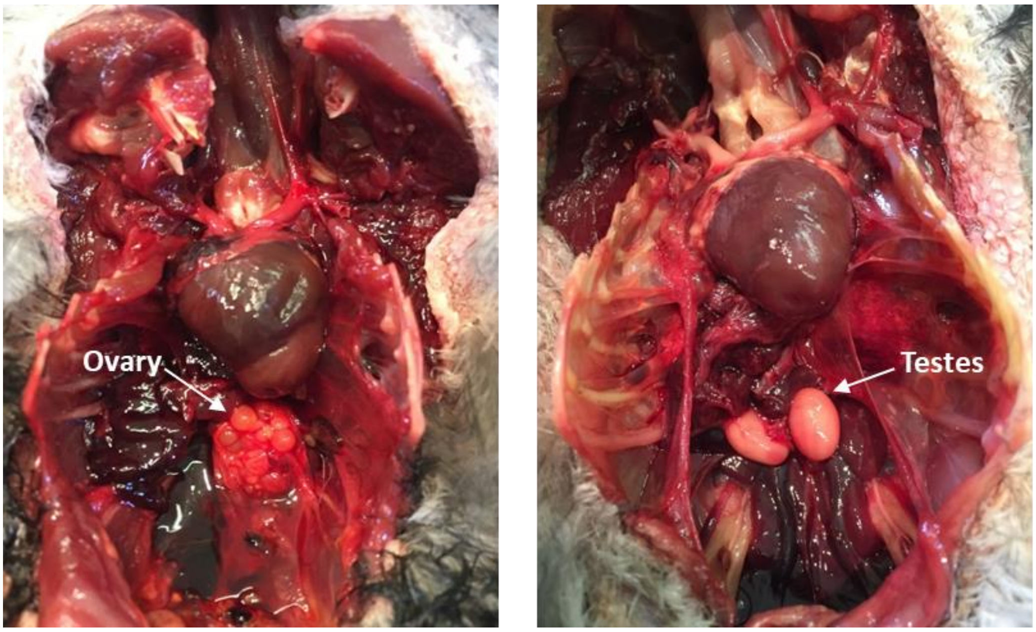


Fig.2.13 Image on left shows the sexual organs of a female carrion crow. Image on the right shows the sexual organs of a male carrion crow.

2.2.4 Aim 4 (Chapter 6): To investigate the consequences of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, sex and age

Chapter 6 was structured around two studies, using the same 38 individuals in both (see section 2.3 for details (Tab.2.3 and 2.4). Study 1 used feather samples seen in section 2.2.2.1 (Fig.2.4): P1, P3, P5, S1, S3, S5, T1, T2, T3. This study was a side-analysis, which investigated differences in feather strength a) across the length of the feather b) between different feathers c) feather regions with and without fault bar occurrence. In order to determine how feather strength differs at sites with and without fault bars, using the methodology outlined in section 2.4.2.4, feather strength measurements were recorded at additional loading points. Loading points were located at regular intervals (2cm) across the length of the feather shaft (Fig.2.14). The first loading point was located at the superior umbilicus, labelled as 0cm (Fig.2.14). Each proceeding loading point was then located at 2cm intervals from this point, recording fault bar presence at each point (Fig.2.14).

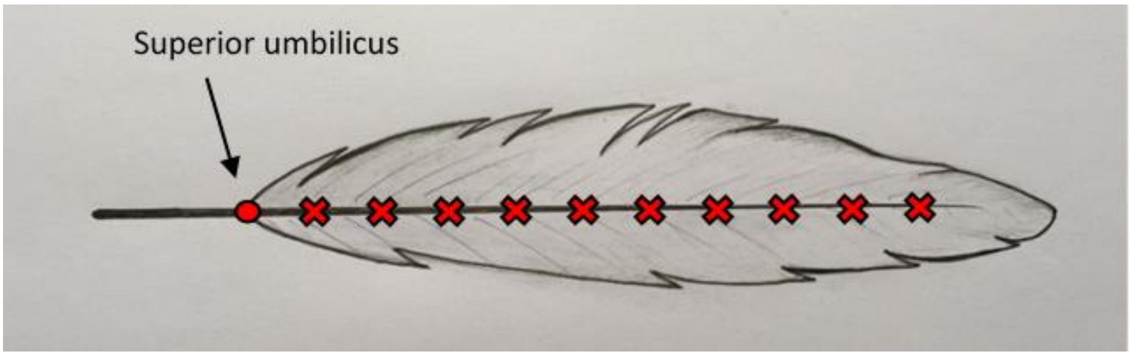


Fig.2.14 Diagram of feather with labelled superior umbilicus and loading points at 2cm intervals.

Study 2 used feathers outlined in section 2.2.3.2 – primary 3, secondary 3, and tail feather 3 (see Fig.2.4 for locations). This study investigated the consequences of poor feather condition (strength) in relation to feather chemical composition at the feather base, endoparasite burden, sex and age. In this, feather strength was tested against the previously mentioned independent variables. Specific statistical analyses were addressed in each data chapter.

2.3 Sample sizes

Tab.2.3 provides sample sizes for each additional measurement, consisting of individuals collected over 2017 and 2018. The age study had the lowest sample size due to this study method being implemented at the end of 2017.

Tab.2.3 Sample sizes (number of carrion crows) for feather quality variables (fault bar measures and feather strength) in relation to additional measurements (chemical profile of feathers, endoparasite burden, sex and age).

Measure	Number of individuals	Category breakdown	N
Chemical profile of feathers	38	N/A	N/A
Endoparasite burden (number of parasite species)	30	0	5
		1	14
		2	6
		3	3
		4	1
		5	1
Sex	33	Male	19
		Female	14
Age	22	1st Yr Juv.	6
		2nd Yr Juv.	7
		Adult	9

Chapter 3: Exploration of fault bar morphology

3.1 Introduction

Resistance to abrasion and strain is an important requirement of feathers, as structural integrity is crucial for energy efficient flight (Corning & Biewener, 1998; Echeverry-Galvis & Hau, 2013; Jovani & Rohwer, 2016). However, structural imperfections are formed if stress is experienced during the growth stage of feathers (Jovani & Diaz-Real, 2012; Kiat & Izhaki, 2016). No further changes are made to the structure of the feather once it is fully grown, as it is no longer in an active state (Jaspers *et al*., 2004). Consequently, the bird's fitness is impacted by poor quality feathers until the next moult cycle. The occurrence of feather defects on the remiges/flight feathers is particularly detrimental to survival, as breakage of these feathers compromise flying ability (Bortolotti *et al*., 2002). This is due to increased wing loading as a result of a reduction in wing area (Navarro & González-Solís, 2007, Velando, 2002). This then increases chances of predation and impacts the ability to travel to areas for foraging (Erritzøe, 2006; Møller *et al.*, 2009). Ultimately, this also impinges on individual success during mate choice due to lack of fitness (Erritzøe, 2006; Møller *et al.*, 2009).

When particular structural characteristics are evident, poor feather quality can be identified. For instance, structural weakness in the form of fault bars (Fig.3.1), also known as stress marks or fret marks, may lead to ragged edges and/or feather breakage due to lack of keratin (Dawson *et al*., 2001; Kose *et al*., 1999; RSPCA, 2013). Riddle (1908) was one of the first scientists to describe fault bars and there is now broad literature on the subject (Erritzøe, 2006; Jovani & Rohwer, 2016; Møller *et al*., 2009). Fault bars were described by Riddle as the "total or partial absence" of barbs, extending across the feather-vane at approximately 90° from the shaft and always running parallel with one another (Riddle, 1908). Murphy *et al*., (1989) have since offered a similar description in the study of white-crowned sparrows (*Zonotrichia leucophrys gambelii*), describing fault bars as a "barbule-free segment of a barb".

Riddle (1908) stated that there are five types of feather faults, with varying severity. Descriptions of which are outlined in Tab.3.1, with further detail provided in section 1.1.2 of the introductory chapter. In addition to Riddle's five types of feather defect, feather holes have also been identified in connection to feather quality (Riddle, 1908; Vagasi, 2014). In light of the different varieties of feather fault seen in feathers, the descriptions of which were used to assess feather damage in carrion crows.

Tab.3.1 Descriptions of different feather faults with reference to illustrations within the introductory chapter.

Fault type	Description	Fig. ref
1	A "total or partial absence" of barbules, extending across the feather-vane at approximately 90° from the shaft and always running parallel with one another (Riddle, 1908)	1.5
	A loss of barbules, peculiar cornification and massing of barbs, in addition to a loss of pigment in the shaft (Riddle, 1908)	1.6
	Barbule-free segment of a barb (Murphy, Miller & King, 1989)	1.7
2	An area of feather which entirely lacked barbs and barbules (Riddle, 1908).	1.8
3	A "very minute depression" across the feather surface which appear in the same direction as the first type of feather defect (Riddle, 1908).	Could not be represented in a drawing
	Comparable to pallid bands - weakened areas across the width of the feather vane and have reduced melanin (Ross *et al.*, 2015).	1.9
4	A weakened/ kinked area in the barb, where the shaft/rachis is constricted and weakened (Riddle, 1908). Can be perceived as a deeply constricted feather pin.	1.10
5	A longitudinal fault bar on one side of the feather vane (Riddle, 1908).	1.11
	Comparable to a 'fault spot', where translucent patches run longitudinally across the feather (Erritzøe, 2006)	1.12

The most recent studies rely on visual descriptions of feather faults, with little known about the underlying morphological changes. Different levels of damages have been postulated but have not been shown on a detailed level, requiring further investigation. Fault bars are often classified using different levels of severity, classifying them as light, medium and strong (Sarasola & Jovani, 2006). These categories are particularly important to consider, as heavier fault bars are more likely to result in feather damage than medium and light fault bars (Sarasola & Jovani, 2006). However, some studies rely on counting fault bars that are "clearly visible", without accounting for variations in width (Møller *et al.*, 2009). In light of current literature in assessing fault bars, it is apparent that there are limited studies which look into the microscopic structure of fault bars, aligning them with macroscopic features.

3.1.1 Formation Theories

There are different hypotheses about the formation of fault bars. The earliest of which describes a lack of nutrient supply to the growing feather at specific points of formation, with reference to alterations in blood pressure (Riddle, 1908). Soon after, Duerden (1909) explored Riddle's theory, concluding that barbule malformation could also result from mechanical damage rather than just a failure of nutrient delivery. More recently, Murphy *et al*. (1989) suggested that unusual muscle contractions around the soft feather shaft during feather growth result in barbule damage. Stress events leading to the production of fault bar occurrence have also been researched, finding faults to be caused by an acute (short-term) stressor rather than chronic (long-term) stress (Jovani & Diaz-Real, 2012). From this, they suggested that fault bar strength/width is not related to the duration of the stress, but to the intensity of the stress (Jovani & Diaz-Real, 2012). Due to the conflicting opinions around the formation of fault bars, the findings of this study will be discussed in relation to each hypothesis.

3.1.2 Study aims and hypotheses

The first aim of this study was to investigate the visual and microscopic characteristics of fault bars. This was conducted in order to identify how fault bars in carrion crows relate to Riddle's (1908) five types of feather faults and the microscopic description linked to each.

The second aim of this study was to assess the relationship between fault bar severity/width and barbule damage to determine the accuracy of using fault bar width as a proxy for damage. Fault bars were initially categorised into light, medium and strong and then studied on a microscopic level to identify how damage manifests in the barbules. Wider fault bars were expected to show more damage to the barbules than light and medium fault bars. The results of each study aim were then discussed in relation to fault bar formation theories.

The third aim of this study was to identify further evidence of structural damage to the feather caused by fault bars. In this, the feather shaft was examined for damage along with the feather pins. Due to personal experience of carrion crow feathers with fault bars, the feather pins and the feather shaft are thought to be damaged at the site of fault bars. This hypothesis stems from a statement made by Riddle (1908) where heavy fault bars have been "observed in the feather-germ, both in their initial stages and immediately before the breaking away of the containing sheaths and the unfolding of the feather-elements". Therefore, this study aimed to observe fault bars in developing pin feathers.

3.2 Methodology

3.2.1 Aim 1: Macroscopic and microscopic characteristics of fault bars

The aim of this study was to investigate the macroscopic and microscopic characteristics of fault bars. This was conducted in order to identify how fault bars in carrion crows relate to Riddle's (1908) 5 types of feather faults. Fig 3.1 shows images of each fault bar category, illustrating varieties of fault bars that occur on carrion crow feathers. Assessment of differences seen between different fault bar widths was viewed on a microscopic level to identify how damage manifests in the barbules.

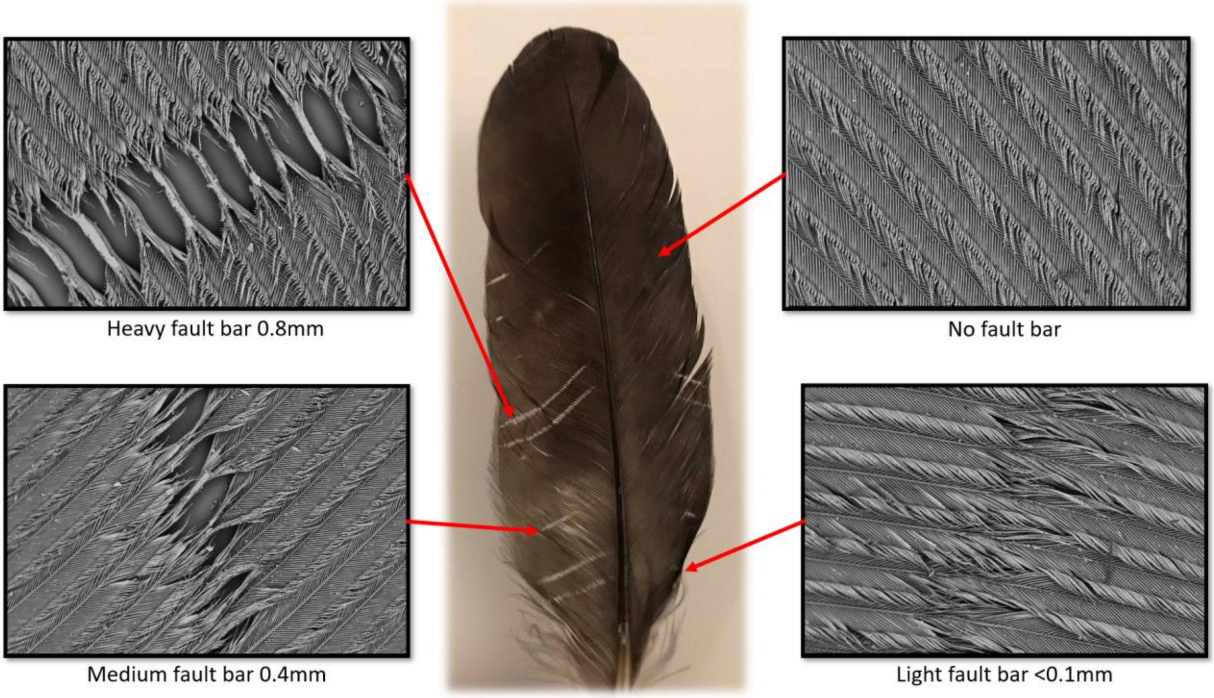


Fig.3.1 Carrion crow *Corvus corone* feather displaying different levels of fault bar severity.

Ten flight feathers (N = 10 individual carrion crows) containing examples of all fault bar categories (outlined above; Sarasola & Jovani, 2006; Fig.3.1) on a single feather were selected and photographed. Fault bars were categorised as ‘light’, ‘medium’ and ‘heavy’, with slight differences to the original method (see section 2.2.1 for further details).

Four samples were cut from each feather representing the following: feather vane with no fault bars present, a light fault bar, medium fault bar and heavy fault bar. Samples were photographed using a Scanning Electron Microscope (SEM) at 150X magnification (see section 2.2.1 for further details). Images were then analysed visually for characteristic examples of feather damage for each fault bar category. This was shown using identification of different types of structural damage with a colour-labelled legend.

The relationship between fault bar severity/width and damage to the barbules was then assessed. In order to achieve this, two variables were created: visual width of fault bar (mm) and the average width of barbules (mm). Shapiro-Wilk testing identified the visual width of fault bars to be non-parametric and the average width of barbules to be parametric. Due to the mix of normality, Spearman’s rank correlation was used to determine the relationship between these variables (Field, 2013).

3.2.2. Aim 2: To identify further evidence of structural damage caused by fault bars, focusing on the feather shaft and growing pin feathers

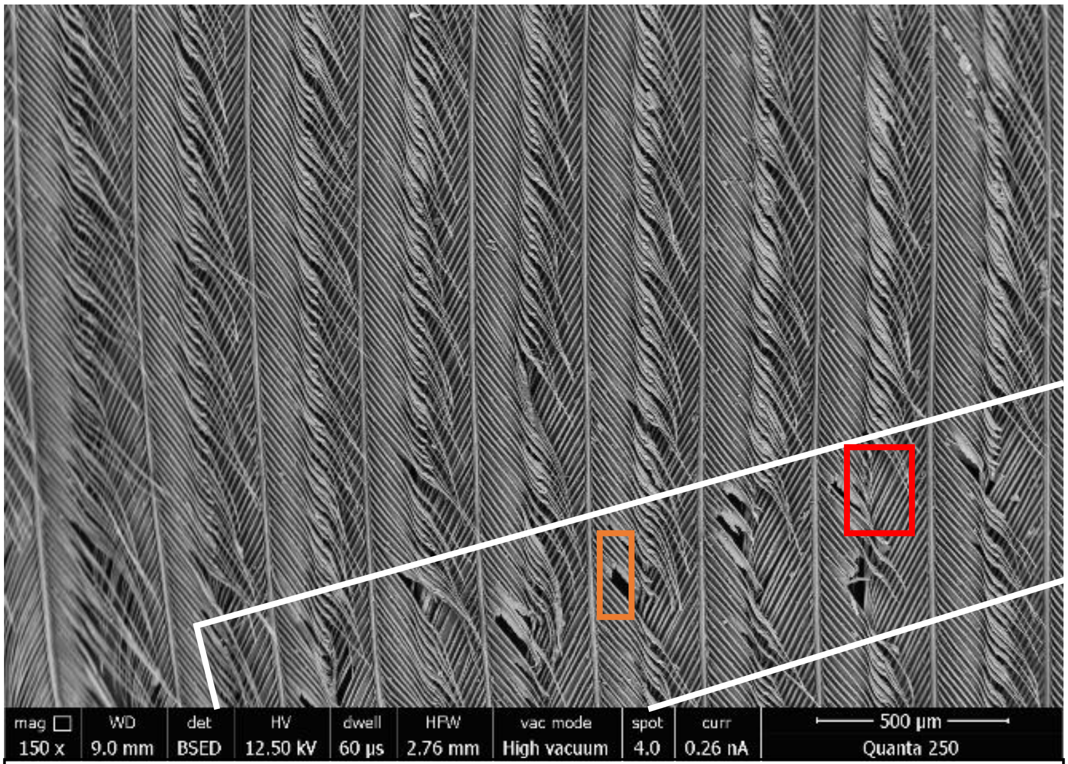
Damage to the feather shaft and feather pin at the site of fault bars was also assessed in order to evaluate their impact on feather structure. Standard photographic images were taken of feather shafts at the sight of a fault bar in addition to SEM imagery at 50X and 100X magnification (see section 2.2.1 for methodology details). Different feathers were used for this aim due to the destructive nature of cutting samples from the feather (N=4). As stated in the methodology chapter (2.2.1), later images of feather shaft damage were collected using SEM equipment at Staffordshire University and can be identified using labelling of ‘low vacuum’. Standard photographic images were taken of pin feathers to capture possible evidence of fault bar occurrence.

3.3 Results

3.3.1 Aim 1: Macroscopic and microscopic characteristics of fault bars

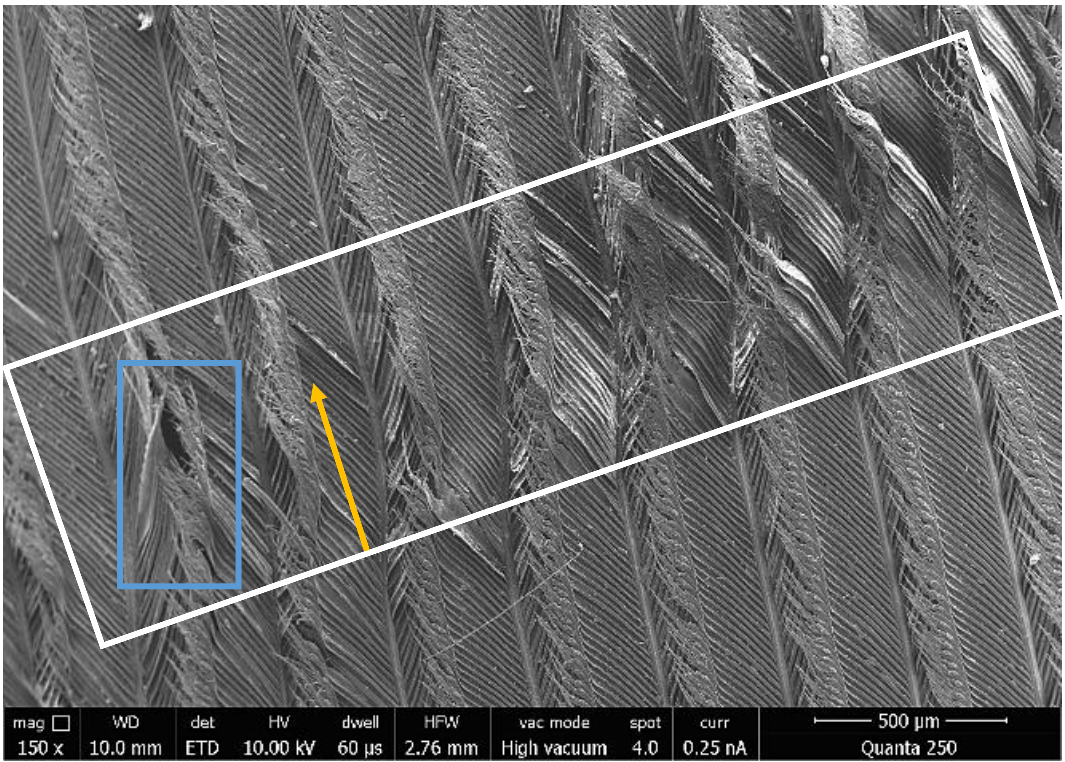
3.3.1.1 Microscopic characteristics of light fault bars

Light fault bars were measured to be <0.01mm. Three images are displayed below, offering examples of differing severity within this category (Fig.3.2 – 3.4). Fig.3.2 illustrated areas of missing hooklets and partially absent barbules. In comparison, Fig 3.3 showed slight crimping in the affected area, with some evidence of a kinked barbs. A disconnection of misaligned hooklets can also be seen here (Fig.3.3). Lastly, Fig.3.4 showed some areas of missing hooklets.



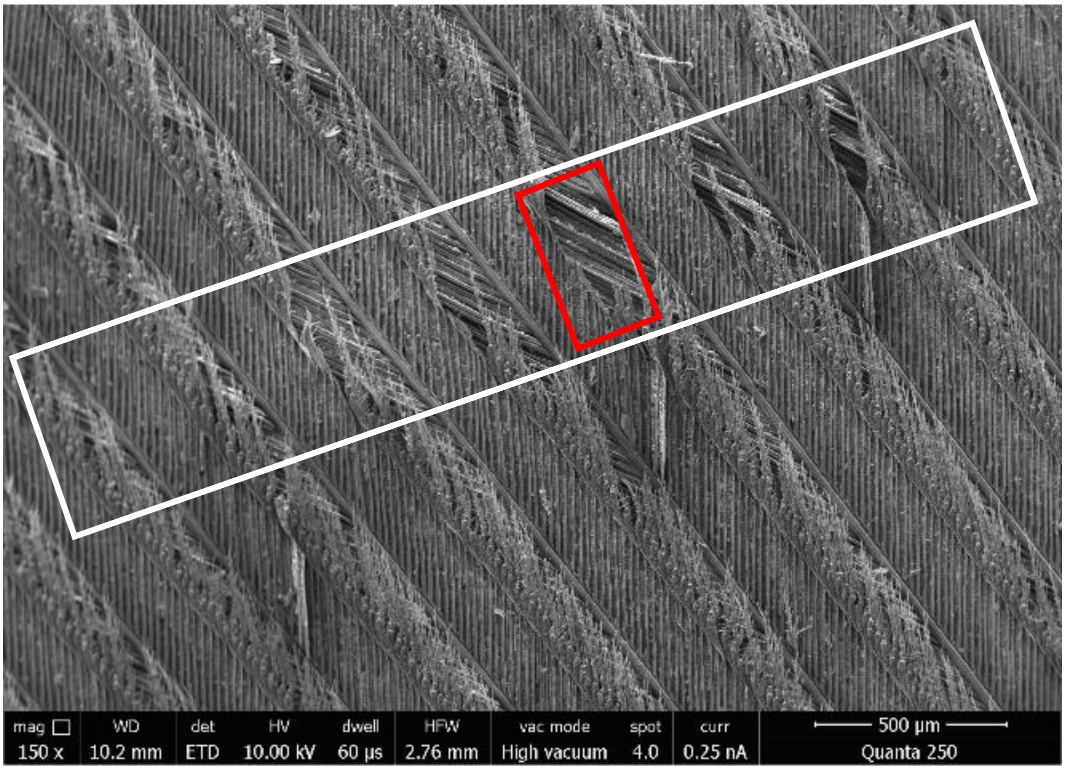
White box = section impacted by fault bar
Red box = Absent hooklets
Orange box = Absent barbules

Fig.3.2 Light fault bar <0.1mm in carrion crow feather.



White box = section impacted by fault bar
Blue box = Misaligned hooklets
Yellow arrow = Kinked barb

Fig.3.3 Light fault bar <0.1mm in carrion crow feather.

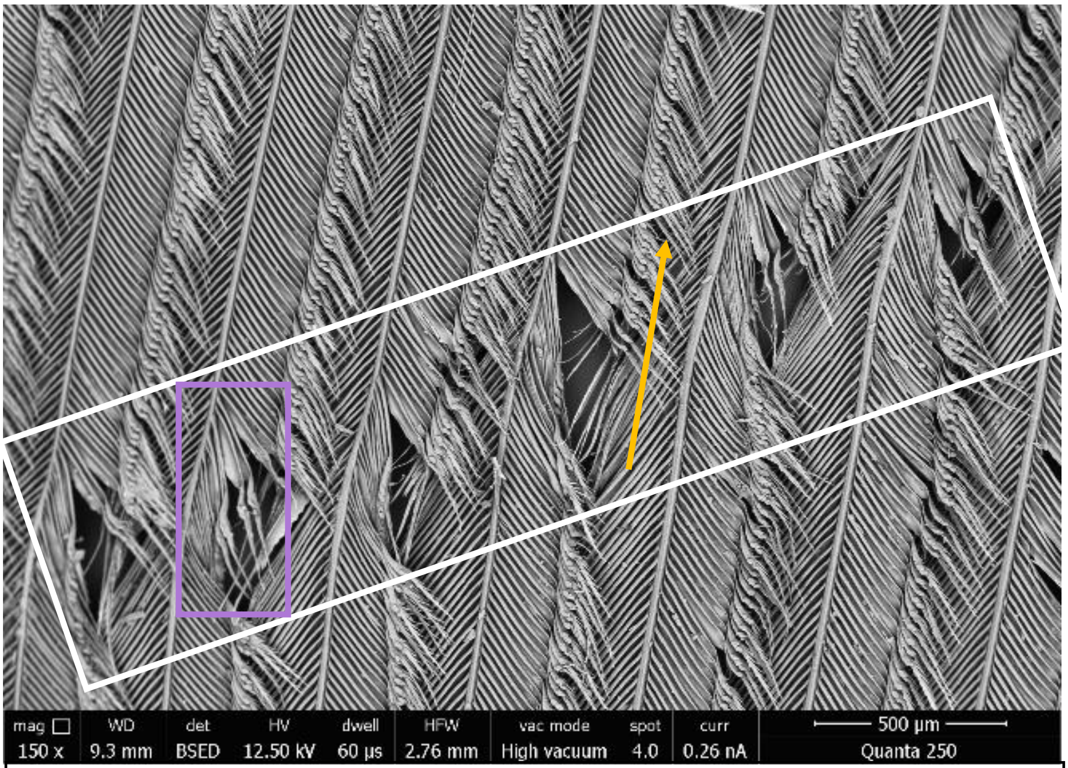


White box = section impacted by fault bar
Red box = Absent hooklets

Fig.3.4 Light fault bar <0.1mm in carrion crow feather.

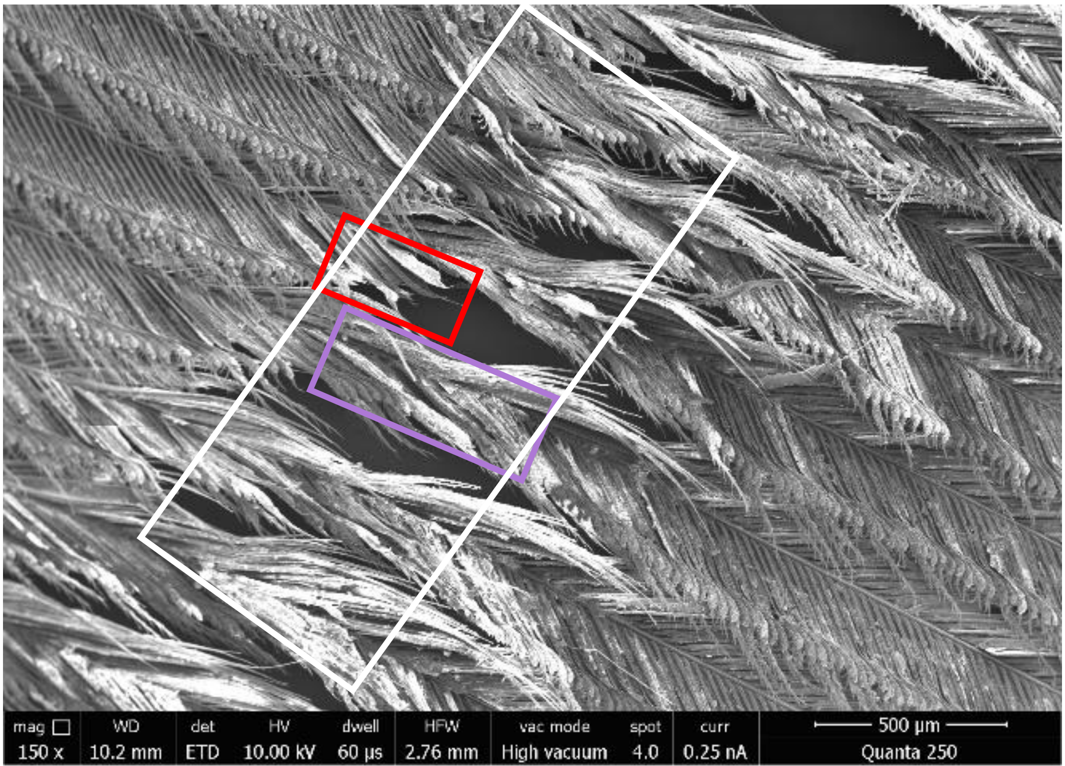
3.3.1.2 Microscopic characteristics of medium fault bars

Medium fault bars were measured to be 0.1-0.59mm. Three images are displayed below, offering examples of differing severity within this category (Fig.3.5 – 3.7). Fig.3.5 shows a crimped/squeezed region with no missing structures, measured at 0.3mm to the eye. Evidence of slightly kinked barbs are seen in this example (Fig.3.5). The barbules and hooklets on the right-hand side of each barb appear to be misaligned and pushed towards the barb. Fig.3.6 shows a fault bar of 0.5mm, showing more severe squeezing. This image illustrates a misalignment of barbules on both sides of the barb in addition to some missing hooklets. Fig.3.7 shows complete constriction of the affected area at 0.4mm to the eye. This shows sections of barbules and hooklets lying flat against the barb. It is difficult to distinguish if any barbules are missing in this example due to the compact nature of the affected section.



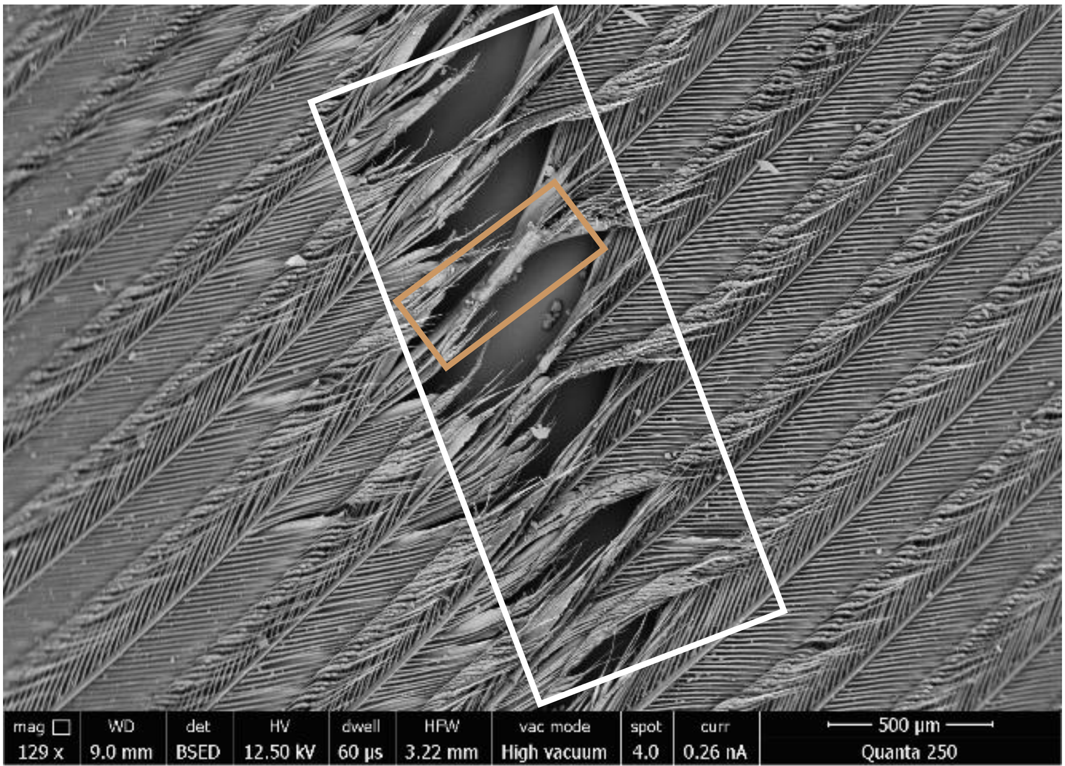
White box = section impacted by fault bar
Purple box = Misaligned barbules
Yellow arrow = Kinked barb

Fig.3.5 Medium fault bar 0.3mm wide in carrion crow feather.



White box = section impacted by fault bar
Red box = Absent hooklets
Purple box = Misaligned barbules

Fig.3.6 Medium fault bar 0.5mm wide in carrion crow feather.

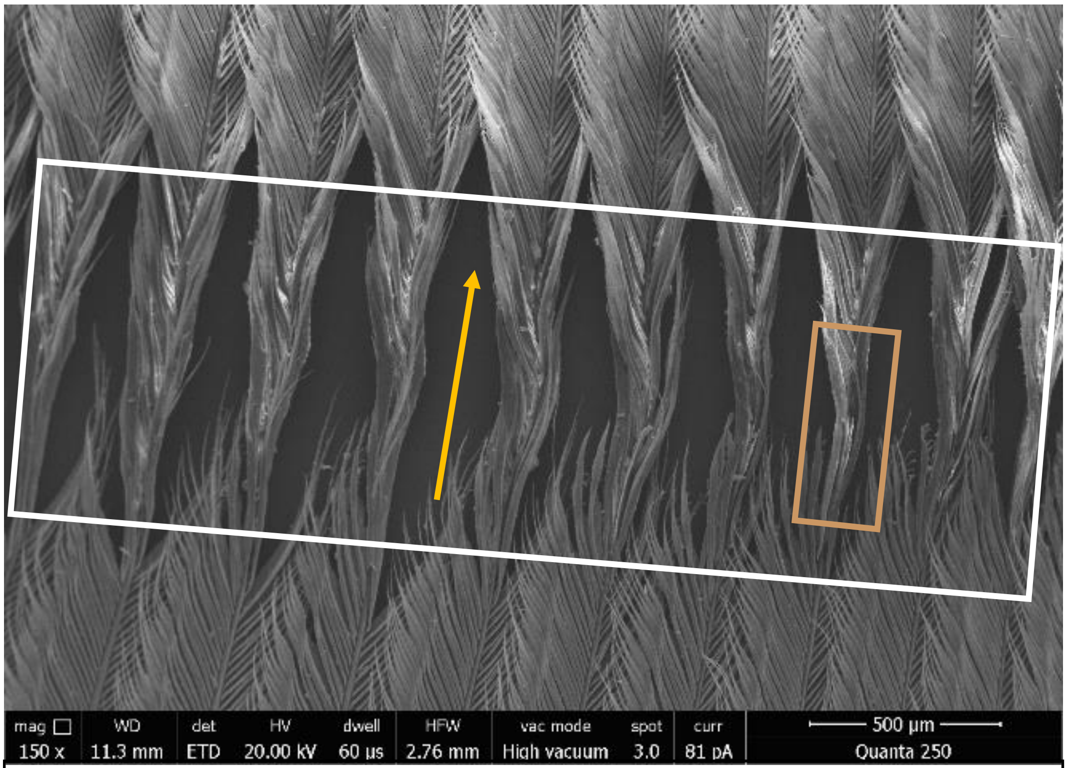


White box = section impacted by fault bar
Brown = Heavy constriction

Fig.3.7 Medium fault bar 0.4mm wide in carrion crow feather.

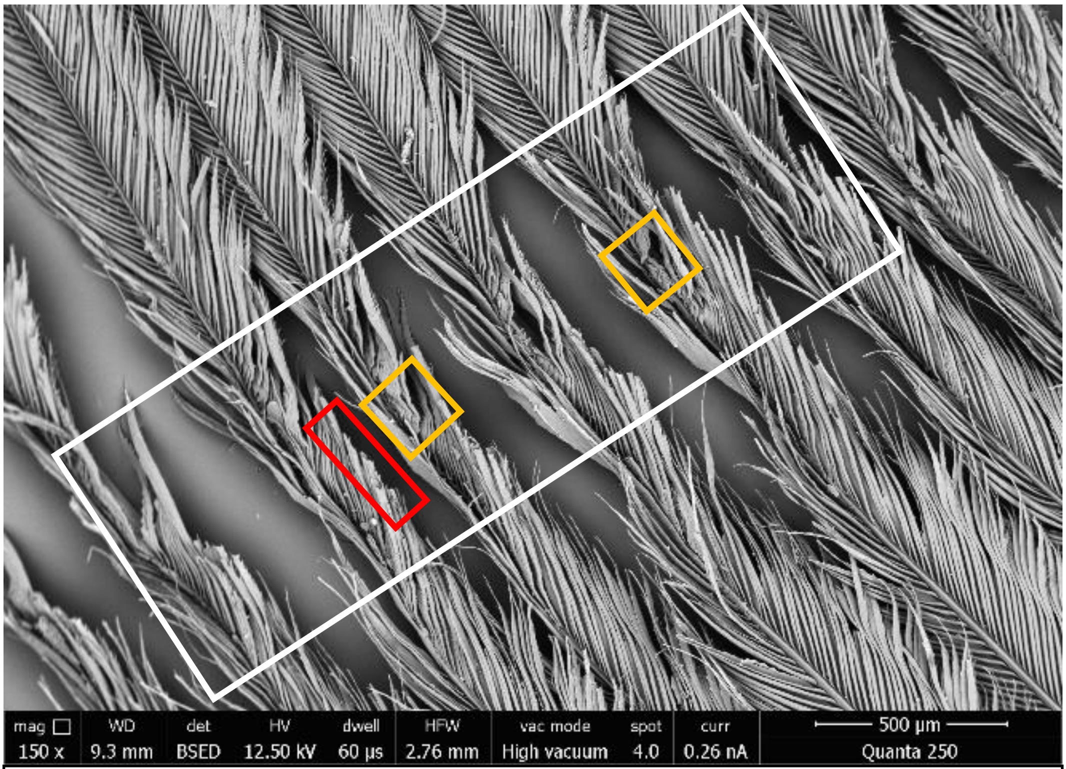
3.3.1.3 Microscopic characteristics of heavy fault bars

Heavy fault bars range between 0.6 to 1.1mm. Three images are displayed below, offering examples of differing severity within this category (Fig.3.8 – 3.10). Fig.3.8 shows a 0.8mm fault bar with kinked barbs and constriction across a wide area. Fig.3.9 shows a 1.1mm fault bar, with less constriction but more damage to the structures than the previous example. This damage consisted of kinked barbules and missing hooklets. Fig.3.10 shows a 0.9mm fault bar with very tightly constricted barbules. The barbule is very compact compared to the second image. There is also evidence of missing hooklets seen here (Fig.3.10).



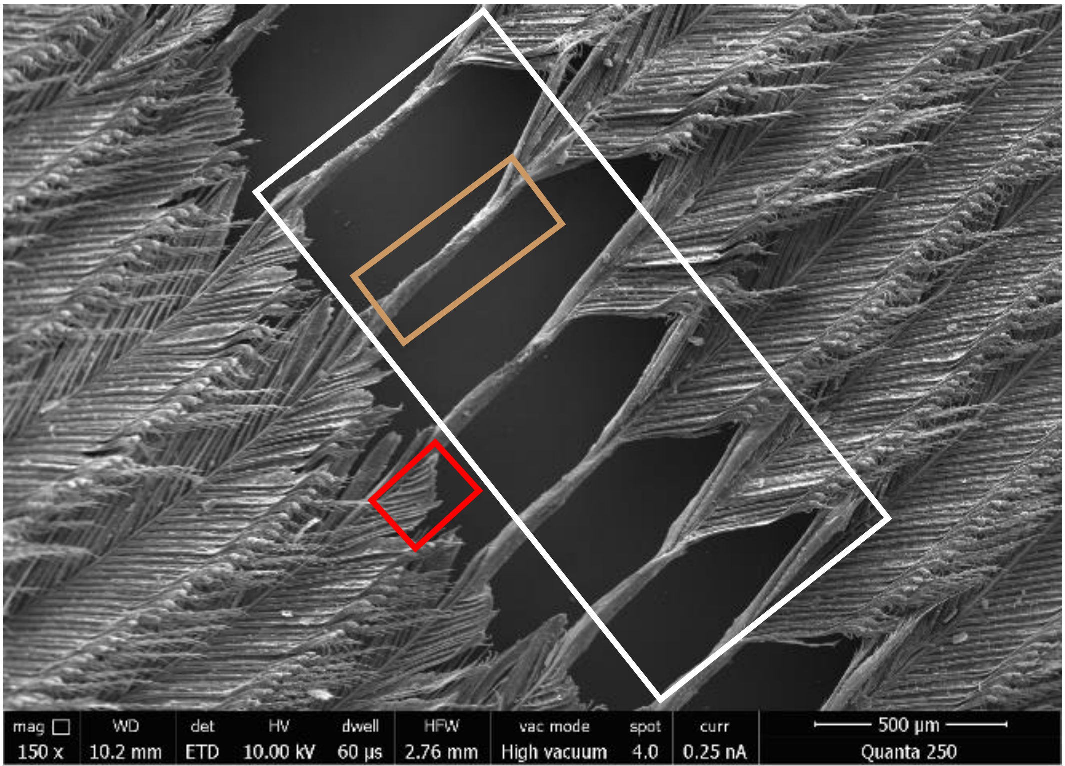
White box = section impacted by fault bar
Yellow arrow = Kinked barb
Brown = Heavy constriction

Fig.3.8 Heavy fault bar 0.8mm wide in a carrion crow feather.



White box = section impacted by fault bar
Red box = Absent hooklets
Yellow box = Kinked barbule

Fig.3.9 Heavy fault bar 1.1mm wide in a carrion crow feather.



White box = section impacted by fault bar
Red box = Absent hooklets
Brown = Heavy constriction

Fig.3.10 Heavy fault bar 0.9mm wide in a carrion crow feather.

3.3.1.4 Relationship between average fault bar width and average barbule width

The aim of this section was to investigate whether the visual width of fault bars is an accurate representation of feather damage. A significant, strong, negative relationship was found between the visual width of fault bars and the average width of barbules ($r_s(40) = -0.915$, $p<0.001$; Fig.3.11). This shows that heavy fault bars have narrow barbules in comparison to less severe fault bars, explaining 92% of the variation. This shows that the visual width of the fault bar is an accurate representation of feather damage.

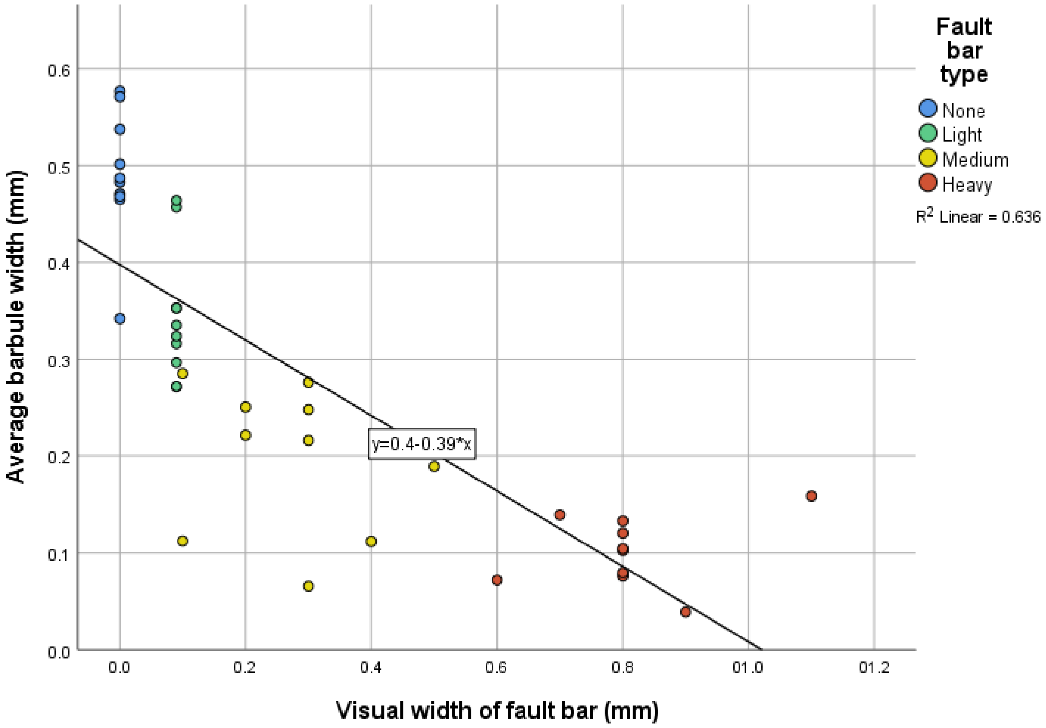


Fig.3.11 Average width of barbules (mm) in relation to visual width of fault bar (mm) according to fault bar type: no fault bar, light fault bar, medium fault bar, heavy fault bar (N=10 per category).

3.3.2 Aim 2: To identify further evidence of structural damage caused by fault bars

3.3.2.1 Damage to the feather shaft at the site of fault bars

Damage to the feather shaft and feather pins at the site of fault bars were assessed in order to evaluate their impact on feather structure. Fig.3.12 shows a series of carrion crow feathers showing a progression of damage to the feather shaft at the site of fault bars. Fig.3.12A illustrates the lowest level of shaft damage, with minute indentations found on the surface of feather shaft. This occurred in a variety of fault bar severities. Further damage is seen in Fig.3.12B, where the feather shaft appears to be narrowed, as if pinched. Splitting of the feather shaft can also be seen here, demonstrating the severity of this damage. Fig.3.12C shows a slightly more damaged feather shaft, where there is a distinct lack of pigment at the site of the fault bar. Fig.3.12D shows the most severe example of feather shaft damage, with indentation, narrowing and lack of pigment. This example appears to be a combination of examples B and C, illustrating a variety of damage characteristics.

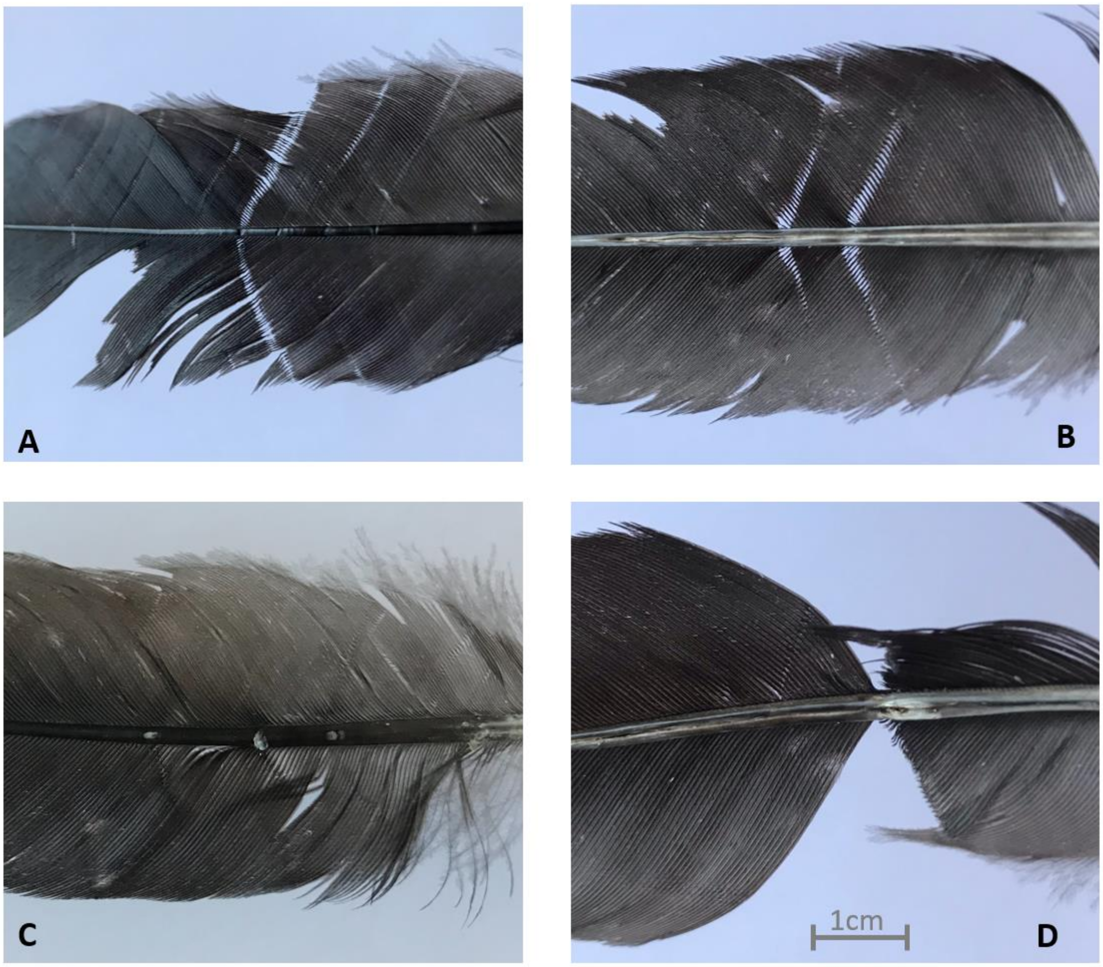


Fig.3.12 Carrion crow feathers showing damage to the feather shaft at the site of fault bars: (A) surface of feather shaft indented (B) narrowing and splitting of the feather shaft (C) lack of pigment (D) indentation, narrowing and lack of pigment.

Fig.3.13 shows a scanning electron microscope image of feather shaft damage seen in example D, displaying disruption to the surface layers. To explore feather shaft damage further using SEM, two additional feathers were photographed under low vacuum. Fig.3.14 (x50 magnification) and Fig.3.15 (x100) show damage at the site of a fault bar measuring 0.59mm. This appears as a crack in the surface layer of shaft. Fig.3.16 (x50) and Fig.3.17 (x100) illustrate a fault bar of 0.63mm, with disruption to the surface layer and slight narrowing of the shaft at the affected area.

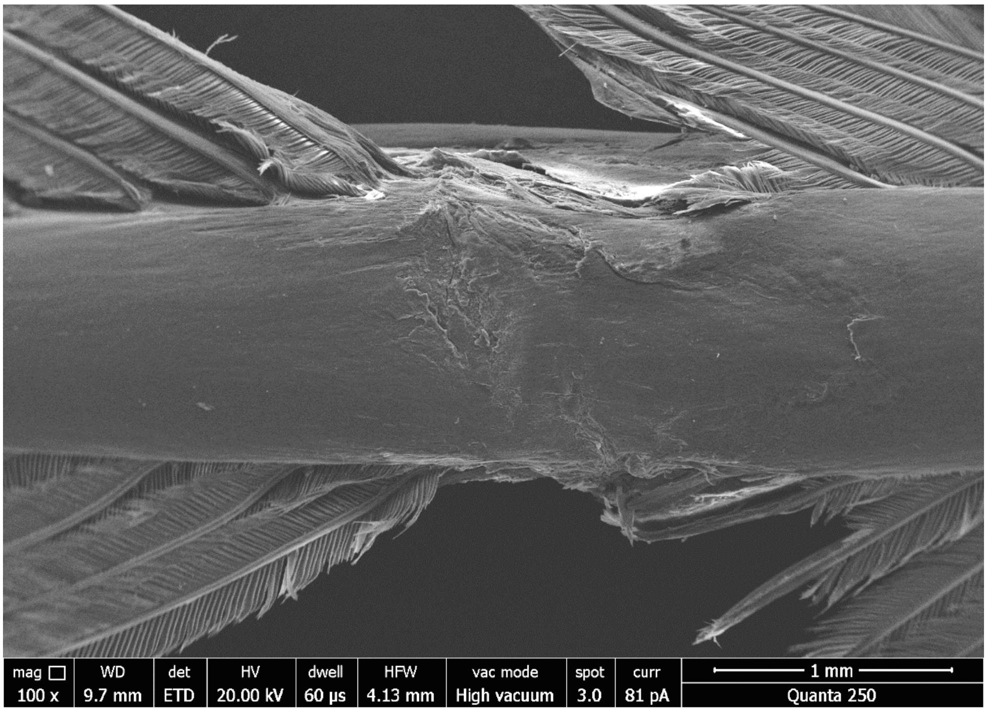


Fig.3.13 Scanning electron microscope image of a feather shaft damaged by fault bar occurrence in a carrion crow feather.

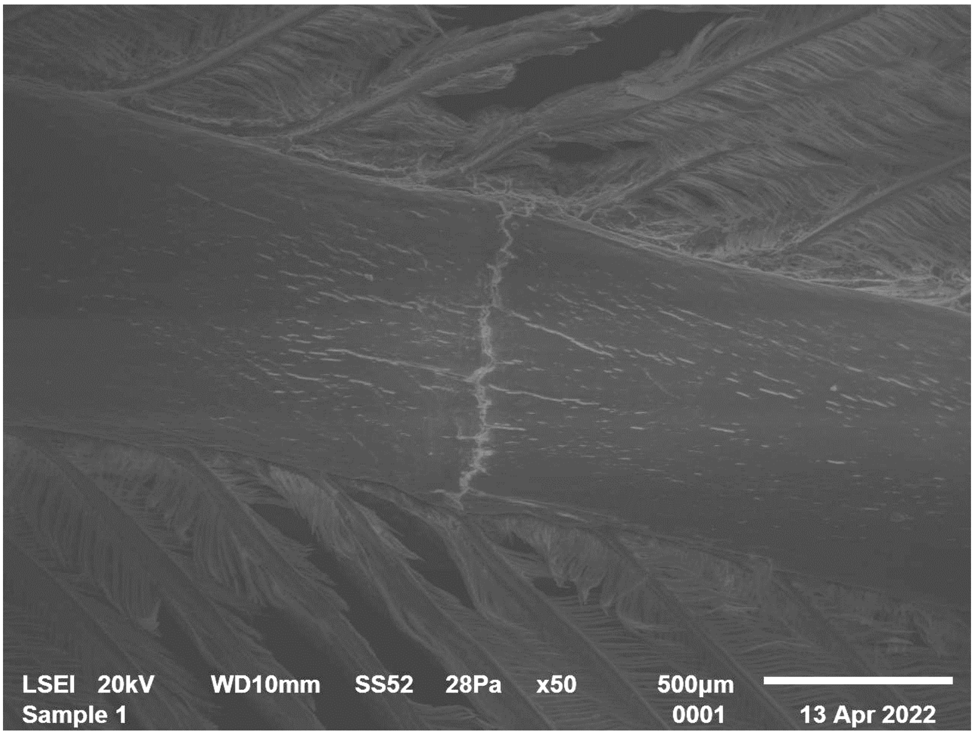


Fig.3.14 Low vacuum scanning electron microscope image at x50 magnification of a feather shaft damaged by a heavy fault bar measuring 0.59mm in a carrion crow feather.

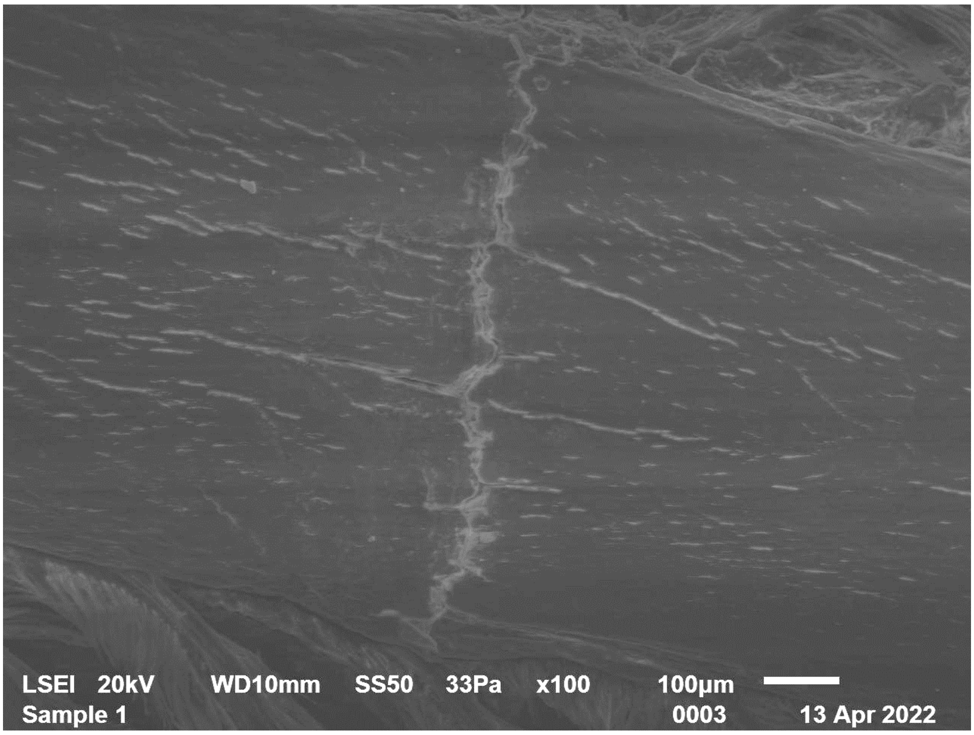


Fig.3.15 Low vacuum scanning electron microscope image at x100 magnification of a feather shaft damaged by a heavy fault bar measuring 0.59mm in a carrion crow feather.

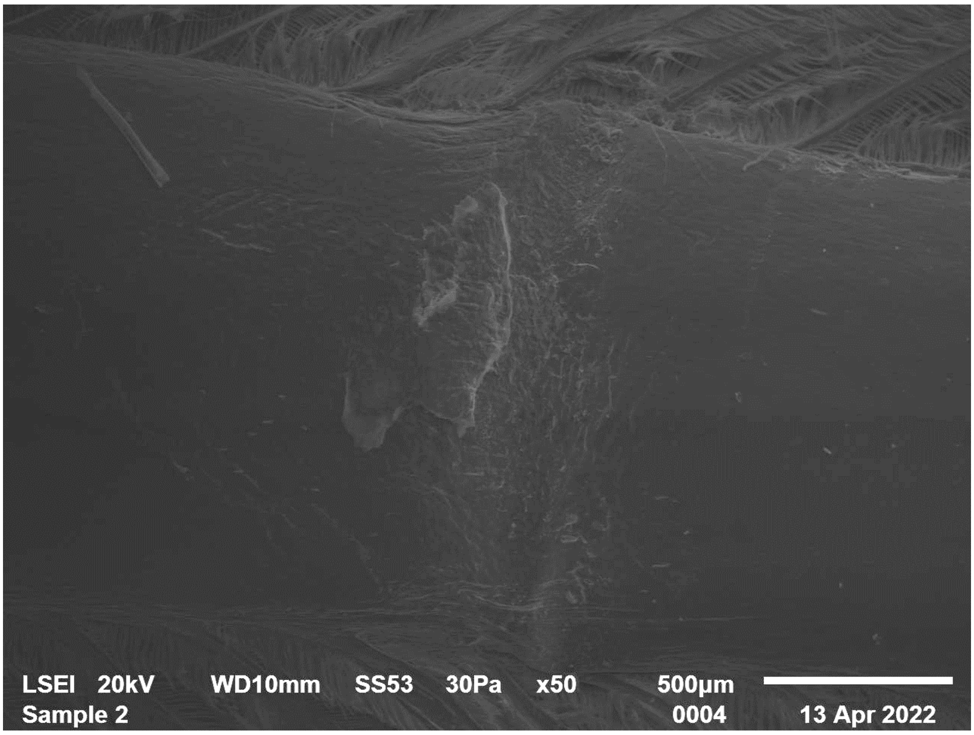


Fig.3.16 Low vacuum scanning electron microscope image at x50 magnification of a feather shaft damaged by a heavy fault bar measuring 0.63mm in a carrion crow feather.

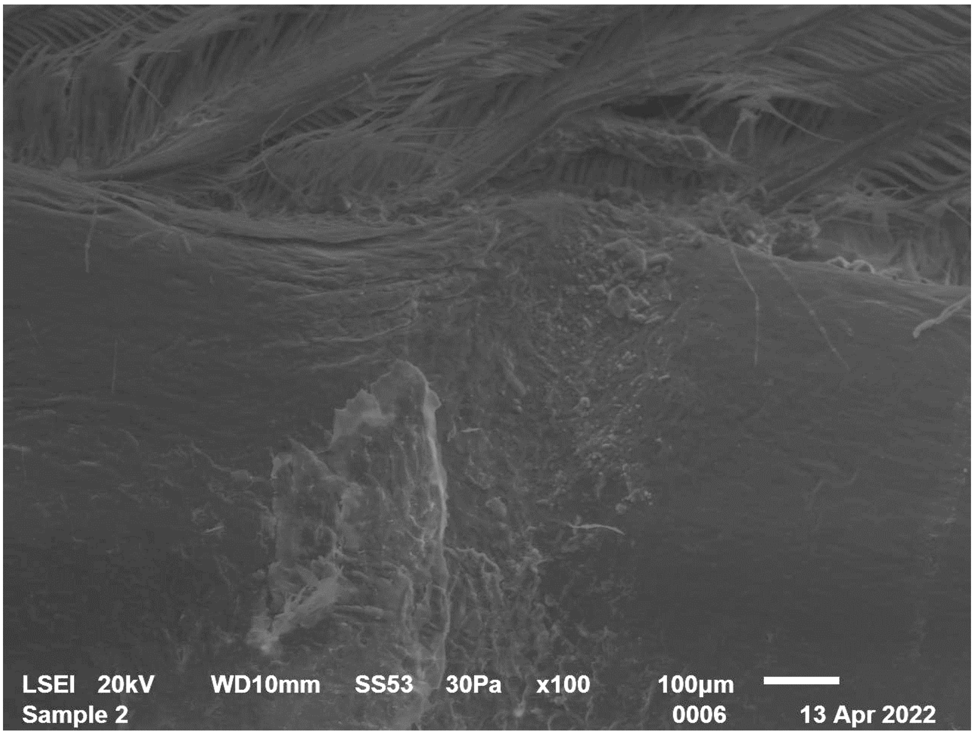


Fig.3.17 Low vacuum scanning electron microscope image at x100 magnification of a feather shaft damaged by a heavy fault bar measuring 0.63mm in a carrion crow feather.

3.3.2.2 Damage to the feather pin at the site of fault bars

As mentioned above, damage to feather pins at the site of fault bars was also assessed to evaluate their impact on feather structure. Fig.3.18 shows a pin feather displaying lines of varying widths across the circumference of the structure. Fault bars are present on the emerging feather vane, indicating to occurrence of faults on this feather. Adjacent feather pins also shared these traits and was seen in a number of carrion crows during the study. One such pin feather with the same characteristics was dissected to reveal a fault bar at the lines depicted on the feather pin. However, this structure was too fragile to photograph due to the nature of opening the developing pin feather. Fig.3.19 and 3.20 provide further evidence of fault bars in pin feathers. Fig.3.20C is particularly interesting as it highlights the narrowing of the feather pin at the site of a fault bar.

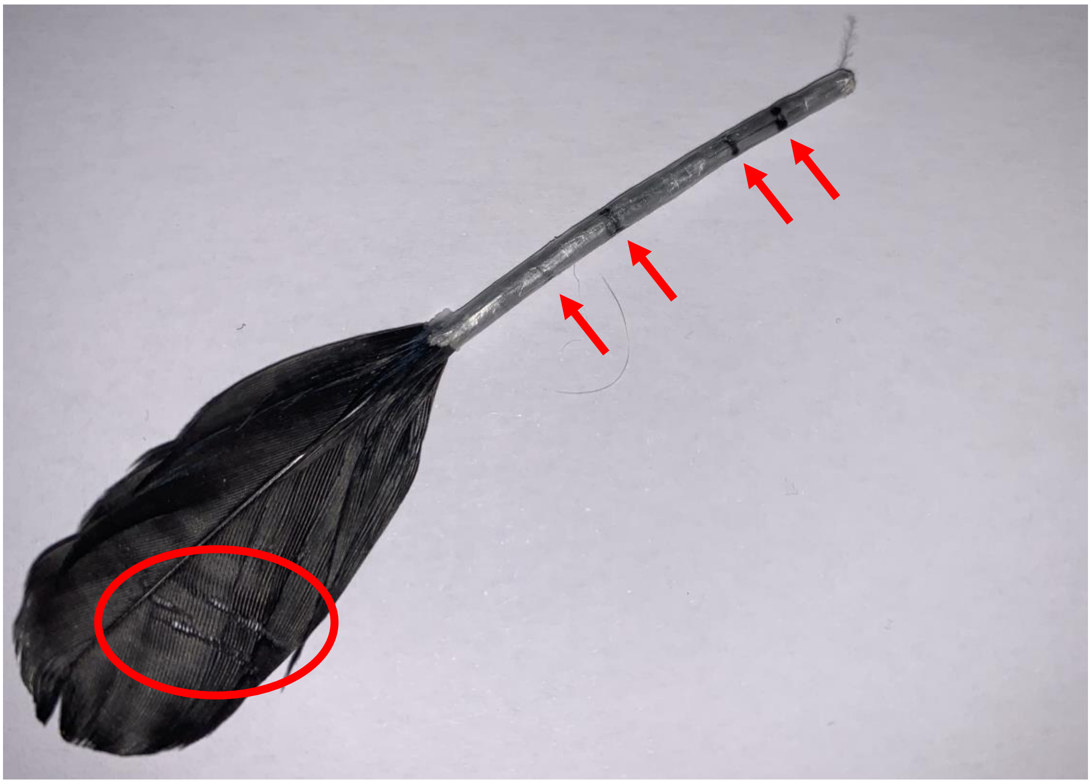


Fig.3.18 Carrion crow pin feather displaying lines of varying widths across the circumference of the structure. Red arrows highlight lines seen in developing pin. Red circle identifies presence of fault bars in the feather structure.

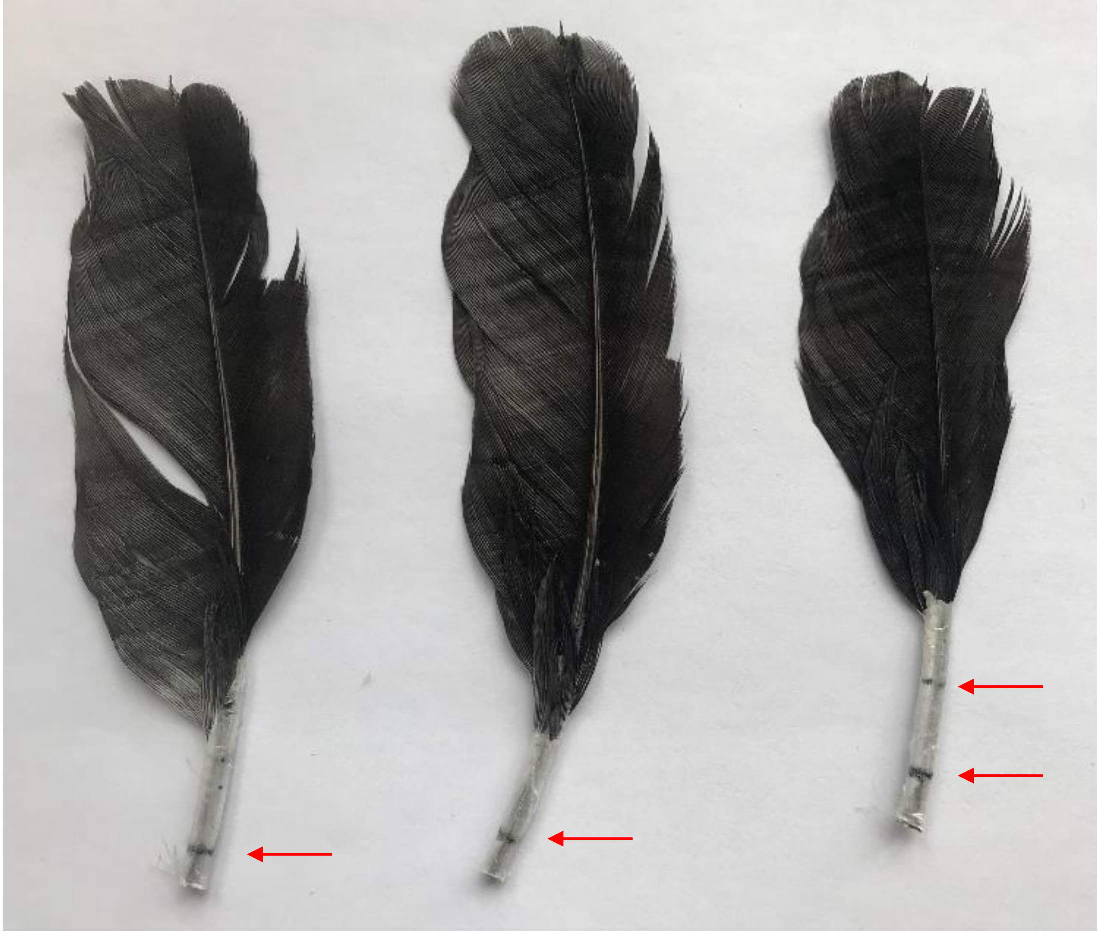


Fig.3.19 Carrion crow pin feathers displaying lines of varying widths across the circumference of the structure.

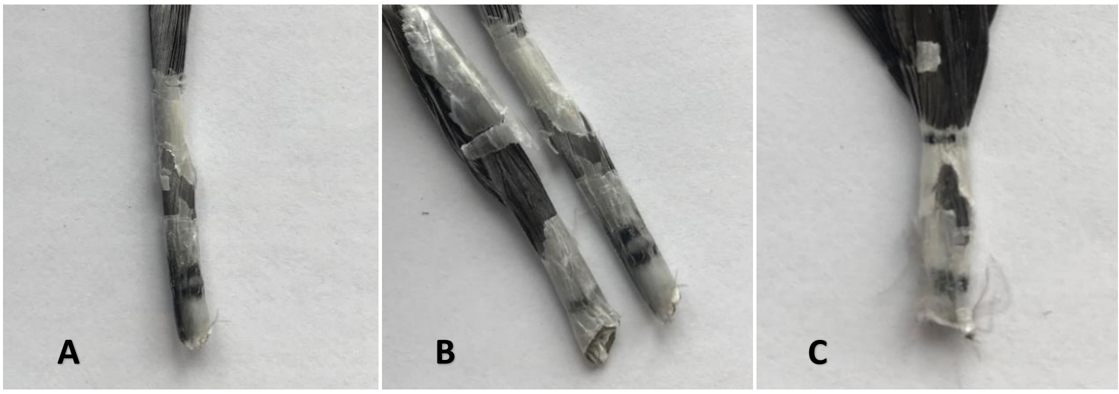


Fig.3.20 Carrion crow pin feathers displaying lines of varying widths across the circumference of the structure.

3.4 Summary of results

- Fault bars found in carrion crow feathers appear characteristically differently to current descriptions. Results found barbules to be constricted rather than displaying areas of missing sections.
- A significant relationship was found between visual fault bar width and average barbule width. This shows that visual fault bar width is an accurate representation of barbule damage.
- Feather shaft damage can be seen at the site of fault bars, revealing the extent of damage caused to the overall feather structure.
- Fault bars can be seen in developing pin feathers.

3.5 Discussion

3.5.1 Aim 1: Macroscopic and microscopic characteristics of fault bars

The macroscopic and microscopic characteristics of fault bars in carrion crows were first assessed in relation to current literature. Fault bars were described by Riddle as the "total or partial absence" of barbs, extending across the feather-vane at approximately 90° from the shaft and always running parallel with one another (Riddle, 1908). Murphy *et al*. (1989) have since offered a similar description in the study of white-crowned sparrows (*Zonotrichia leucophrys gambelii*), describing fault bars as a "barbule-free segment of a barb" (Fig.1.7). In comparison to these descriptions of the commonly known 'fault bar', there appears to be morphological differences seen in fault bars studied in carrion crow feathers. Scanning electron microscope images (SEM) revealed that the barbules were often constricted rather than absent where fault bars occur. This may be due to improved SEM technology, as the images captured by Murphy *et al*. were published in 1989. Moreover, the findings in this study appear to be very similar to an illustration provided at the end of Riddle's publication (Fig.1.6), describing a "very peculiar cornification and massing of barbs". However, he also described this occurrence as a "loss of barbules in the fault bar region" (Riddle, 1908), which is similar to the description provided by Murphy *et al*. (1989). The concept of "missing" barbules in current literature is therefore questioned in this study, offering an alternative hypothesis in relation to the macroscopic and microscopic characteristics of fault bars. However, it should be noted that the occurrence of fault bars may vary between species. For example, fault bar occurrence has been documented in a range of passerine and non-passerine species including white-crowned sparrows *Zonotrichia leucophrys gambelii* (Murphy *et al*., 1988; Murphy *et al*., 1989), ospreys *Pandion haliaetus* (Machmer *et al*., 1992), American kestrel *Falco sparverius, Linnaeus* (Bortolotti *et al*., 2002), white storks *Ciconia Ciconia* (Jovani & Blas, 2004) and many more (Serrano & Jovani, 2005; Sarasola & Jovani, 2006; Pap *et al*., 2007; Møller *et al*., 2009; Strochlic & Romero, 2008; Jovani, Montavole & Sabate, 2014). Therefore, further research must be conducted using current technology to confirm the occurrence of which across a range of species.

In light of the findings here, it is interesting to discuss a concept provided by Riddle (1908) in relation to the differences in susceptibility to damage between barbs and barbules. In this, he stated that barbules "suffer more than the barbs under reduced feeding, etc." due to their less favourable position in relation to capillary blood supply (Riddle, 1908). He explained that the barb-forming region is closer to the capillaries, enabling it to benefit from some of the newly formed cells of the cylinder-cell layer (Riddle, 1908). Barbules, in comparison have reduced cell-division and growth in the fault bar region (Riddle, 1908). This concept is supported by the fact that barbules and their linking mechanisms are the last structures to form and may also be more susceptible to damage than barbs due to their delicate structure (Lucas & Stettenheim, 1972; Yu *et al*., 2004). This explanation aids in understanding the impact of feather damage on the different parts of the feather structure, shedding light on the barbule malformations seen in this study.

The light fault bars assessed in this study were similar to those described in Riddle's (1908) third type of feather fault. This was described as a "very minute depression" across the feather surface, which appear in the same direction as the first type of feather defect (Riddle, 1908). Riddle's description is very similar to that of 'pallid bands', described by Ross *et al*. (2015) as weakened areas with reduced melanin (Fig.1.9). However, examples seen here do not appear to reflect the description of a pallid band due to the presence of melanin. In contrast, medium and heavy fault bars appeared similar to the description given in Riddle's (1908) fourth type of feather fault. This was described as an area where barbs are weakened or kinked, with the shaft and/or rachis constricted and weakened (Riddle, 1908). Riddle's description here is interesting and suggests that all sections of the barb are present but impacted by constriction.

In order to further explore the macroscopic and microscopic characteristics of fault bars, various fault bars widths were assessed in relation to the level of visual damage to the barbules using SEM imagery. Results found visual assessment of fault bars to be an accurate barbule damage, identifying this simplistic measure as a proxy for structural damage. A very high correlation coefficient of over 90% was seen here; therefore, the visual measurement reflects the microscopic damage very well. Visual fault bar width has been used to represent feather damage in many other studies such as Sarasola and Jovani (2006). This result also shows that barbules were more damaged, i.e., squeezed tighter together in wider fault bars. This relates to an existing hypothesis outlined by Murphy *et al*. (1989), where barbule damage is described as the result of muscle contractions around the soft feather shaft during feather growth (Murphy *et al*., 1989). If muscle constriction were to be the cause of this occurence, the differences in fault bar severity could be linked to the power of the muscle constriction at the time of formation. For example, shock events e.g. predators could result in very tight, abrupt muscle contractions around the feather follicle. In contrast, unsuccessful begging for food, for example, may link to a wider ‘V-shaped’ squeeze. If timeframe were to be a factor in fault bar occurrence, there would be four possible outcomes - Fig.3.15 shows an illustration of this concept. Fig.3.15A&B show tight barbule constriction, in contrast to Fig.3.15C&D which show loose barbule constriction. Fig.3.15A&C show stress over a long period of time (green arrows), whereas Fig.3.15B&D show stress over a short period of time. The high correlation coefficient of over 90% between fault bar width and microscopic barbule squeeze indicates that in most cases, examples A and D occurred in carrion crows. This also supports Jovani & Diaz-Real’s (2012) study which found that fault bar strength/width was not related to the duration of the stress, but to the intensity of the stress. A new explanation is possible for this correlation. In this, tighter muscular constriction results in the flattening of barbules over a larger surface area of the shaft, appearing as a wider fault bar. In contrast, a lighter muscular squeeze results in bending the barbules less, therefore occupying less space along the shaft.

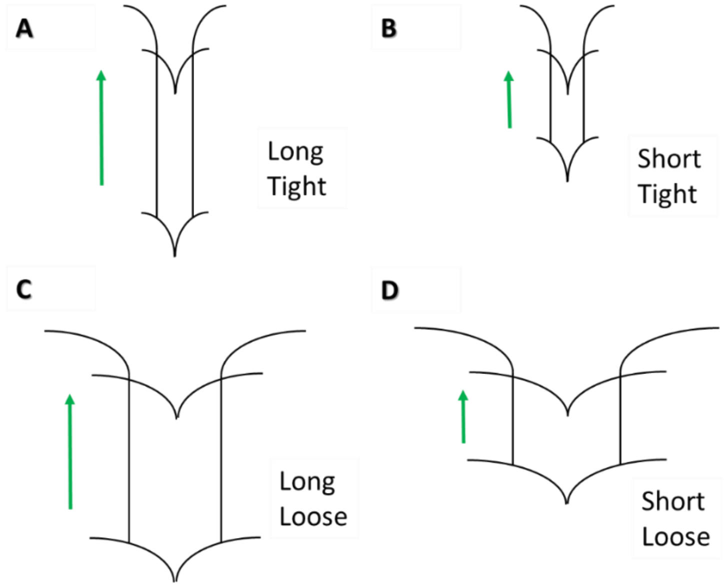


Fig.3.15 A & B show tight barbule constriction. C & D show loose barbule constriction. A & C show stress over a long period of time (green arrows). B & D show stress over a short period of time.

In order to confirm the connection between muscle constriction, stress factors and fault bar production, assessments are required at the time of feather formation. These types of experiments have been found to be challenging due to the stressful nature of handling individuals for measurement/observation. Therefore, an alternative method may need to be devised. Moreover, it would be interesting to determine at what point a fault bar is created in the feather pin development. The findings of this study support the muscle constriction hypothesis in many ways; however, the concept of blood and nutrient supply should still be acknowledged. In fact, there may be multiple factors occuring together, which result in the formation of a fault bar. In response to this notion, the chemical profile of feathers will be assesed in later chapters, focusing on the occurrence of nutritional elements across a range of feathers with and without fault bars.

Fault bars may occur differently in other species of bird, which may have a different variety of stressors. For instance, crows may have less predation than smaller bird species, which has been found to play a role in fault bar production (Møller *et al.*, 2009). Secondly, the role of food restriction events in feather growth and quality of different species is unknown, as body size and metabolic rate vary accordingly. Thirdly, crows nest high in the trees (Holyoak, 1967); therefore, they may be subject to wind exposure, requiring muscle constrictions to prevent falling from the nest. This rarely happens in small birds, as their nests are built closer to the ground (Peters, 2007). Lastly, nestling competition may play also role. For example, a study conducted on the upland buzzard (*Buteo hemilasius*), found that sibling competition was a greater source of stress than variations in relative nutritional condition (Yosef *et al.*, 2013). This may also relate to clutch size, as a larger number of siblings would result in higher levels of competition. Again, stress intensity has been found to impact fault bar width rather than the length of time spent under stress (Jovani & Diaz-Real, 2012). Therefore, this implies that sudden events such as a threat of predation or a strong gust of wind may produce wider fault bars than that of milder stress events such as unsuccessful begging for food or sibling rivalry. Therefore, the frequency and prevalence of different stressors may have a large impact on fault bar occurrence and severity in different species.

3.5.2 Aim 2: To identify further evidence of structural damage caused by fault bars

Damage to the feather shaft at the site of fault bars was assessed, showing evidence of damage in the form of indentations and narrowing of the feather shaft. When explored further, cracking and abrasion to the feather shaft surface layer was seen. This suggests that the entire feather structure can be affected at the location of a fault bar and does not merely affect the feather vane. Therefore, if muscular constriction occurs at a particular time of feather growth, the tightening around the developing pin feather may result in fault bar occurrence in addition to shaft damage at this point. The level of shaft damage would, therefore, depend on the severity of the fault bar.

In addition to these findings, lack of pigment was also seen on the feather shaft in some of these locations. This occurrence is difficult to justify, appearing as abrasions to the surface. Scanning electron microscope images of feather shaft damage were displayed to further assess this occurrence. This illustrated disruption to the surface layers, supporting the idea that the shaft surface appeared abraded at the site of fault bars. This area appears to lack pigmentation, which has also been described by Riddle (1908) and can be seen in Fig.1.6, described as ‘almost total absence of pigment in the shaft’ at the site of the fault bar. Alterations in pigmentation could be explained by reflections of light. On the other hand, Riddle (1908) suggested that a lack of nutrition results in a “suspension of pigment production during the fault-bar producing period”.

A further hypothesis was tested in this study aim, presenting evidence to support Riddle's (1908) suggestion that fault bars can be observed in developing pin feathers. This evidence can be seen in section 3.3.2.2, displaying lines of varying widths across the circumference of the pin feather structure. Fault bars were present on the emerging feather vane of Fig.3.18, indicating the occurrence of faults on this feather. Adjacent feather pins also shared these traits and was seen in a number of carrion crows during the study. A pin feather displaying the same characteristics showed a fault bar at the lines depicted on the feather pin. This occurrence has not been documented in prior research, suggesting a novel understanding of their presentation during feather growth. This visual display is potentially easier to detect in darker feathers; however, this conclusion cannot be drawn without comparison to feathers of paler pigmentation. Moreover, the visible fault bar line around the circumference of the pin feather may support the concept of constriction during feather growth. However, further research to reveal a clearer understanding of this occurrence is required.

3.6 Conclusion

The findings of this chapter revealed that fault bars seen in carrion crow feathers appear characteristically differently to the commonly used descriptions offered by Riddle (1908) and Murphy *et al*. (1989). Results found barbules to be constricted rather than displaying areas of missing sections. A significant relationship was found between visual fault bar width and average barbule width. This shows that visual fault bar width is an accurate representation of barbule damage in general. Potential fault bar production mechanisms were then discussed in relation to existing hypotheses relating to a lack of blood and nutrient supply (Riddle, 1908) and damage caused by muscular constriction (Murphy *et al*., 1989). The high correlation coefficient of over 90% between fault bar width and microscopic barbule squeeze indicated that in most cases, two scenarios occur in carrion crows: 1) tight constriction/severe stress 2) loose constriction/minor stress. However, the concept of blood and nutrient supply should still be acknowledged. In fact, there may be multiple factors occurring together, which result in the formation of a fault bar. In order for this formation theory to be proven, further research is required to identify the validity of the interpretations made.

Feather shaft damage was seen at the site of fault bars, revealing the extent of damage caused to the overall feather structure. This occurs predominantly in heavy fault bars; however, slight indentations on the feather shaft can also be seen at the location of less severe fault bars. The occurrence of shaft damage in light fault bars may be the result of minor stress events, in comparison to more severe stress events which may lead to shaft damage at the site of heavier fault bars. Fault bars were also documented in developing pin feathers for the first time, displayed as dark lines of varying thickness around the circumference of the pin.

The findings in this study also lead to questions around the causes of fault bar production in carrion crows. Moreover, fault bars may occur differently in other species of bird, which may have a different variety of stressors. For example, there may be differences in predation risk, food availability, food quantity requirements, nest location and exposure to poor weather conditions, in addition to sibling competition and clutch size. Further research into species-specific stressors is required to draw the correct conclusion in relation to the causes fault bar production. Some potential causes of fault bar production in carrion crows will be assessed in later chapters, consisting of the chemical profile of feathers, parasite burden, sex and age.

Chapter 4: An assessment of the relationship between different feather quality measures across different feather types

4.1 Introduction

Feather damage has a negative impact on bird flight and survival (Bortolotti *et al*., 2002; Corning & Biewener, 1998; Echeverry-Galvis & Hau, 2013; Jovani & Rohwer, 2016) and social signalling (Doucet, 2002; Lee *et al*., 2012; Yin *et al.*, 2006). Moreover, feather quality has a large bearing on individual success during mate choice, due to their demonstration of fitness (Erritzøe, 2006; Møller *et al.*, 2009). Feather quality can be measured in a variety of ways, including factors such as iridescence (Griffith, Parker & Olson, 2006; McGraw, 2003; Meadows, Roudybush & McGraw, 2012; Pacyna *et al*., 2018), strength (Dawson *et al.,* 2000; DesRochers *et al*., 2009; Pap *et al.,* 2013) and the occurrence of snapped (Dawson, Bortolotti & Murza, 2001; Kose *et al*., 1999) and white feathers (Bonser, 1995; Kaiser, 2008). This study aims to evaluate how poor feather quality assessed with fault bars correlates with other measures of quality. The feather quality measurements used in this study have rarely been investigated together, leading to a new approach to this subject.

4.1.1 Factors influencing flight efficiency

4.1.1.1 Fault bars

The presence of fault bars and other structural defects are often used to determine feather quality, as these structural weaknesses often lead to ragged edges and/or feather breakage due to lack of keratin (Dawson *et al*., 2001; Kose *et al*., 1999). The method in which fault bars are measured varies slightly across studies. Tab.4.1 illustrates a variety of methods used to measure fault bars, primarily based on counting the frequency of occurrences in feathers. However, some studies (Jovani & Blas, 2004; Jovani, Montavole & Sabate, 2014; Sarasola & Jovani, 2006) also acknowledge the variation of widths in fault bars, classifying them as light, medium and strong (descriptions of which can be found in section 2.2.1). This is a particularly important factor to consider, as heavier fault bars are more likely to result in feather damage than medium and light fault bars (Sarasola & Jovani, 2006). Therefore, counting the occurrence of fault bars irrespective of its width may dismiss the differences in potential feather damage. The previous chapter supports this notion, finding differences in barbule damage across a range of fault bar severities. In this, a highly significant negative relationship was found between the average visual width of fault bars and the average width of barbules.

This shows that barbules are more ‘squeezed’/damaged in wider fault bars. In light of differences seen in measuring fault bars, this study will test the relationship between the average fault bar width and number of fault bars on a feather to see whether these measures are interchangeable. Little is known about how these two measures are related and if they can be used interchangeably. For example, do heavy fault bars go hand in hand with a high number of fault bars on the feather or do they measure something different? In the first case, studies using either measure can be compared, whereas in the latter case, comparisons across studies using different methods are difficult. Consequently, this study aims to establish the relationship between these measures and identify an appropriate variable for further analysis of fault bars. It should also be noted that fault bar location is often documented in previous studies, as this ultimately impacts the likelihood of feather breakage. This particular element will be assessed in later chapters where feather strength is recorded in relation to fault bar occurrence.

Tab.4.1 Methods for assessing fault bars in a range of studies across a different topics and species.

Method	Topic	Species	Authors and date of publication
Number of fault bars	Malnutrition during postnuptial molt	White-crowned sparrows *Zonotrichia leucophrys gambelii*.	Murphy, King, Lu (1988)
SEM images of fault bar structural characteristics	Nutritional factors	White-crowned sparrows *Zonotrichia leucophrys gambelii*.	Murphy, Miller & King (1989)
Number of fault bars	Fault bar occurrence in nestlings	Ospreys *Pandion haliaetus*	Machmer *et al.* (1992)
Fault bars counted and location recorded - snapped and missing	Stress during feather development predicts fitness potential	American kestrel *Falco sparverius, Linnaeus*	Bortolotti, Dawson & Murza (2002)
Number of categorised fault bars (light, medium or strong) in addition to location.	Adaptive allocation of fault bars	White storks *Ciconia Ciconia*	Jovani & Blas (2004)
Fault bar occurrence and position	Age-related environmental sensitivity and weather mediated nestling mortality	White storks *Ciconia ciconia*	Jovani & Tella (2004)

Number of fault bars	Adaptive fault bar distribution in a long-distance migratory, aerial forager passerine?	Barn swallow *Hirundo rustica*	Serrano & Jovani (2005)
Number of categorised fault bars (light, medium or strong) in addition to evidence of barbule breakage	Risk of feather damage explains fault bar occurrence in a migrant hawk	Swainson's hawk *Buteo swainsoni*	Sarasola & Jovani (2006)
Number of fault bars	Evolution of partial moult	Great tit *Parus major*	Pap *et al. (*2007)
Number of fault bars and location	Frequency of fault bars in feathers of birds and susceptibility to predation	Goshawk *Accipiter gentilis L.*, 23 prey species	Møller, Erritzøe & Nielsen (2009)
Number of fault bars	Effects of chronic psychological and physical stress on feather replacement	European starlings *Sturnus vulgaris*	Strochlic & Romero (2008)
Number of categorised fault bars (light, medium or strong)	Fault bars and bacterial infection	Urban feral pigeons *Columba livia*	Jovani, Montavole & Sabate (2014)

4.1.1.2 Feather strength

Central factors in feather function are strength and flexibility; allowing feathers to withstand different air pressures during flight (Bachmann *et al*., 2012; DesRochers *et al.,* 2009). The structural integrity of these feathers can be compromised by growth defects, often resulting in feather breakage which negatively impacts on flight ability and survival (Erritzøe, 2006). Therefore, if poor feather quality affects the mechanical properties of feathers, it could consequently reduce individual fitness (Corning & Biewener, 1998; DesRochers *et al.,* 2009). Corning & Biewener (1998) suggested that flexural stiffness is more important than strength to feather shaft performance during flight. This may be explained by the fact that flight feathers must bend to endure aerodynamic forces within allowable flexural strains (Wang & Meyers, 2017). Different feather types are known to have different strengths, in accordance with their particular function (Ginn & Melville, 1983; Videler, 2007). Primary feathers are the strongest and largest flight feathers/remiges, providing thrust during flapping flight (Ginn & Melville, 1983; Videler, 2007). Similarly, secondary remiges are also crucial for flight and provide lift (Ginn & Melville, 1983; Videler, 2007). Tail feathers have a generally directional purpose, with a strong and robust structure to resist lift forces and avoid damage from contact with the ground (Balmford *et al*., 1994; Corning & Biewener, 1998; Fitzpatrick & Price, 1997; Norberg, 1994; Thomas, 1997; Tubaro, 2003). Therefore, feather type is an important factor to consider when considering the role of strength in feather quality. It must also be noted that feather strength varies across the feather length (Butler & Johnson, 2004; Sullivan *et al*., 2017). Feathers are stiffer and less tolerant of stress at the base and become gradually more flexible and resistant to stress towards the distal end of the feather (Butler & Johnson, 2004; Sullivan *et al*., 2017). Therefore, this must also be taken into account when considering feather strength as a measure of general quality. Little is known about the relationship between feather strength and other measures of quality currently; therefore, this study aims to investigate this to provide further insight into this topic.

4.1.1.3 Feather breakage

As mentioned previously, poor feather quality often leads to breakage (Dawson *et al*., 2001; Kose *et al*., 1999; RSPCA, 2013). When a single feather is broken, the cost of failure, in terms of survival, should be much less than that of a bone or limb (Corning & Biewener, 1998). However, this cost is increased by the fact that broken feathers cannot be repaired and are generally replaced only annually in the wing moult (Corning & Biewener, 1998). Feathers can break for a number of reasons, for instance, predator threats, impact damage and degradation from poor structural quality (Dawson *et al*., 2001; Kose *et al*., 1999). Moreover, particular feather types may be more susceptible to damage; for example, tail feathers may be more susceptible to breakage due to contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Moreover, Jovani & Rohwer (2016) discussed feather breakage in relation to the 'fault bar allocation' hypothesis. In both raptors (swainson's hawk *Buteo swainsoni*) and cranes (sandhill - *Grus canadensis*), fault bars were less common in primaries than in rectrices or secondaries, but they were more likely to result in feather breaks in the primaries when they occurred there (Sarasola & Jovani, 2006; Jovani *et al*., 2010). This pattern suggests that natural selection has suppressed the production of fault bars in feathers where damage would seriously lower survival. Current studies on feather breakage in relation to fault bar occurrence generally tend to focus on feather vane damage (Sarasola & Jovani, 2006; Jovani *et al.*, 2010); therefore, further information is required to truly understand this connection.

4.1.2 Factors influencing social signalling

4.1.2.1 Feather colouration: Melanin

Feather colouration is costly in nature, serving as an honest display of fitness to conspecifics for mate choice (Griffith, Parker & Olson, 2006; McGraw, 2003; Meadows *et al.*, 2012; Pacyna *et al.*, 2018). Melanin pigments have been found to be used in thermoregulation and abrasion resistance (Bonser, 1995), whereas structural colours serve an ornamental function, playing a role in camouflage, courtship, individuality, colour signalling and communication (Doucet; 2002; Lee *et al.*, 2012; Yin *et al.*, 2006). Studies such as Harper (1999) have also confirmed that dull feathers are a good indicator of individuals in poor physiological condition. Melanin is also regarded as an important factor in providing increased resistance to abrasion, lowered barb breakage in feathers and decreased wear (Burtt, 1986; Bonser, 1995; Kose *et al.*, 1999). However, recent studies have since questioned this hypothesis, suggesting that further evidence is required to support this (Butler & Johnson, 2004). This is due to the lack of consideration of barb position along the rachis, a potentially confounding variable (Butler & Johnson, 2004).

4.1.2.2 Feather colouration: Iridescence

The presence of iridescence is dependent on the layering and angle of keratin amongst the melanin pigments (Brink & Van Der Berg, 2004; Doucet *et al.*, 2006; Maia *et al.*, 2009). Iridescent feathers are thought to be an honest signal of individual quality due to their costly production, resulting in playing a prominent role in sexual displays (Griffith, Parker & Olson, 2006; Pacyna *et al.*, 2018). Ornamental feathers impose a handicap for the bearer, as these feathers are more susceptible to bacterial degradation (Javůrková *et al.*, 2019; Ruiz-Rodriguez, 2015). Fitter male individuals are able to maintain good feather quality as they have a larger uropygial gland which prevents bacterial growth (Leclaire *et al.*, 2014; Møller *et al.*, 2009). Therefore, good feather quality is an honest signal of immunocompetence against bacteria (Leclaire *et al.*, 2014; Møller *et al.*, 2009). Iridescent feathers have also been found to have decreased hydrophobicity, again, emphasising the fitness costs associated with this variety of feather colouration (Eliason & Shawkey, 2011). When evaluating colouration in the form of iridescence as a feather quality factor, it is important to consider the colour of the feather in question. For example, black feathers generally show weaker iridescence than feathers of lighter colour (Lee *et al.*, 2012; Doucet *et al*., 2006). Nonetheless, within a species, poor quality feathers often lack lustre, appearing dull with an absence of iridescence (Harper, 1999).

4.1.2.3 Aberrant white feathers

Poor feather quality can also be identified through the presence of feathers with an abnormal lack of melanin, which is typically referred to as 'leucism' (van Grouw, 2013). Melanin is absent from areas in which it is normally deposited. Feathers with white colouration are more susceptible to damage than those of normal melanin deposition (Bonser, 1995). Due to a lack of melanin, white feathers in general become more susceptible to UV damage and, therefore, deteriorate at a faster rate than dark feathers (Kaiser, 2008). Consequently, the aberrant white feathers may compromise the wing, as they are suggested to be a weak area in the overall wing structure. The cause of aberrant white feathers is generally thought to be associated with genetic factors (Harrison, 1957a,b; Sage, 1954; Sage, 1962; van Grouw & Hume, 2016); however, it has now been found to link more commonly with the physical condition and age of individuals (Slagsvold, Rofstad & Sandvik*,* 1988; van Grouw, 2018) and can also include environmental conditions e.g. food availability (Rollin, 1964; van Grouw, 2018). The occurrence of leucism as an indicator of feather quality will be assessed further in this study, to determine it's relationship to other factors.

4.1.3 Study aims and hypotheses

This chapter intended to investigate two hypothetical scenarios: a) Poor condition during feather growth has multiple effects on feather structure, leading to correlation of quality measures b) Alternatively, fault bars could be seen as brief periods of poor environmental conditions, which do not affect the rest of the feather structure as a whole. In order to reach this conclusion, the following study aims were outlined:

1. To determine the relationship between the number of fault bars and the severity (width) of fault bars on a feather to evaluate different methods used in current literature.

2. To test the ‘fault bar allocation hypothesis’ outlined by Jovani and Blas (2004) in relation to the number/width of fault bars in different feather types.

   Hypothesis: The number/width of fault bars was expected to be greater in the tail feathers, with the lowest number/width found in the primary feathers.

3. Expanding on the second aim, predictions of occurrence of other feather quality measures were formulated in response to the limited information known about the distribution of other quality measures across different feather types. **Hypotheses** for each feather quality variable are as follows:

- Feather strength was expected to reflect the differences in function between feather types (Ginn & Melville, 1983; Videler, 2007). Tail feathers were expected to be of comparable strength to primary feathers due to their need to resist lift forces and avoid damage from contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Secondary feathers were expected to be the weakest feather type due to their generally smaller size (Ginn & Melville, 1983).

- The number of snapped feathers was expected to be highest in the tail feathers, due to being in regular contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Moreover, in line with the ‘fault bar allocation hypothesis’ (Jovani & Blas, 2004), tail feathers have a high number of fault bars compared to the flight feathers, which may consequently result in a higher occurrence of breakage.

- Carrion crows are a monomorphic species (Dawson *et al.*, 2001) and appear to have a uniform appearance in respect to the colouration of different feather types; therefore, average feather iridescence was expected to be consistent across feather types. This is particularly important in conveying honesty signals to conspecifics (Meadows *et al.*, 2012).

- The occurrence of aberrant white feathers was expected to differ between feather types in line with the ‘fault bar allocation hypothesis’ (Jovani & Blas, 2004), finding fewer white feathers in the wing feathers than the tail. Where this results is not found, the notion that aberrant white feathers have a hereditary cause would be supported (Harrison, 1957a,b; Sage, 1954; Sage, 1962; van Grouw & Hume, 2016).

4. To determine the relationship between different feather quality indicators. Previous studies rarely considered several measures for feather quality, typically focusing on one method.

5. To select feather quality variables for use in further analysis of the causes and consequences of poor feather quality (to be conducted chapters 5 and 6).

4.2 Methodology

4.2.1 Study variables

The relationship between average fault bar width and the average number of fault bars was first assessed (aim 1). This was conducted in order to select the best variable for the depiction of fault bars. Please refer to section 2.2.2.2.1 for methodology details on fault bar measurements. To test the ‘fault bar allocation hypothesis’ (Jovani & Blas, 2004), differences in the number/width of fault bars were assessed within separate feather categories: primary, secondary and tail (aim 2). This hypothesis was then extended to further measures of feather quality, testing each of the variables across the different feather types: average feather strength, the number of snapped feathers, average feather iridescence and the number of white feathers (aim 3). To determine the relationship between different feather quality indicators, relationships between the feather variables mentioned above were determined (aim 4). Feather quality variables for use in further analysis were then selected to determine the causes and consequences of poor feather quality in later chapters (aim 5).

The average feather strength (N/mm^2) variable was used to gain information on how tolerance to stress varies in different feathers and how this variable relates to other feather quality measures (see section 2.2.2.5 for further methodology details). Three feathers were selected for measurement within each feather type (see Fig.2.4), calculating an average (mean) measurement for each feather type. This measurement was taken at the base/superior umbilicus (see Fig.2.8) of each feather in order to account for the varying strength of feathers at different points along the shaft (Butler & Johnson, 2004; Sullivan *et al.*, 2017). This measurement position also allowed the use of broken feathers to be used, increasing the sample size (see section 2.2.2.2.2 for details). For the number of snapped feathers variable, three feathers were used within each feather type, counting the number of snapped feathers for each feather type. Please see section 2.2.2.2.2 for further information on this methodology. Average feather iridescence (T/%) was used: For each feather type, the three selected feathers were taped in an overlaid manner to a white sheet of paper to simulate feather arrangement on the body (Maia *et al.*, 2009). This particular measurement was chosen, as other studies have found this to provide a reliable interpretation (Maia *et al.*, 2009). Three measurements were taken at 3 specified measurement points across the overlaid feathers to calculate the mean iridescence (see Fig.2.8). Please refer to section 2.2.2.3 for further methodology information. For the number of white feathers variable, three feathers (Fig.2.4) were used within each feather type, counting the number of white feathers for each feather type. See section 2.2.2.2.2 for further details.

4.2.2 Statistical methodology for selection of dependent variables

The following variables were tested for normality using IBM SPSS Statistics 24, representing each feather type: average number of fault bars, average width of fault bars (mm), average feather iridescence (Transmission %), average feather strength (N/mm^2), number of snapped feathers and number of white feathers (N=47). Kolmogorov-smirnov normality testing found a mix of parametric and non-parametric data; therefore, non-parametric tests were selected for analysis. The first step in this analysis was to identify the most accurate measure of fault bars (aim 1). A spearman's rank correlation was used here to assess the relationship between average fault bar width (mm) and the average number of fault bars. The second step was to test the 'fault bar allocation hypothesis' (Jovani & Blas, 2004) in relation to the number/width of fault bars in different feather types (aim 2). In this, Friedman testing was conducted (Field,

2013) using the three feather types as independent variable (primary, secondary and tail). When a significant result was found, Wilcoxon signed rank testing was conducted to determine specific differences (Field, 2013). The ‘fault bar allocation hypothesis’ was then extended to other feather quality variables, testing each measure between the different feather types (study aim 3). This was conducted in order to account for structural differences between feather types. The last step then compared the selected fault bar variable against all other feather quality variables (study aim 4), using Spearman’s rank correlation coefficient (Field, 2013). This was conducted in order to select dependant variables for the proceeding data chapters (study aim 5).

4.3 Results

4.3.1 Selection of an appropriate variable to represent the occurrence of fault bars (aim 1)

Spearman’s rank correlation found a significant positive relationship between the average width of fault bars (mm) and the average number of fault bars in primary ($r_s(47)=0.828$, $p<0.001$), secondary ($r_s(47)=0.739$, $p<0.001$) and tail feathers ($r_s(47)=0.674$, $p<0.001$). When all feather types were combined for re-analysis, the same relationship occurred ($r_s(141)=0.735$, $p<0.001$). This shows that feathers with higher numbers of fault bars had wider fault bars on average. This also shows that both variables were equal in their representation of fault bars. Therefore, average fault bar width was selected to represent the fault bar variable, as this variable was found to correlate with barbule damage in the previous chapter.

4.3.2 Testing the fault bar allocation hypothesis (aim 2)

The mean width of fault bars was significantly different across the three feather types – primary, secondary and tail ($\chi^2(2) = 24.780$, $p<0.001$; Friedman, N=47). More specifically, the mean width of fault bars in tail feathers (0.23 +/- 0.021 mm) was found to be significantly wider than those in primary feathers (0.15 +/- 0.020 mm; Wilcoxon; Z=-4.341, $p<0.001$) and secondary flight feathers (0.14 +/- 0.020 mm; Z=-3.525, $p<0.001$) (Fig.4.1). Primary and secondary flight feathers did not differ significantly in the mean width of fault bars (Z=-0.720, p=0.471; Fig.4.1).

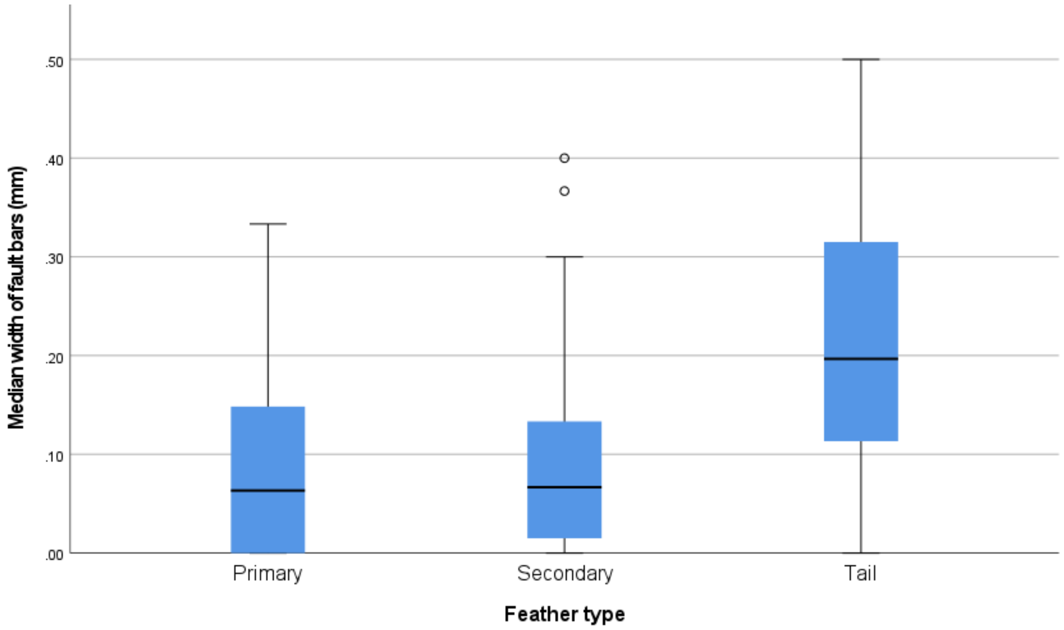


Fig.4.1 Median width of fault bars in primary, secondary and tail feathers (N=47 per feather type). Boxes represent median values with upper and lower quartiles. Error bars represent standard error, with outliers marked as circles.

4.3.3 Expanding the 'fault bar allocation hypothesis' to other feather quality measures (aim 3)

4.3.3.1 Average feather strength (N/mm^2)

Mean feather base strength was significantly different across the three feather types – primary, secondary and tail ($\chi^2(2) = 12.359$, p=0.002; Friedman, N=47). More specifically, feather strength was significantly higher in tails than primary feathers (Z=-2.386, p=0.016) and secondary feathers (Z=-3.923, p<0.001) (Fig.4.2). Primary feather strength was found to be significantly higher than secondary feathers (z=-2.125, p=0.034; Fig.4.2).

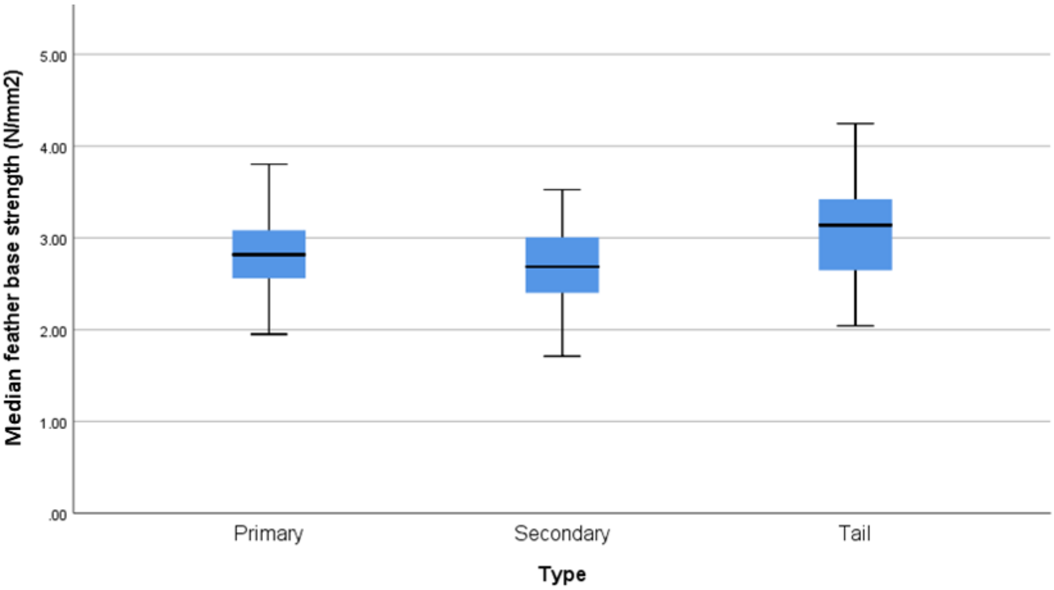


Fig.4.2 Median feather strength (N/mm^2) of primary, secondary and tail feathers (N=47 per feather type). Boxes represent median values with upper and lower quartiles. Error bars represent standard error.

4.3.3.1 Number of snapped feathers

The number of snapped feathers was significantly different across the three feather types – primary, secondary and tail ($\chi^2(2) = 6.269$, p=0.043; Friedman, N=47). More specifically, across all feathers used in this study (n=423), significantly higher numbers of snapped feathers were found in primary feathers than secondary feathers (Wilcoxon, Z=-2.714, p=0.012). The number of snapped feathers in tail feathers did not differ significantly to the number of snapped feathers in primary (Z=-0.713, p=0.501) and secondary feathers (Z=-0.846, p=0.473) (Fig.4.3). The large number of outliers may be explained by the fact that a large number of snapped feathers were found in relatively few individuals: primary (24 snapped feathers in 13 individuals), secondary (15 snapped feathers in 9 individuals), tail (21 snapped feathers in 10 individuals). Generally, the same individuals had snapped feathers across more than one feather type.

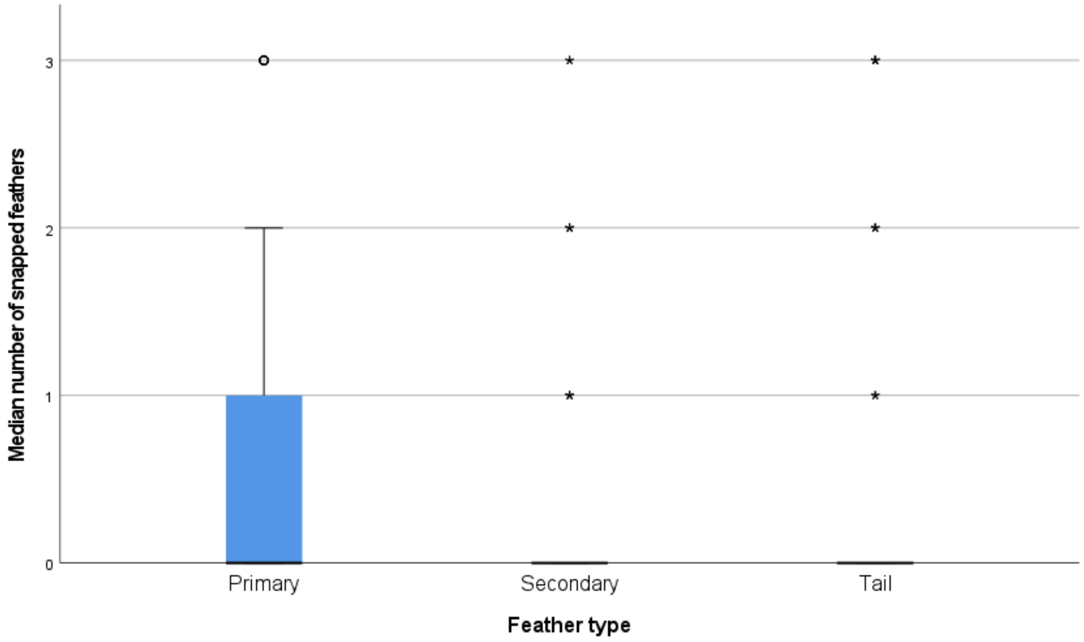


Fig.4.3 Median number of snapped feathers in primary, secondary and tail feathers (N=47 per feather type). Boxes represent median values with upper and lower quartiles. Error bars represent standard error, with outliers marked as circles.

4.3.3.3 Average feather iridescence (Transmission/%)

No significant difference was found in average feather iridescence between feather types – primary, secondary and tail (χ2(2) = 2.851, p=0.244; N=47; Fig.4.4).

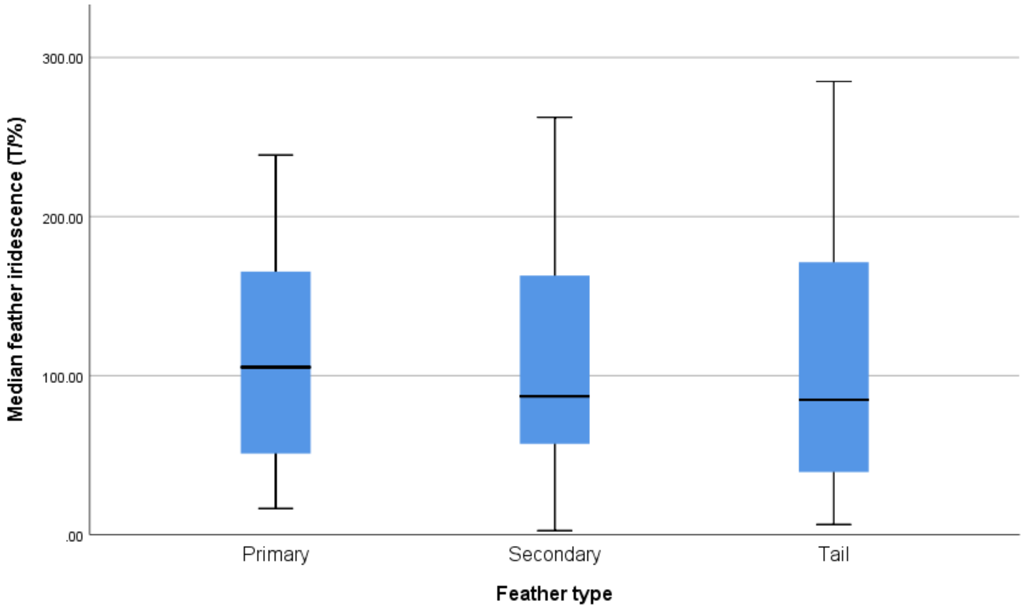


Fig.4.4 Median feather iridescence (T/%) in primary, secondary and tail feathers (N=47 per feather type). Boxes represent median values with upper and lower quartiles. Error bars represent standard error.

4.3.3.4 Number of white feathers

The number of white feathers was significantly different across the three feather types – primary (mean of 1.13 ± 0.201 standard error), secondary (1.11 ± 0.205) and tail (0.23 ± 0.115) ($\chi^2(2) = 26.169$, $p<0.001$; Friedman, N=47). More specifically, across all feathers in this study (n=423), significantly lower numbers of white feathers were found in tail feathers than primary (Z=-3.236, p<0.001) and secondary feathers (Z=-3.126, p=0.001) (Wilcoxon, N=47). The number of white feathers did not differ significantly in primary and secondary feathers (Z=-0.264, p=0.984). As explained in the snapped feather results, a large number of white feathers were also found in relatively few individuals: primary (53 white feathers in 20 individuals), secondary (52 white feathers in 19 individuals), tail (11 white feathers in 4 individuals). Generally, the same individuals had white feathers across more than one feather type.

4.3.4 Relationships between feather quality measures (aim 4)

Spearman's rank correlation tested the relationship between the following variables, within each feather type: average width of fault bars, average feather iridescence (Transmission/%), average feather strength (N/mm^2), number of snapped feathers and number of white feathers (Tab.4.2).

Tab.4.2 Spearman's rank correlation outputs for feather quality measurements across different feather types (n=47). Emboldened values indicate p value. Non-emboldened values indicate r value. Yellow highlighter represents significant values.

Feather Type		FB	Strength	Snapped	Iridescence	White
Primary	**FB**	1.000	-0.156	0.200	-0.278	0.077
		.	**0.350**	**0.222**	**0.087**	**0.641**
	Strength	-0.156	1.000	-0.265	0.246	-0.055
		0.350	.	**0.108**	**0.136**	**0.743**
	Snapped	0.200	-0.265	1.000	-0.679	0.489
		0.222	**0.108**	.	**<0.001**	**0.002**
	Iridescence	-0.278	0.246	-0.679	1.000	-0.432
		0.087	**0.136**	**<0.001**	.	**0.006**
	White	0.077	-0.055	0.489	-0.432	1.000
		0.641	**0.743**	**0.002**	**0.006**	.
Secondary	**FB**	1.000	0.043	-0.129	-0.213	0.096
		.	**0.798**	**0.435**	**0.193**	**0.563**
	Strength	0.043	1.000	-0.26	0.331	-0.214
		0.798	.	**0.114**	**0.042**	**0.196**
	Snapped	-0.129	-0.260	1.000	-0.462	0.247
		0.435	**0.114**	.	**0.003**	**0.129**
	Iridescence	-0.213	0.331	-0.462	1.000	-0.392
		0.193	**0.042**	**0.003**	.	**0.013**
	White	0.096	-0.214	0.247	-0.392	1.000
		0.563	**0.196**	**0.129**	**0.013**	.
Tail	**FB**	1.000	0.124	0.423	-0.224	0.263
		.	**0.457**	**0.007**	**0.17**	**0.106**
	Strength	0.124	1.000	0.187	0.068	0.079
		0.457	.	**0.261**	**0.687**	**0.637**
	Snapped	0.423	0.187	1.000	-0.442	0.239
		0.007	**0.261**	.	**0.005**	**0.142**
	Iridescence	-0.224	0.068	-0.442	1.000	-0.075
		0.17	**0.687**	**0.005**	.	**0.648**
	White	0.263	0.079	0.239	-0.075	1.000
		0.106	**0.637**	**0.142**	**0.648**	.
All combined	**FB**	1.000	0.150	0.134	-0.243	-0.027
		.	**0.112**	**0.149**	**0.008**	**0.772**
	Strength	0.150	1.000	-0.091	0.161	-0.168
		0.112	.	**0.335**	**0.087**	**0.074**
	Snapped	0.134	-0.091	1.000	-0.528	0.341
		0.149	**0.335**	.	**<0.001**	**<0.001**
	Iridescence	-0.243	0.161	-0.528	1.000	-0.275
		0.008	**0.087**	**<0.001**	.	**0.003**
	White	-0.027	-0.168	0.341	-0.275	1.000
		0.772	**0.074**	**<0.001**	**0.003**	.

4.3.4.1 Relationship between average fault bar width and average feather strength

The average width of fault bars (mm) did not correlate with average feather strength (N/mm^2) in any of the feather types. Therefore, due to repetition of results, measures for individual feather types were combined to re-assess this relationship across all feather types. Results of this combined test found no significant correlation between average fault bar width and average feather strength across all feather types combined (rs(141)=0.150, p=0.112).

4.3.4.2 Relationship between average fault bar width and the number of snapped feathers

Tab.4.2 shows that average fault bar width was not correlated with the number of snapped primary or secondary feathers. However, a significant positive correlation was found between the average width of fault bars and the number of snapped feathers in the tail ($r_s(141)=0.423$, p=0.007; Fig.4.5). Fig.4.5 shows large variation in the category of no snapped feathers.

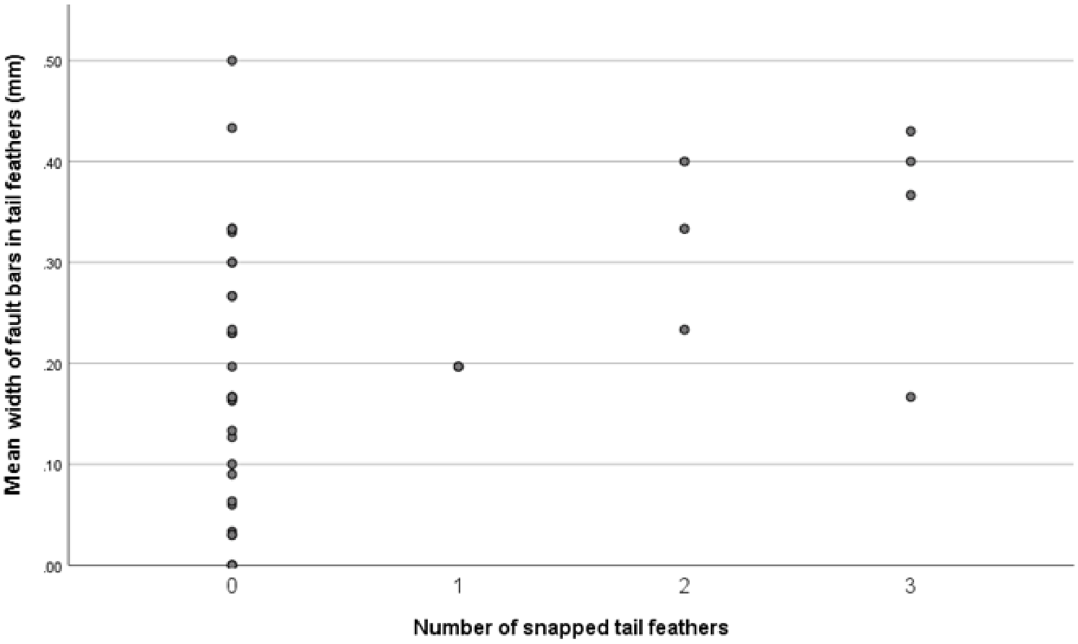


Fig.4.5 Mean fault bar width (mm) in relation to number of snapped tail feathers (N=47). Each data point represents an average of 3 feather measurements. Line of best fit unavailable.

4.3.4.3 Relationship between average fault bar width and average feather iridescence

Tab.4.2 shows a non-significant relationship between average fault bar width and average feather iridescence (T/%) across all feather types. Therefore, due to the repetition of results, all feather types were combined to re-assess this relationship. Results of this combined test found a significant negative correlation between average fault bar width and average feather iridescence across all feather types combined ($r_s(141)=-0.243$, $p=0.008$) (Fig.4.6). This indicated that dull feathers were associated with wider fault bars across all feather types combined.

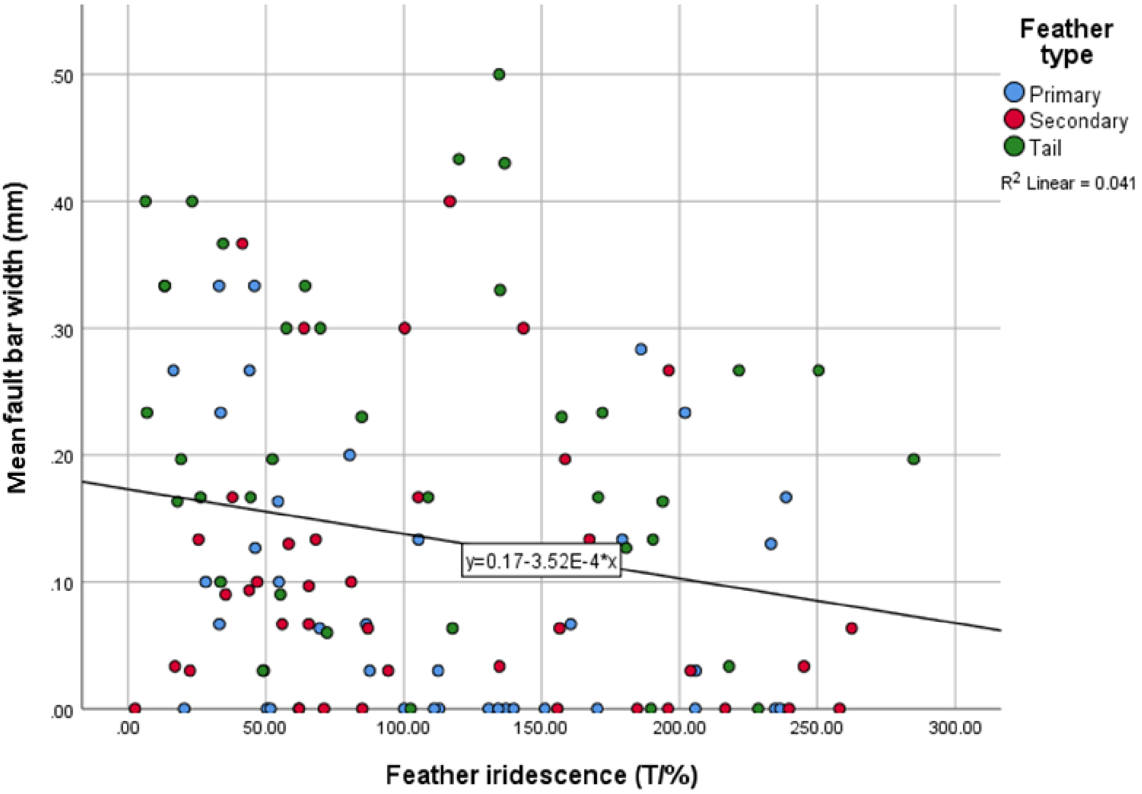


Fig.4.6 Mean fault bar width (mm) in primary, secondary and tail feathers in relation to average feather iridescence (T/%) (N=47 per feather type). Each data point represents an average of 3 feather measurements. Line of best fit represents all feather types collectively.

4.3.4.4 Relationship between average width of fault bars and the number of white feathers

The average width of fault bars did not correlate with the number of white feathers in any of the feather types. Therefore, to increase sample sizes, all feather types were combined to re-assess this relationship. Results of this combined test found no significant correlation between average fault bar width and number of white feathers across all feather types combined ($r_s(141)=-0.027$, $p=0.772$).

4.3.3.5 Relationship between average feather strength and the number of snapped feathers

Average feather strength did not correlate with the number of snapped feathers in any of the feather types. Therefore, to increase sample sizes, all feather types were combined to re-assess this relationship. Results of this combined test found no significant correlation between average feather strength and number of snapped feathers across all feather types combined ($r_S(141)=-0.091$, $p=0.335$).

4.3.4.6 Relationship between average feather strength and iridescence

A significant positive relationship was found between average feather strength and average feather iridescence in secondary feathers ($r_S(39)=0.331$, $p=0.042$; Tab.4.1; Fig.4.7). However, this relationship was not found in primary or tail feathers.

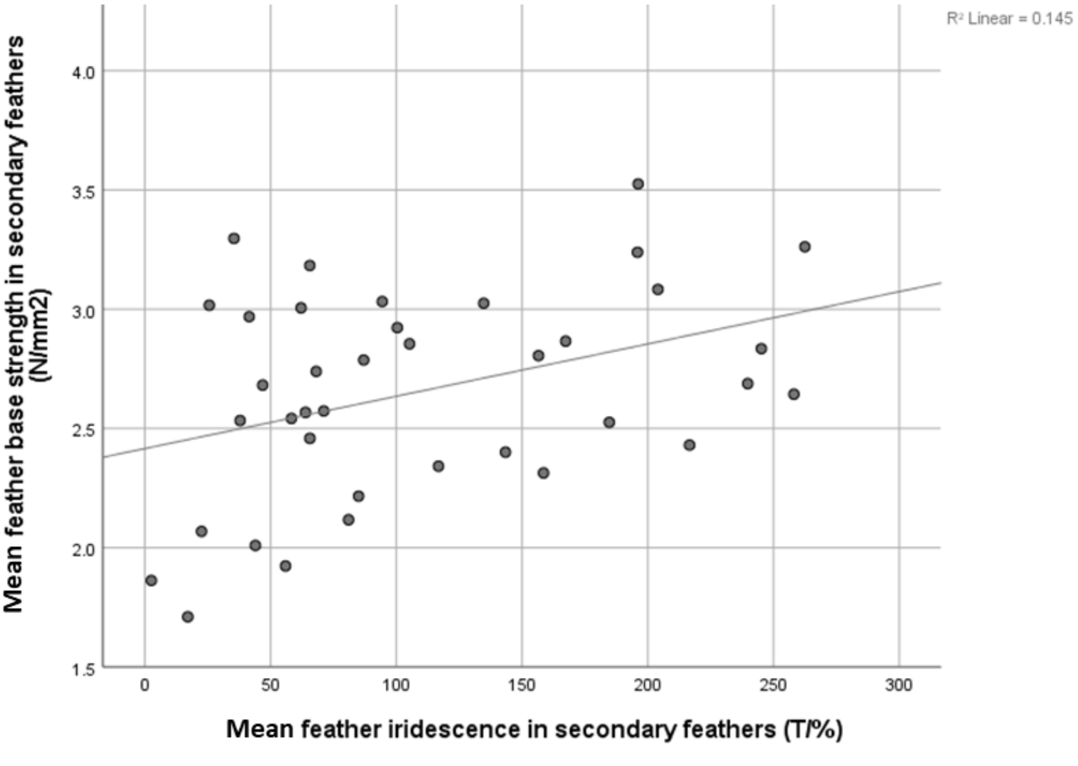


Fig.4.7 Mean feather strength (N/mm^2) in relation to mean feather iridescence (T/%) of secondary feathers (N=47) with line of best fit. Each data point represents an average of 3 feather measurements.

4.3.4.7 Relationship between average feather strength and the number of white feathers

Average feather strength was not found to correlate with the number of white feathers in any of the feather types. Therefore, due to repetition of results, all feather types were combined to re-assess this relationship. Results of this combined test found no significant correlation between the number of white feathers and average feather strength across all feather types combined ($r_s(141)=-0.168$, $p=0.074$). However, this result did approach significance with a positive trend, suggesting that on average, leucism may occur in stronger feathers.

4.3.4.8 Relationship between number of snapped feathers and average feather iridescence

A significant negative relationship was found between the number of snapped feathers and average feather iridescence in all feather types. Due to repetition of results, all feather types were combined to re-assess this relationship. Results of this combined test found a significant negative correlation between the number of snapped feathers and average feather iridescence across all feather types combined ($r_s(141)=-0.528$, $p<0.001$; Fig.4.8). This suggests that dull feathers have a higher chance of snapping. However, large variation was seen in individuals with no snapped feathers, accounted for by a larger sample size in this category (25/39).

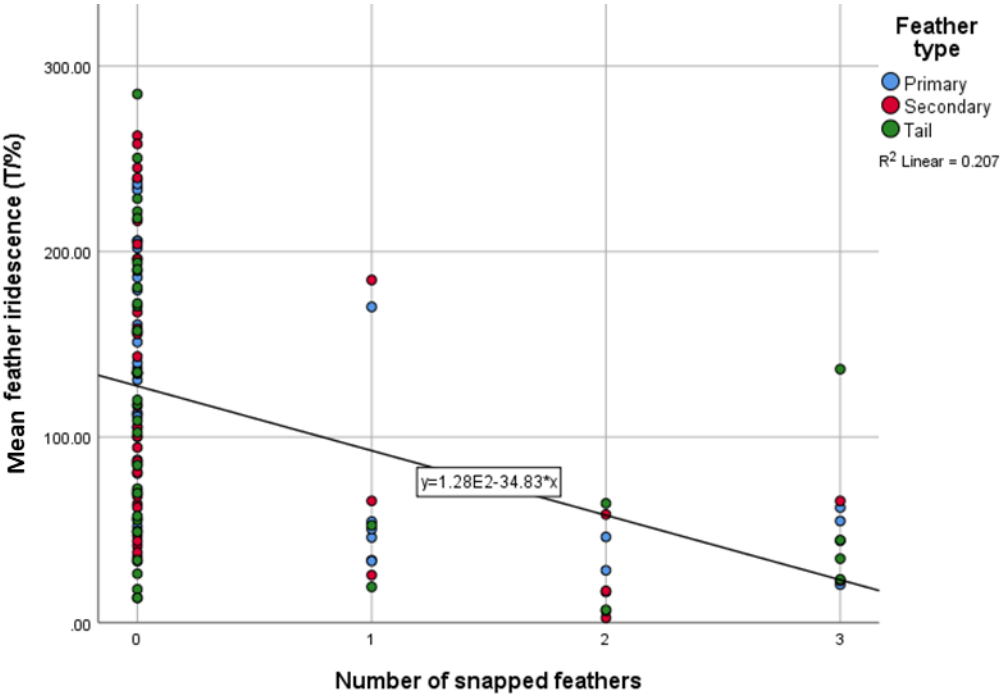


Fig.4.8 Number of snapped feathers in primary, secondary and tail feathers in relation to mean feather iridescence (T/%) (N=47 per feather type). Each data point represents an average of 3 feather measurements. Line of best fit represents all feather types collectively.

4.3.4.9 Relationship between the number of snapped and white feathers

A significant positive correlation was found between the number of snapped and white feathers in primary feathers ($r_s(47)=0.489$, $p=0.002$; Fig.4.9). No significant relationship was found between the number of snapped and white feathers in secondary or tail feathers (Tab.4.2).

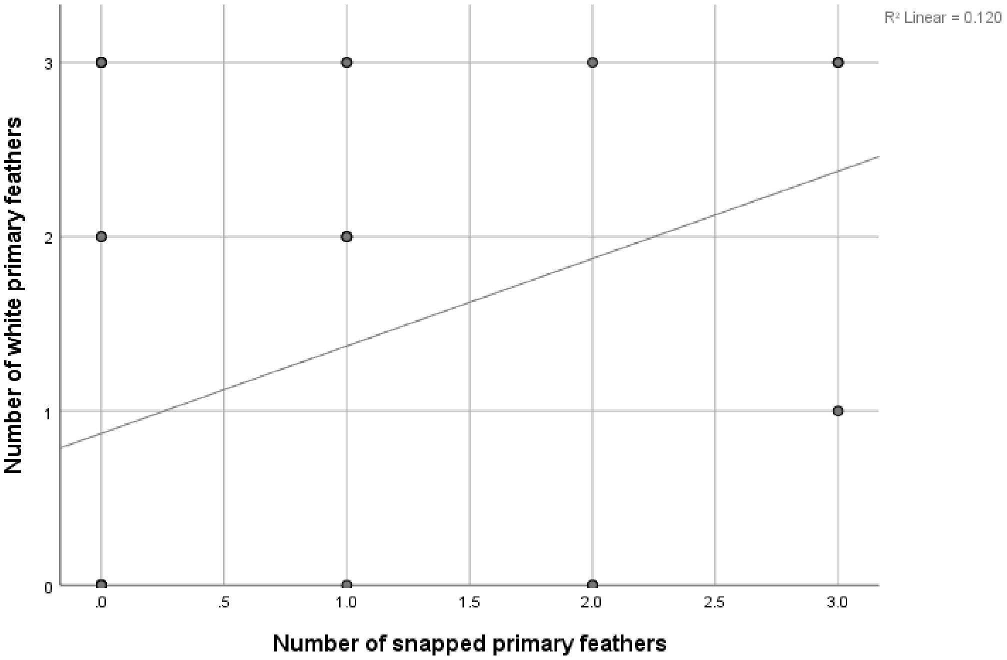


Fig.4.9 Number of snapped feathers in relation to the number of white feathers found in primary feathers (N=47).

4.3.4.10 Relationship between average iridescence and the number of white feathers

A significant negative correlation was found between average feather iridescence and the number of white feathers in primary ($r_s(141)=-0.432$, $p=0.006$) and secondary feathers ($r_s(141)=-0.392$, $p=0.003$). In contrast, no significant relationship was found between these variables in the tail feathers. This suggests that dull wing feathers are more likely to have occurrences of leucism.

4.4 Discussion

4.4.1 Selection of appropriate fault bar measure variable (aim 1)

The first aim of this study was to determine the most accurate measure of fault bars, accounting for different methods used in current literature. Results found that feathers with higher numbers of fault bars had wider fault bars on average. This also shows that both variables were equal in their representation of fault bars. Moreover, this result also allows comparison of findings across previous methods and studies. In previous studies, fault bar width has been categorised into 'light, medium and heavy' in accordance with a popular method outlined by Sarasola and Jovani (2006). Therefore, this is the first time that the raw width measurement of fault bars has been used. It is interesting that a higher number of fault bars also means on average heavier fault bars. This raises questions as to what this could mean from a causal point of view, i.e. in relation to the different formation hypotheses put forward in the previous chapter. This suggests that carrion crows experience either infrequent, minor stress events or a high occurrence of severe stress events. This is an interesting concept and may offer up new information about individual fitness and natural selection in carrion crows. Previous studies have found that a high number of fault bars occurred in individuals of lower fitness (Bortolotti *et al*., 2002; Machmer *et al*., 1992; Blanco & de la Puente, 2002; Jovani & Rohwer, 2016; Møller, 1989). It has been argued that in some circumstances, fault bars may merely be an identifier of individuals in poor condition with low survival chances due to factors unrelated to feather malformation (Jovani & Rohwer, 2016). This result points towards the idea that individuals that suffer from a large amount of stress may be individuals of low fitness. Nevertheless, it should also be noted that the individuals used in this study were admitted to a rehabilitation centre and may not be representative of the overall population. In light of the findings in this aim, average fault bar width was selected to represent the fault bar variable, as this variable was found to correlate with barbule damage in the previous chapter.

4.4.2 Variation in feather quality measures across different feather types (aims 2 and 3)

The second aim of this study was to determine whether fault bars differ between feather types according to the 'fault bar allocation hypothesis' (Jovani & Blas, 2004). This hypothesis states that fault bars occur on feathers that are least important for flight, resulting in the majority of fault bars being located on the tail feathers, with the lowest numbers in the primary feathers (Jovani & Blas, 2004). Therefore, the widest fault bars were expected to be found on the tail feathers, with narrower fault bars in primary feathers. Results supported this hypothesis, finding significantly wider fault bars in tail feathers than wing feathers. Moreover, primary and secondary feathers were found to have comparable fault bar widths. This shows that carrion crows have evolved to allocate nutrients to the most important feathers in order to minimise the direct fitness costs of fault bars (Jovani *et al*., 2010; Jovani

& Blas, 2004). This hypothesis has been supported by many other studies, being confirmed in a range of different species (Bortolotti *et al*., 2002; Jovani *et al*., 2010; Jovani & Blas, 2004; Murphy *et al*., 1989; Sarasola & Jovani, 2006; Slagsvold, 1982b). For example, Jovani *et al* (2010) found significantly lower numbers of fault bars in feathers with high damage risk in sandhill crane (*Grus canadensis*). This finding also adds another aspect to this field of knowledge, in that birds may divert the heaviest damage to less important feather types.

Feather strength was expected to reflect the differences in function between feather types (Ginn & Melville, 1983; Videler, 2007). Primary feathers are the strongest and largest flight feather, providing thrust during flapping flight (Ginn & Melville, 1983; Videler, 2007). In comparison, secondary flight feathers are more important for providing lift (Ginn & Melville, 1983; Videler, 2007). Results found secondary feathers to have significantly lower stress tolerance than primary feathers. This may be explained by the fact that secondary feathers are closer to the body, resulting in lower exposure to wing forces and damage (Ginn & Melville, 1983). Tail feathers were expected to be of comparable strength to primary feathers due to their need to resist lift forces and avoid damage from contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Results found tail feathers have significantly higher stress tolerance than primary and secondary wing feathers. In line with the 'fault bar allocation hypothesis' (Jovani & Blas, 2004), the highest number of fault bars were found in the tail due to its lower importance. This suggests that in addition to its minor role in flight, the majority of fault bars may also be allocated to the tail due to its high stress tolerance.

In regard to assessing the number of snapped feathers, tail feathers were expected to have the highest number of breakages due to having higher numbers of fault bars (Jovani & Blas, 2004), in addition to being in contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Results did not support this hypothesis, finding the number of snapped tail feathers found to be comparable to primary and secondary feathers. Moreover, significantly higher numbers of snapped feathers were found in primary feathers than secondary feathers. It is important to acknowledge that a large number of outliers occurred in the data due to a large number snapped feathers being found in relatively few individuals: primary (24 snapped feathers in 13 individuals), secondary (15 snapped feathers in 9 individuals), tail (21 snapped feathers in 10 individuals). Generally, the same individuals tended to have snapped feathers across more than one feather type. However, some caution was taken with the findings of this variable. This is due to the nature of the subjects used in this study, which were all admitted to a rehabilitation centre for a range of reasons. For example, admissions may have been involved in a collision or entanglement, leading to feather breakage. Consequently, feather breakage in the samples used may have occurred in circumstances that are unrelated to feather quality. Moreover, primary feathers are more likely to break during handling/entanglement as they are located at the outmost areas of the wing, whereas secondaries are closer to the body and may be more protected by the other feathers.

Carrion crows are a monomorphic species (Dawson *et al*., 2001) and appear to have a uniform appearance in respect to the colouration of different feather types; therefore, average feather iridescence was expected to be consistent across feather types. This is particularly important in conveying honesty signals to conspecifics (Meadows *et al*., 2012). Results supported this hypothesis, confirming that average feather iridescence (T/%) was consistent across feather types, suggesting that communication of fitness to conspecifics is uniform across the body in carrion crows. This is important to acknowledge due to the limited information on the impact of iridescence in sexual selection.

The occurrence of aberrant white feathers was expected to differ between feather types in line with the 'fault bar allocation hypothesis' (Jovani & Blas, 2004), finding fewer white feathers in the wing feathers than the tail. Results did not support this hypothesis, finding significantly lower numbers of white feathers in the tail in comparison to primary and secondary feathers. In this, primary and secondary feathers had comparable occurrences of white feathers. This shows that wing feathers were more susceptible to lack of pigmentation. This, again, may be linked to the importance of having a strong tail, due to being in contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003). Moreover, this result also suggests that white feathers compromise the wing structure less than fault bars, raising questions on whether or not white feathers really compromise the wing. This may, therefore, indicate a hereditary cause of aberrant white feathers in carrion crows (Harrison, 1957a,b; Sage, 1954; Sage, 1962; van Grouw & Hume, 2016).

4.4.3 Aim 4: Relationships between feather quality measures

The fourth aim of the study was to explore relationships between different feather quality measures. The first relationship assessed was between the average width of fault bars and the average strength of the feather, finding no significant correlation. This suggests that fault bars are not a reflection of the structural core strength of the feather.

Results found that a higher number of snapped tail feathers occurred when wider fault bars were present. This is interesting as the tail feathers were found to have the widest fault bars, which indicates that feather breakage appears to be associated with severe fault bars. However, as discussed previously, the true cause of the feather breakage cannot be identified in the study samples, especially in tail feathers which are regularly in contact with the ground.

A significant relationship was found between average fault bar width and average feather iridescence across all feather types. Iridescent feathers are thought to be an honest signal of individual quality, with dull feathers found to be a good indicator of poor physiological condition (Eliason & Shawkey, 2011; Harper, 1999; Ruiz-Rodriguez, 2015). This suggests that the entire feather is compromised by the occurrence of fault bars. Conversely, the causes of fault bar occurrence and low iridescence in carrion crows may not be the same. Regardless, it suggests that average fault bar width may be an accurate indicator of feather quality and may also indicate individuals in poor physiological condition. It is also interesting to reflect on the connection between iridescence and it's tight link to the evolution of feather colouration (Lee *et al*., 2016). A study on eight species of *Corvidae* (not including carrion crow) found three distinct colouration schemes in feathers (1) brown or black matte colours were produced in barbs and barbules; (2) blue, bluish grey and white non-iridescent structural colours were produced in the barbs and (3) structural iridescent colours were produced only in distal barbules (Lee *et al*., 2016). Therefore, in light of the barbule damage discussed in the previous chapter, it raises questions about the general barbule arrangement in carrion crow feathers. In order to understand the signalling function of iridescent feathers in carrion crows, studies into the evolutionary history of plumage colouration must include a wider variety of species of *corvidae*.

No relationship between average fault bar width and aberrant white feathers was found. This is interesting due to the varying causes of this defect. This occurrence is generally thought to be associated with genetic factors (Sage, 1962); however, it has now been found to link more commonly with the physical condition and age of individuals and can also include environmental conditions e.g. food availability (van Grouw, 2018). This leads to further questions around the occurrence of leucism in carrion crows, especially in relation to their depiction of poor feather quality. This also suggests that there may be different causes affecting fault bars and white feathers, which queries the role of white feathers in the 'fault bar allocation' hypothesis (Jovani & Blas, 2004). Aim 2 also found that wing feathers were more susceptible to lack of pigmentation. Therefore, fault bars and white feathers appear to be 'allocated' to different feathers, which again gives the impression that white feathers are not detrimental the quality of carrion crow feathers.

No relationship was found between average feather strength and the number of snapped feathers. This is interesting as weaker feather were expected to be more susceptible to breakage. However, as mentioned previously, the cause of feather breakage is unknown and may have occurred through factors that are not associated with feather integrity. Stronger feathers were associated with higher average feather iridescence in secondary feathers only. This is interesting, as secondary feathers were found to be weaker than the other feather types. This may be coincidental; however, owing to generally smaller feather sizes in secondaries. The relationship between average feather strength and the number of white feathers was close to significant in all feather types combined. Aberrant white feathers are commonly seen as weaker (Bonser, 1995; Kaiser, 2008), whereas this suggests the opposite. This could potentially relate back to the possibility of causal hereditary conditions (Harrison, 1957a,b; Sage, 1954; Sage, 1962; van Grouw & Hume, 2016) in carrion crows and also supports the findings discussed above (no relationship between fault bar width and white feathers). In regard to the general lack of relationship between feather strength and the other quality measures, it can be thought that feather strength offers different information on feather quality than the other measures. Therefore, this variable will be used in future chapters as a unique area of research.

High numbers of snapped feathers were found in individuals with dull feathers across all feather types. This result was expected due to the honest signalling of feather iridescence. Moreover, high numbers of snapped feathers were also associated with a high number of white feathers on average across all feather types combined. This suggests that aberrant white feathers are more prone to breakage. However, it is important to, again, note that the cause of feather breakage is unknown. Therefore, this conclusion cannot be drawn without further research.

4.4.4 Aim 4: Selection of dependant variables for further analyses (titles of which stated below)

The fourth aim of the study was to select dependant variables for further analyses, based on the results of aims 2 and 3.

4.4.4.1. Investigation into the causes of fault bar production in relation to chemical profile of feathers, parasite burden, sex and age (Chapter 5)

The average width of fault bars (mm) was selected as the dependant variable for this study aim. This dependant variable was used to explore the causes of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, age and sex. In addition to this study aim, this chapter aimed to explore the chemical composition of feathers in fault bars of different widths, specifically in relation to nutritional elements.

4.4.4.2. Consequences of poor feather quality in the form of feather strength in relation to the chemical profile of feathers, endoparasite burden, sex and age (Chapter 6)

Average feather strength was selected as the dependant variable for this study aim. It was not found to correlate with any other variable; therefore, offers different information than the variables selected above. This dependant variable was used to explore the consequences of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, age and sex. In addition to this study aim, this chapter aimed to investigate differences in feather strength in relation to fault bar presence. The purpose of this aim was to determine how fault bars impact a feather’s tolerance to stress at particular locations on the feather.

4.5 Conclusion

A key finding in this study was the relationship between the average width of fault bars and average feather iridescence across all feather types. This information strengthens our knowledge of how dull feathers portray honest communication signals of low fitness. Moreover, average feather strength was found to be an independent measure of quality, with generally no relationships found with the other quality measures. This shows that feather strength offers different information of feather quality than the other measures, resulting in this factor being used for separate analysis.

The average width of fault bars and the average feather strength variables will, therefore, be used as dependant variables for the preceding data chapters. The average fault bar width variable will be used to investigate the causes of fault bar production in relation to chemical profile of feathers, parasite burden, sex and age (Chapter 5). Furthermore, average feather strength will be used to investigate the consequences of poor feather quality in relation to the chemical profile of feathers, endoparasite burden, sex and age (Chapter 6).

Chapter 5: The causes of fault bar production in relation to the chemical profile of feathers, endoparasite burden, sex and age

5.1 Introduction

The structural integrity of feathers can be compromised by stress during the growth stage, resulting in fault bar formation and reduced flight efficiency (Corning & Biewener, 1998). Fault bars are weak, translucent bands that appear through the width of the feather vane (Erritzøe, 2006; Jovani & Rohwer, 2016). The causes of fault bar production are poorly understood, with two hypotheses currently in discussion. The first of which relates to a lack of nutrient supply to the growing feather at specific points of formation, with reference to alterations in blood pressure (Riddle, 1908). In response to Riddle's theory around nutrient supply, nutritional deficiency is, therefore considered. For example, nutritional elements such as calcium, zinc and iron have been found to be important regulators of melanin biosynthesis (McGraw, 2003; Niecke, Rothlaender & Roulin, 2003). Furthermore, calcium induces an aggregation of melanin, which increases feather stability (Bonser, 1995; Niecke *et al.*, 1999; Okazaki *et al.*, 1985).

More recently, Murphy *et al.* (1989) suggested that stress led to unusual muscle contractions around the soft feather shaft during feather growth resulting in barbule damage (Murphy *et al.*, 1989). A wide variety of stressors have been researched, including hereditary traits and psychological trauma from human handling, environmental factors, parasite burden and disease (Erritzøe, 2006). The hypotheses around fault bar formation in relation to nutrient supply and muscle contraction can be developed further. The above scenario predicts that fault bars are a result of the muscle contractions damaging the barbules. At the same time, squeezing of the blood vessels may temporarily hinder nutrient supply, which would result in a different chemical signature at a fault bar site as compared to a site without fault bars. Alternatively, the squeezing of the blood vessel could leave permanent damage and compromise the chemical composition of the entire feather.

When considering Riddle's (1908) ideas around limited nutrient supply playing a role in fault bar formation, it is interesting to view this in regard to parasite burden. Parasite burden has a significant impact on individual performance, as parasites compete with the host for nutrients, triggering costly immune responses (Hudson *et al.*, 1998; Møller, 1997; Reed *et al.*, 2012; Sheldon & Verhulst, 1996). In addition to this factor, age was also considered as a potential contributor to variations in nutrition and stress. As stated previously, young birds are particularly vulnerable to fault bar production, as the feather growth period is very sensitive and is also an important time for general body growth (Hawfield, 1986; Serrano & Jovani, 2005). Due to this, nestlings are vulnerable to fault bar production, even under low levels of stress (Erritzøe, 2006). Therefore, this study aims to compare differences in fault bar occurrence between first year juveniles, second year juveniles and adults, to further explore this relationship. Unfortunately, nestlings were excluded from the study due to this age group displaying feathers in pin formation, which were unfeasible for feather measurements. Therefore, age was assessed from the point of fledgling (first year juvenile), second year juvenile and adult in the following results. Carrion crows perform a partial post-juvenile moult, meaning that they have the same flight feathers from when they are nestlings to when they moult at the end of their second year (Ginn & Melville, 1983). Therefore, as the feathers become worn and abraded in the second year, they become less tolerant to stress. Therefore, feathers of first and second year old crows reflect a) conditions in the nest (yearlings) and b) wear and tear in second year birds as compared to annually replaced feathers in adults.

Differences between sexes have also been found in the susceptibility to fault bar production, which have been linked to differences in factors such as parental investment and migration distances (Dawson *et al.*, 2001; Slagsvold, 1982b). These studies showed that sex can play an important role in the propensity to producing fault bars, depending on the species of bird.

The hypothesis around fault bar formation in relation to nutrient supply and stress factors requires further investigation. The findings presented in chapter 3 supported the muscle constriction hypothesis (Murphy *et al.*, 1989) in many ways; however, the concept of blood and nutrient supply (Riddle, 1908) could not be dismissed. In fact, there may be multiple factors occuring together, which result in the formation of a fault bar. In response to this notion, the chemical profile of feathers was assesed in this chapter, focusing on the occurrence of nutritional elements across a range of feathers with and without fault bars. It can be questioned whether the chemical composition of feathers differ at sites containing fault bars, especially in relation to fault bars of different severity. In contrast, does a lack of nutrient supply impact the entire feather, rather than the specific areas of damage?

5.1.1 Study aims and hypotheses

In summary, the feather structure can be compromised during growth (Corning & Biewener, 1998), which has been discussed in relation to nutrient supply (Riddle, 1908) and the power of muscle constriction (Murphy *et al.*, 1989). In light of Riddle's (1908) hypothesis, it could be suggested that specific nutrients may play a role in the early stages of feather formation; therefore, this factor was considered. Moreover, I suggested two scenarios for how muscle contraction can affect nutrient supply. The chemical composition of specific feather sections are, therefore, analysed in order to explore these two scenarios: 1) nutrient supply impacts the feather at locations of fault bar occurrence 2) nutrient supply impacts the entire feather. Growth defects have also been discussed in relation to a variety of different stressors, e.g. hereditary traits, psychological trauma from human handling, environmental factors, parasite burden and disease (Erritzøe, 2006). In response to these ideas, the following factors will be explored:

Aim 1: In-depth assessment of feather chemical composition. Investigation of differences in chemical composition of feathers at fault bar sections of different severities in relation to fault bar free sections to test the nutrient supply hypothesis (Riddle, 1908). This represents scenario 1, where nutrient supply is temporarily reduced for as long as the muscle contraction lasts. This was tested using feathers showing all three types of fault bar (within-feather comparison; n=10).

- **Objective 1.1:** (Scenario 1) Chemical composition differs between sites with and without fault bars. Moreover, wide fault bars were expected to have a different chemical composition than feather sections with narrow fault bars.

Hypothesis 1.1: Chemical composition differs between sites with no fault bars and sites with fault bars of different severity. This leads to a direct link between feather damage and chemical composition (Riddle, 1908).

Aim 2: Investigate the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age.

- **Objective 2.1:** To investigate whether the much cruder but easier analysis of the shaft base produces similar results as the detailed study above. This would allow using the shaft base as a proxy for feather quality (n=38). This objective also tests scenario 2 that the entire feather is chemically compromised.

Hypothesis 2.1: (Scenario 2) Alternatively to hypothesis 1.1, feathers with fault bars were expected to have a different chemical composition at the feather base than feathers without fault bars. This leads to the conclusion that fault bars are not linked solely to chemical composition but caused by other factors, with the overall feather being impacted (Duerden, 1909).

- **Objective 2.2:** To investigate the relationship between sex and average fault bar width in feathers (please note that fault bar width was chosen to represent feather damage in chapter 3, as it was found to correlate positively with the number of fault bars).

Hypothesis 2.2: Due to carrion crows being monomorphic and non-migratory, fault bar width was not predicted to vary between sexes (Dawson *et al*., 2001; Slagsvold, 1982b).

- **Objective 2.3:** To investigate the relationship between the number of endoparasite species present within an individual and average fault bar width in feathers.

Hypothesis 2.3: Due to the trigger of costly immune responses caused by endoparasites (cestodes, flukes and acanthocephalan), individuals with high numbers of endoparasite species were expected to have feathers with wider fault bars (Freed *et al*., 2005; Jovani *et al*., 2014; Møller *et al*., 1996).

- **Objective 2.4:** To investigate the relationship between age and average fault bar width in feathers.

Hypothesis 2.4: Due to their susceptibility to fault bar production, first and second year juveniles were expected to have wider fault bars on average compared to adults, which also correlates with a higher number of fault bars (Hawfield, 1986; Serrano & Jovani, 2005).

5.2 Study Methodology

5.2.1 Assessment of feather chemical composition

For both Aims 1 and 2, the chemical composition of feather samples was gained using Scanning Electron Microscope Energy-Dispersive X-ray spectroscopy (SEM-EDS) (please refer to section 2.2.3.1 for more details). Chemical elements measured for both study aims were as follows – carbon, calcium, chlorine, oxygen and sulphur.

5.2.1.1 Aim 1: In depth analysis of relationships between chemical composition and fault bars

Ten carrion crows were used in this study, with one wing feather selected per individual. This feather was selected based on the occurrence of a range of different fault bar severities presented together. This provided consistency in the sampling of these fault bars and acted as a control measure for the study. Four samples were taken from each feather, representing the following categories: 1. No fault bar present 2. Light fault bar 3. Medium fault bar 4. Heavy fault bar (please refer to section 2.2.1 for further guidance on these categories). Distance from the feather base was then measured for each sample to determine the position of each sample. This is an important measure, as studies have shown that the chemical composition of feathers is non-uniformly distributed throughout the length of the feather (Howell *et al.*, 2017).

In the morphology chapter (see section 3.3.3.5), a significant negative relationship was found between the visual width of fault bars (mm) and the average width of barbules (mm). This shows that heavy fault bars had narrow barbules in comparison to less severe fault bars. The average width of barbules (mm) was used to represent feather quality for this study aim, to take advantage of data collected on an accurate, microscopic level. The morphology chapter showed differences in the severity of barbule 'squeezing', which will be compared to the chemical composition. Please note that this study was conducted on a smaller sample size than aim 2, the main analysis. This is due to the intensive nature of data collection for this study.

The average barbule width in each feather was assessed by measuring the width of each barbule at 150 x magnification and calculating the mean across all barbules for each measuring point (1 – 4). The mid-point of each ‘squeezed’ section was used for this measurement (see section 2.2.1; Fig.2.3).

5.2.1.2 Aim 2: Investigate the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age

In order to make use of a larger data set, this study used the measure of average (mean) width of fault bars on a given feather, rather than the in-depth measure of barbule width for each fault bar. Thirty-eight carrion crows were used in this study, with the following feathers used for analysis: primary feather 3, secondary 3 and tail feather 3. These feathers were chosen in order to represent the central area of each feather type on the wing (see Fig.2.4 for locations). Primary and secondary feather data were combined for this data set, due to having statistically comparable fault bars widths, to be labelled ‘wing feathers’. Tail feathers were analysed separately due to their differences in function and fault bar burden.

In contrast to aim 1 and to address objective 2.1., chemical composition measurements were only taken at the base of the feather. The location of this fragment was chosen in order to maintain consistency due to the use of snapped feathers. This then identified whether the much cruder but easier analysis of the shaft produces similar results as the detailed study above. This would allow using the shaft as a proxy for feather quality in future studies.

In addition to the average chemical composition of the feather base (discussed above), three further variables were used for this study. Sex (female/male) (objective 2.2.) was identified during post-mortem, identifying urogenital structures for each sex (section 2.2.3.2.3; Panto, 2017). Endoparasite burden was measured using the number of endoparasite species (objective 2.3) found in the gut and trachea of each bird (section 2.2.3.2.2). Age (objective 2.4) was categorised with reference to inner mouth colouration and descriptions of feather characteristics, using three age categories (first year juvenile, second year juvenile and adult) (section 2.2.3.2.3; Svensson, 1992). It is important to note that nestlings were not included in this study due to the presence of pin feathers, which were unfeasible for feather measurements.

5.3 Statistical methodology

5.3.1 Aim 1: Relationship between chemical composition and fault bars (n=10)

The dependant variable for this study was average barbule width (mm) at four locations (no fault bar, light fault bar, medium fault bar and heavy fault bar). Independent variables for this study consisted of the proportion of chemical elements within feather samples - Carbon C, Calcium Ca, Chlorine Cl, Oxygen O and Sulphur S. Outliers were excluded using the identification of high z-scores (van den Berg, 2022). In this, $|z| \geq 3.29$ indicates an outlier (van den Berg, 2022). When outliers were removed for analysis, this was clearly stated in the relevant result sections.

Chemical data was transformed using Square-root arcsine transformation due to the data being proportional. In addition, feather position can affect chemical composition (Howell *et al.*, 2017). To consider this, in each of the 10 feathers, feather position was measured at each sample site (1 – no fault bar, 2 – light fault bar, 3 – medium fault bar, 4 – heavy fault bar). Linear regression analysis was used to test for a relationship between feather position and proportion of each chemical, separately. When the results of this test were significant, positive and negative deviation from the control line (areas of no fault bar occurrence) were calculated (Field, 2013). This resulted in the use of data across light, medium and heavy fault bars (n=30). When the results of the feather position linear regression were not significant, the original chemical data were used without considering location for future analyses. This data set used areas of feather with no fault bars, light, medium and heavy fault bars (n=40).

To investigate effects of chemical composition on fault bars, average barbule width (mm) of each feather sample was used as dependent variable in a generalised linear mixed model (GLMM). Shapiro-Wilk testing identified this variable to be parametric. The main analysis used for this aim was a generalised linear mixed model (GLMM) with an identity link function. The traditional GLM assesses the impact of one or multiple predictor variables on an outcome variable (Frey, 2018). In addition, GLMMs are extensions of GLMs where the data are repeated measures (Sinharay, 2010). Independent variables were fault bar type (1 – no fault bar, 2 light, 3 medium, 4 - heavy) and proportion of each chemical for each fault bar type. Average barbule width (mm) was then tested in relation to the proportion of each chemical in relation to each sample (n=40). Akaike (AIC) values were used to determine the best model, identifying the lowest values to be the best data fit (Field, 2013). Different combinations of independent variables were assessed to identify those with the best fit (Field,2013).

5.3.2 Aim 2: Investigate the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age (n=30)

It should be noted that aims 1 and 2 used different measures for feather damage. Aim 1 used average barbule width, where narrow/squeezed barbules represented more severe damage. The dependant variables used study aim 2 were the average (mean) width of fault bars in carrion crow wing and tail feathers. Here, wider fault bars represent more severe damage. Shapiro-Wilk testing identified both dependant variables to be non-parametric; therefore, data was transformed using Log10, resulting in normally distributed data. For both wing and tail feathers, a series of linear regressions were conducted to determine the relationship between the average width of fault bars (mm) and a range of independent variables: the chemical composition of the feather shaft, endoparasite burden, sex and age. The chemical data was transformed using Square-root arcsine transformation due to being proportionate, as discussed above.

Linear regressions were conducted in stages, as sex and age were not available for all measured feathers. While the sample size for chemical data was n=38, the sample size for parasite burden was n=30, for sex n=33 and for age n=22. Therefore, the first linear regression excluded age to take advantage of the larger sample size available in the other variables (n=30). The second linear regression incorporated age in addition to any variables that had a significant effect in the first analysis (n=22). Stepwise method was chosen for this analysis, to evaluate the order of importance of variables and select useful subsets of variables (Huberty, 1989; Lewis, 2007; Thompson, 1995). This method develops a sequence of linear models that, at each step, considers the removal of each entered predictor (Snyder, 1991; Lewis, 2007; Thompson, 1989). Stepping method criteria threshold was increased to p=0.099 to increase the likelihood of including variables that may have a significant effect once included.

5.4 Results

5.4.1 Aim 1: Relationship between chemical composition and fault bars (n=10)

5.4.1.1 Chemical composition changes across the feather length

A significant negative relationship was found between feather position (cm) and quantity of carbon (%)($F_{1,48}$= 4.176, p=0.047). Position explained 80% of the variation in carbon. Fig.5.1 shows that there was significantly more carbon at the base of the feather, decreasing towards the tip.

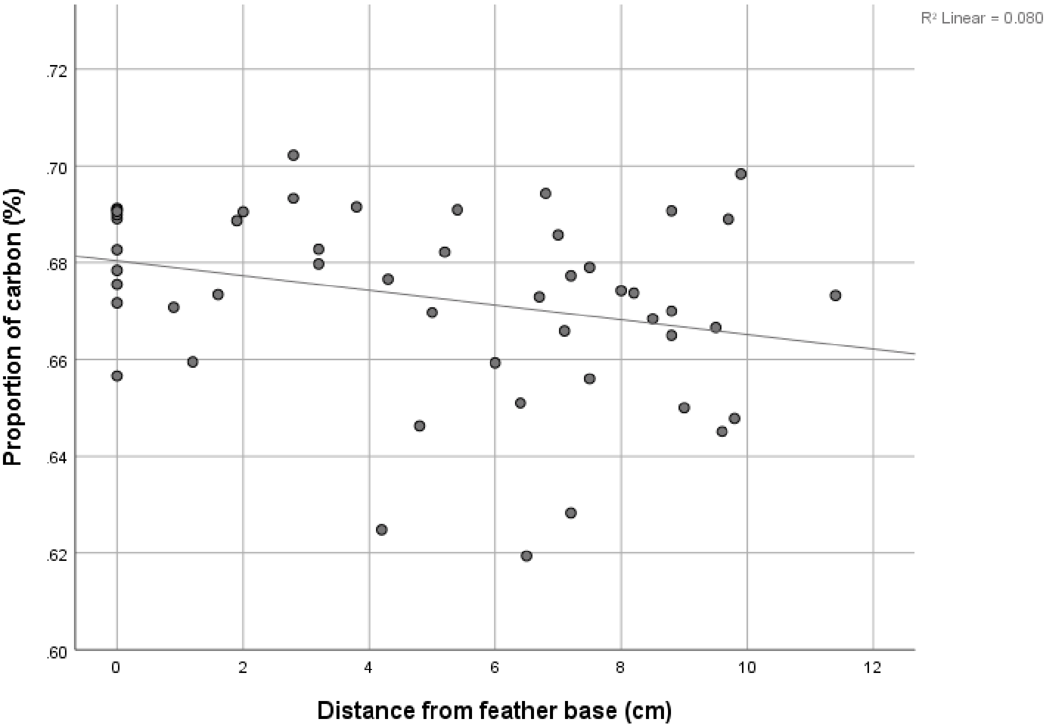


Fig.5.1 Proportion of carbon in relation to the distance of sample from the feather base (cm) (n=40).

A significant relationship was found between feather position and quantity of calcium (%) ($F_{1,48}$= 10.654, p=0.002). Fig.5.2 shows that there was significantly more calcium towards the tip of the feather, explaining 18% of the variation.

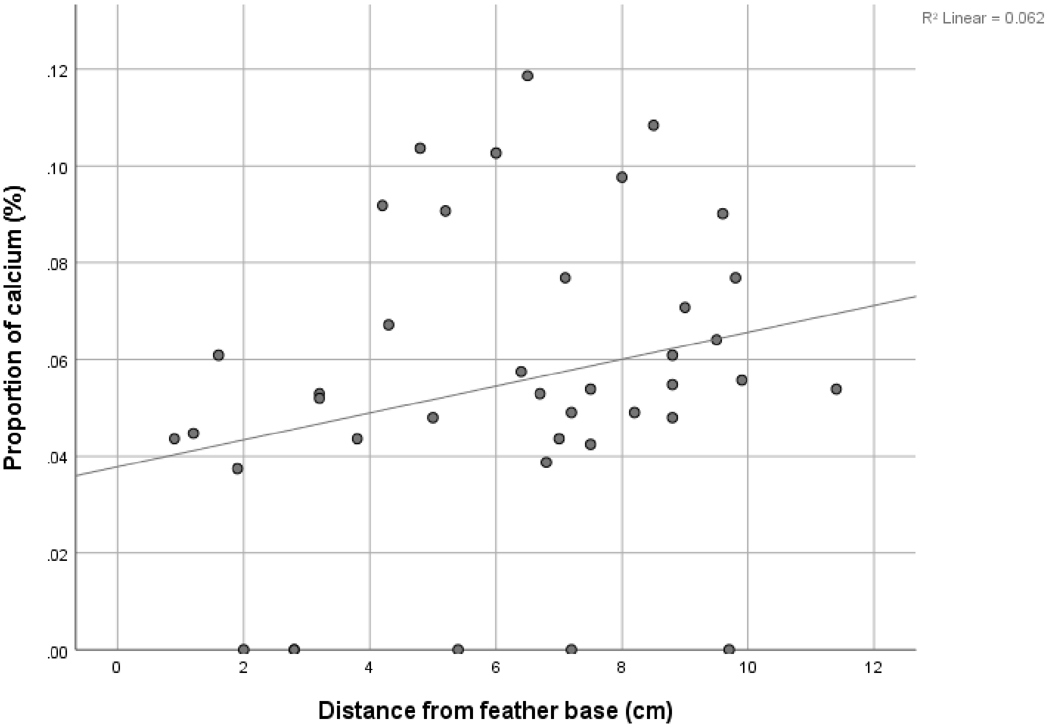


Fig.5.2 Proportion of calcium in relation to the distance of sample from the feather base (cm) (n=40).

No significant relationship was found between feather position and quantity of chlorine (%) ($F_{1,48}$= 0.003, p=0.954) or oxygen (%) ($F_{1,48}$= 0.219, p=0.642). Both variables were used without consideration of feather location in further analyses. In contrast, a significant relationship was found between feather position and quantity of sulphur ($F_{1,48}$= 13.670, p=0.001). Fig.5.3 shows that there was significantly more sulphur towards the tip of the feather, explaining 22% of the variation.

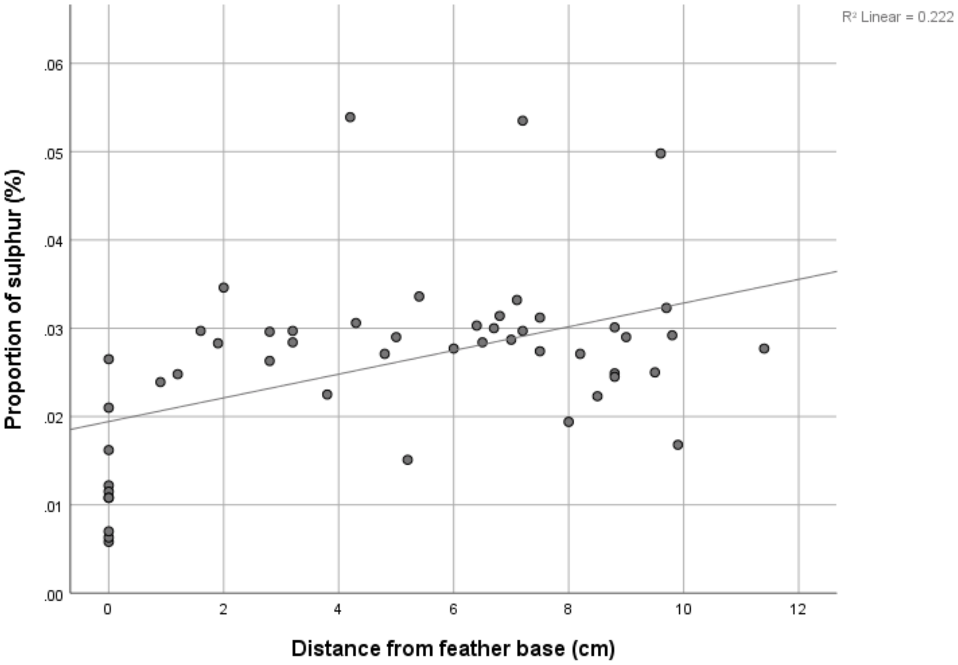


Fig.5.3 Proportion of sulphur in relation to the distance of sample from the feather base (cm) (n=40).

5.4.1.2 Differences in the chemical composition of feathers at fault bar sections of different severities in relation to fault bar free sections

Please note that this study uses average barbule width (mm) as a measure of feather damage. Narrow/squeezed barbules equate to more severe damage in this study aim. Chemical composition analyses were conducted in two groups. Group 1 consisted of the proportion of carbon, calcium and sulphur. These chemicals were found to have a relationship with feather position; therefore, positive/negative deviation from the control measure (region with no fault bars) were used for this data set (n=30). Group 2 consisted of the proportion of chlorine and oxygen. These chemicals were not found to have a relationship with feather position; therefore, original data was used with the inclusion of the control measure (no fault bars).

Group 1: The best model included the proportion of calcium, sulphur and carbon (Tab.5.1). The GLMM found a significant effect between average barbule width and the proportion of calcium (F=4.346, t=2.085, p=0.047) in feathers in relation to fault bar type (Fig.5.4). Fig.5.4 shows that a lower proportion of calcium is associated with more severe fault bars. In contrast, no significant effect was found between average barbule width and the proportion of sulphur (F=0.553, t=-0.744, p=0.464) or carbon (F=0.139, t=0.373, p=0.712) in feathers in relation to fault bar type.

Tab.5.1 Generalised linear mixed model output testing the chemical composition of feathers at fault bars of different severities (n=30) using residuals of chemical composition in relation to baseline values generated from locations without fault bars to consider location of fault bars. Akaike and delta values provided for best fit information of independent variables entered: proportion of calcium, sulphur and carbon.

Model	Chemical	Akaike (AIC)	Delta (difference)
1	Calcium Sulphur Carbon	-47.806	
2	Calcium Sulphur	-45.370	2.436
3	Calcium	-43.898	3.908
4	Sulphur	-41.645	6.161
5	Carbon	-40.208	7.598

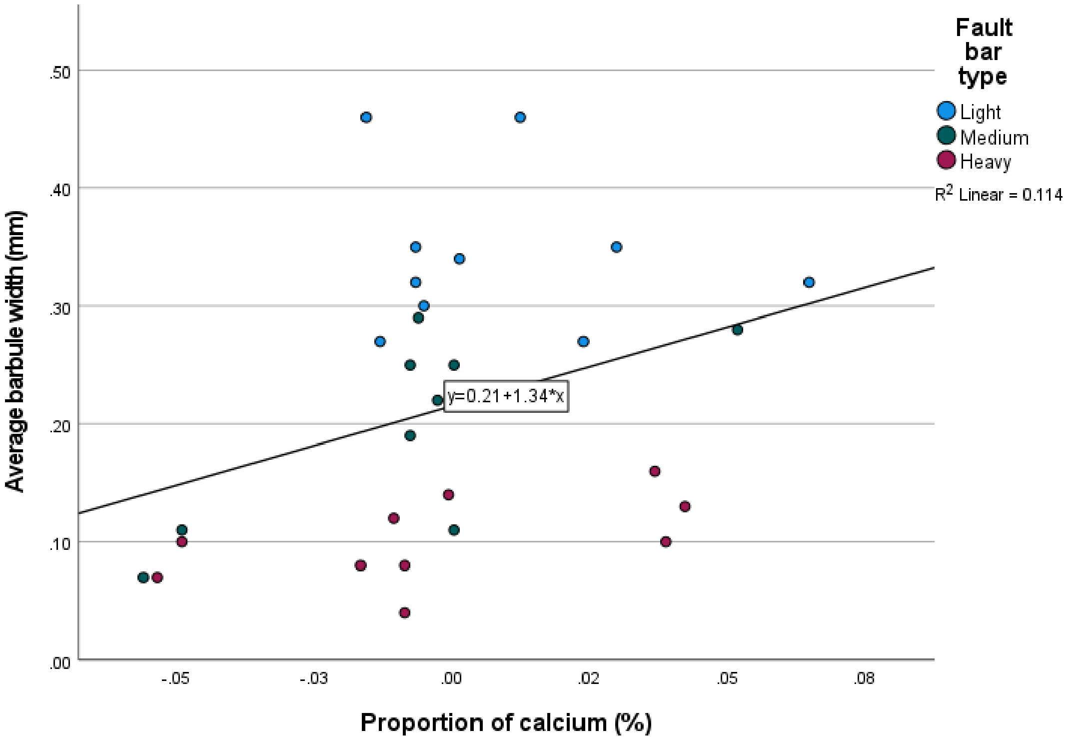


Fig.5.4 Proportion of calcium (%) of feather sections in relation to average barbule width (mm) (n=30). Width is inversely related to squeeze i.e. lower values equate to heavier squeeze/damage. Please note that transformed calcium data is displayed, in addition to being calculated against a control line (areas of no fault bar occurrence).

Group 2: The best model included the proportion of oxygen and chlorine (Tab.5.2). The GLMM found no significant effect between average barbule width and the proportion of oxygen (F=2.452, t=1.566, p=0.126) or chlorine (F=2.173, t=1.474, p=0.149) in feathers in relation to fault bar type.

Tab.5.2 Generalised linear mixed model output testing the chemical composition of feathers at fault bar free sections and fault bars of different severities (n=40). Akaike and delta values provided for best fit information of independent variables entered: proportion of oxygen and chlorine.

Model	Chemical	Akaike (AIC)	Delta (difference)
1	Oxygen Chlorine	-35.080	
2	Oxygen	-32.240	2.840
3	Chlorine	-30.513	4.567

5.4.2 Aim 2: Investigate the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age (n=30)

Please note that this study uses average fault bar width (mm) as a measure of feather damage. Wider fault bars equate to more severe damage in this study aim.

5.4.2.1 Wing feathers

Stepwise linear regression analysis was conducted to assess the relationship between average fault bar width (mm) in wing feathers and the following factors: chemical composition, endoparasite burden and sex (n=30). The number of endoparasite species remained in the model, explaining 12.8% of the variance ($F_{1,27}$=3.978, p=0.056). This result was close to significance, suggesting a negative trend between number of endoparasite species and the average width of fault bars. Fig.5.6 shows low numbers of parasite species in more individuals with severe fault bars. Tab.5.3 shows full results of the excluded variables.

Tab.5.3 Linear regression output for excluded variables, assessing the relationship between average fault bar width (mm) in wing feathers and the following factors: chemical composition (proportion of carbon, calcium, chlorine, oxygen and sulphur), endoparasite burden (number of parasite species) and sex (n=30).

	t	Sig.
Carbon	0.103	0.919
Calcium	-0.899	0.377
Chlorine	0.360	0.722
Oxygen	-0.206	0.839
Sulphur	-0.113	0.911
Sex	1.136	0.266

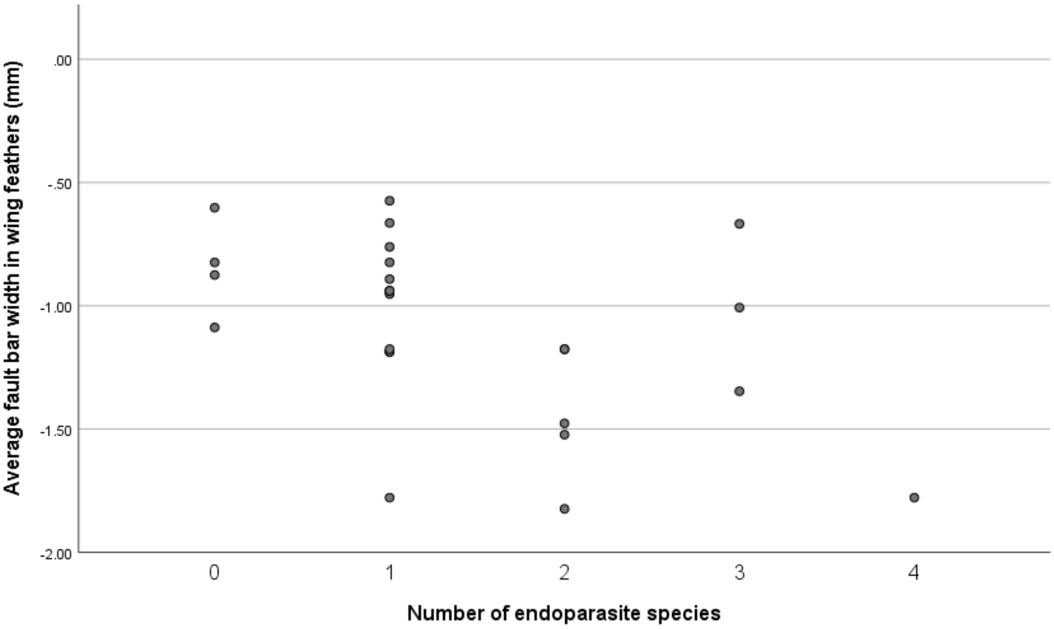


Fig.5.5 Number of endoparasite species in relation to mean fault bar width in wing feathers (mm) (n=30). Higher fault bar width values correspond to more severe fault bars. Please note that transformed data is displayed on both axis.

A second stepwise linear regression analysis was conducted to assess the relationship between average fault bar width (mm) in wing feathers and the following factors: endoparasite burden and age (n=22). The number of endoparasite species remained in the model, explaining 19.4% of the variance ($F_{1,20}$=4.804, p=0.040). Again, Fig.5.6 shows a low numbers of parasite species in more severe fault bars. Age was excluded from the model due to non-significant effect (t=-1.191, p=0.248).

5.4.2.2 Tail feathers

Stepwise linear regression analysis was conducted to assess the relationship between average fault bar width (mm) in tail feathers and the following factors: chemical composition, endoparasite burden and sex (n=30). The proportion of carbon remained in the model, explaining 17.2% of the variance ($F_{1,25}$=5.18552, p=0.032). Fig.5.6 shows a higher proportion of carbon in wider fault bars in the tail feathers. Tab.5.4 shows full results of the excluded variables.

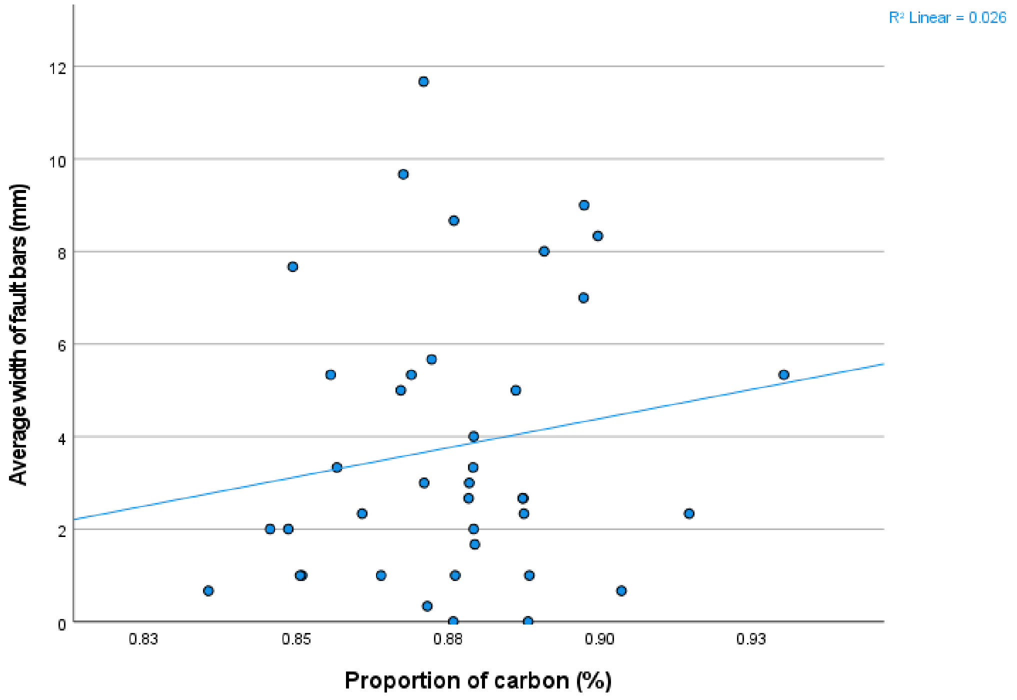


Fig.5.6 Proportion of carbon in relation to mean fault bar width in tail feathers (mm) (n=30).

Tab.5.4 Linear regression output for excluded variables, assessing the relationship between average fault bar width (mm) in tail feathers and the following factors: chemical composition (proportion calcium, chlorine, oxygen and sulphur), endoparasite burden (number of parasite species) and sex (n=30).

	t	Sig.
Calcium	-0.737	0.468
Chlorine	1.188	0.247
Oxygen	0.214	0.833
Sulphur	1.396	0.175
Parasites	-0.036	0.972
Sex	0.772	0.448

A second stepwise linear regression analysis was conducted to assess the relationship between average fault bar width (mm) in tail feathers and the following factors: proportion of carbon and age (n=22). Age and the quantity of carbon had a significant effect on average fault bar width, explaining 34.9% of the variance ($F1_{,19}$=4.819, p=0.021). Wider fault bars in the tail feathers were found in younger individuals, explaining 21.7% of the variance ($F_{1,19}$=5.262, p=0.033; Fig.5.7). A higher proportion of carbon was found in wider fault bars in the tail feathers, explaining 13.2% of the variance ($F_{2,18}$=4.819, p=0.021; Fig.5.6).

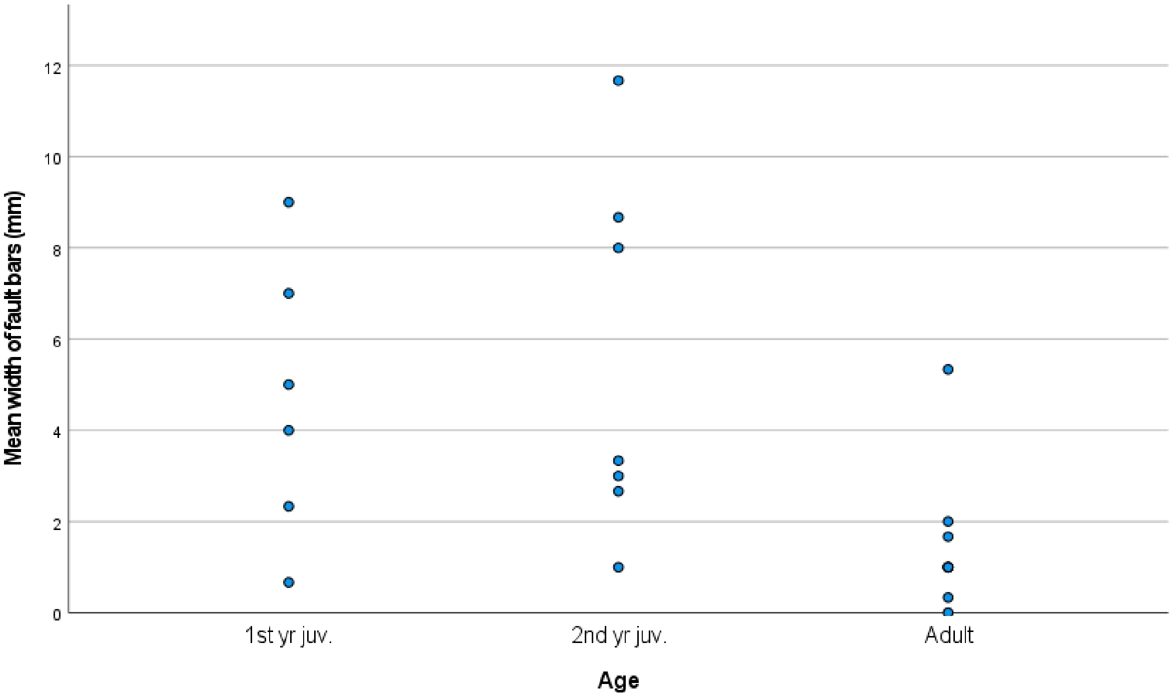


Fig.5.7 Age of carrion crow individuals in relation to mean fault bar width in tail feathers (mm) (n=22). Line of best fit unavailable.

5.5 Summary of results

5.5.1 Aim 1: Relationships between chemical composition and fault bars (n=10)

- Proportion of carbon, calcium and sulphur varied with feather position. A higher proportion of carbon was found at the feather base, with higher proportions of calcium and sulphur found towards the tip.
- Significantly lower proportions of calcium were found in heavier fault bars of wing feathers. No other chemicals were found to vary between areas of different fault bar severity.

5.5.2 Aim 2: Investigate the causes of fault bar production in relation to feather chemical composition at the feather base, endoparasite burden, sex and age (n=30)

- Wide fault bars in the wing feathers were associated with low numbers of endoparasite species.
- Wide fault bars in the tail feathers were associated with a high proportion of carbon in the feather shaft base.
- Wide fault bars in the tail feathers were associated with younger individuals.
- No relationship was found between fault bar occurrence and sex in both wing and tail feathers.

5.6 Discussion

5.6.1 Aim 1: Chemical composition of feathers

5.6.1.1 Chemical composition changes across the feather length

The first step in this study aim was to determine how the chemical composition of a feather varies across the length of the structure. This is an important measure, as studies have shown that the chemical composition of feathers is non-uniformly distributed throughout the length of the feather (Howell *et al.*, 2017). Moreover, this aim acknowledged the different feather positions of fault bar samples used in this study. This found that the proportion of carbon, calcium and sulphur varied with feather position. A higher proportion of carbon was found at the feather base. This could suggest that carbon plays a structural role in thicker, more rigid section of the feather. The feather base is also the least flexible and has a lower tolerance of stress compared to the rest of the feather (Butler & Johnson, 2004; Sullivan *et al.*, 2017). It was suggested by Bortolotti (2010) that some of the most commonly used isotopes in ecology, such as carbon, are important to feather physiology and are mass dependent. However, due to carbon being a basic component of keratin and all other organic molecules, this element cannot be considered as an isolated individual molecule. It should also be noted that the data fit for this result was very low; therefore, conclusions cannot be drawn.

A high proportion of calcium was found towards the tip of the feather, which is the most tolerant area to stress. This suggests that calcium may be more prominent in delicate, flexible parts of the feather. This result was supported by a study on bald eagle (*Haliaeetus leucocephalus*) rectrice feathers, which found a strong dilution effect in calcium as feather mass increased with growth (Bortolotti, 2010, Bortolotti & Barlow, 1986). In contrast, a study on the breast feathers of three seabirds/*Procellariiformes* (flesh-footed *Ardenna carneipes*, streaked *Calonectris leucomelas* and short-tailed (*Ardenna tenuirostris* Shearwaters) found consistently high concentrations of calcium at the calamus towards the skin interface (Howell *et al.,* 2017). Differences in results could be explained by potential differences between species and the use of different feather types.

Similar to calcium, a high proportion of sulphur was also found towards the tip of the feather in carrion crow feathers. This, again, suggests that sulphur may be more prominent in areas that are most tolerant of stress. Sulphur makes up the building blocks of a feather, due to the presence of sulphur-based amino acids in keratin (Bortolotti, 2010). A diet deficient in sulphur-containing amino acids has been discussed in previous studies in relation to the occurrence of pallid bands in feathers (Jovani & Rohwer, 2016; Murphy *et al*., 1988). Pallid bands are weakened areas with reduced melanin across the width of the feather vane (Ross *et al*., 2015). Therefore, the distribution of sulphur along the feather may be associated with particular structural characteristics.

5.6.1.2 Differences in the chemical composition of fault bars at different severities

Different levels of damage to the feather vane were explored in this study aims whilst accounting for the effect of feather on chemical composition. Average barbule width was used to represent feather damage due to wide fault bars presenting narrow/squeezed barbules. Due to the discussion of nutrient supply in feather growth (Riddle, 1908), it was expected that the chemical composition of fault bars would differ according to severity. Results partly supported this hypothesis, finding significantly lower proportions of calcium in heavier fault bars. This implies that feather malformation may be linked to a nutritional deficiency, much like those seen in human nails (Cashman & Sloan, 2010). Calcium is essential for the survival of birds as it is an important element in skeletal mineralisation and eggshell formation (Dawson & Bidwell, 2005; Graveland, 1998; Pacyna *et al*., 2018; Zduniak *et al*., 2014). A study by Dawson and Bidwell (2005) supports the importance of calcium in their study of tree swallows (*Tachycineta bicolor*), finding improved nestling growth and fitness in individuals that were given calcium supplements. They also found that these individuals had higher survival rates after leaving the nest, suggesting that calcium availability impacts fitness

(Dawson & Bidwell, 2005). The results of this study therefore add to the knowledge of calcium and its role in fitness, expanding to feather quality.

A lack of calcium in the diet has also been found to cause physiological issues such as osteodystrophy (metabolic bone disease/MBD) in birds, resulting in brittle bones, deformed limbs and soft beaks (Forbes & Zsivanovits, 2002; Stocker, 2005). When low calcium levels occur, the parathyroid glands secrete parathyroid hormone (PTH), to compensate for the nutritional imbalance (Thomas, 1985). This then leads to a condition called Nutritional Secondary Hyperparathyroidism (SHP) which displays in symptoms such as dull feathers, slow feather growth after moult, feather picking, mental dullness, lethargy and gastrointestinal problems (Thomas, 1985). This is interesting and supports the notion that calcium may indeed impact feather health.

Due to the keratinous structure of feathers, a link between calcium deficiency and feather deformations is logical (Riddle, 1908; Sullivan *et al*., 2016). Calcium is a major regulator of keratinocyte differentiation, which is a crucial process in feather formation (Bikle, Xie & Tu, 2012). Moreover, keratinocytes in low calcium concentrations have been found to proliferate but fail to differentiate into a stratified layer (Bikle *et al*., 2012). Calcium also induces an aggregation of melanin, which has been suggested to result in an increased stability of feathers (Bonser, 1995; Niecke *et al*., 1999; Okazaki *et al*., 1985). This is due to the increased keratin thickness in melanised feathers compared to non-melanised feathers (Butler & Johnson, 2004). Therefore, feathers with limited calcium are less durable and less resistant to mechanical stress (Niecke *et al*., 1999; Pacyna *et al*., 2018). Due to the requirement of nutrient sequestration, melanin is costly to produce, making it an honest display of fitness (Griffith, Parker & Olson, 2006; Pacyna *et al*., 2018). This links to the finding that wider fault bars were associated with dull feathers with a low iridescence (chapter 3). This suggests that low iridescence may be another consequence of nutritional deficiency or stress on the body. Moreover, due to dull feathers acting as an honest display of low fitness to counterparts, individuals with a high number of fault bars/more severe fault bars may be less likely to be chosen for mating.

A potential lack of calcium in the diet of carrion crows leads to questions about the availability of this nutrient in their diet. Wild birds typically gain calcium from food sources such as eggs, snails and mineral deposits in the soil (Mänd, Tilgar & Leivits, 2000). However, a study by Scheuhammer (1991) found that acidification impacts the availability of calcium-rich prey e.g. snails due to leaching of minerals from the soil. Moreover, even in calcium-rich areas, obtaining sufficient levels of calcium can be challenging and time-consuming (Graveland, 1998; Mänd *et al*., 2000). A study by Holyoak (1968) on the gizzard contents of carrion crows revealed a low consumption level of snails and eggs year-round. This provides one explanation for this potential nutritional imbalance, as these food sources are very high in calcium. However, it should be noted that the individuals used in this study are not representative of the entire population as they were admitted to a rehabilitation centre for a range of reasons. Therefore, this study needs to be expanded to account for this.

5.6.2 Aim 2: Causes of fault bar production in relation to nutrient supply, sex, endoparasite burden and age

5.6.2.1 Feather chemical composition in reference to nutrient supply

Aim 1 of the study assessed the variation in chemical composition of feathers across fault bars of different severities. This particular study used the feather vane sections, whereas aim 2 used the feather shaft base. Aim 1 found significantly lower proportions of calcium in more severe fault bars. However, a low proportion of calcium in the shaft was not found in feathers with wider fault bars on average. This implies that the entire feather is not compromised by lack of nutrient supply, alternatively, the suggested calcium deficiency appears to occur in isolated sections of feather vane where fault bars are displayed. This result also shows that the feather shaft base cannot be used as a proxy for the chemical composition of damaged feathers. This is unfortunate as this method allows for the use of snapped feathers and does not require the acknowledgement of differing chemical composition across the feather length. Nevertheless, the extent of calcium deficiency appears to increase with fault bar severity which is interesting as it supports the connection between this element and growth deformity.

Results of this study aim also found wide fault bars to be associated with a high proportion of carbon in tail feathers. Due to the higher occurrence of heavy fault bars in the tail feathers, this is interesting as it suggests that carbon may be distributed to feathers that are more prone to breakage, supporting the ‘fault bar allocation hypothesis’ (Jovani & Blas, 2004). However, as stated previously, carbon is a basic component of keratin and all other organic molecules. Therefore, this element cannot be considered as an isolated individual molecule.

Reflecting on the potential formation theories of fault bars, it is interesting to consider the role of nutrient supply in the blood during feather growth. Failure of nutrient delivery was discussed by Riddle (1908) and was later followed up by Duerden (1909) who concluded that barbule malformation could also result from mechanical damage. These two conflicting theories were discussed in chapter 3, suggesting a combination of the two occurrences to be significant in the formation of fault bars. When the feather is growing from the papilla of the follicle, it has an active blood supply and is often referred to as a ‘blood feather’ (Bennett & Baumgartner, 2015). Therefore, nutrients are delivered to the growing feather structure through the blood (Lillie, 1940). The follicle grips the feather at the calamus by muscular contraction of the follicular muscle (Bennett & Baumgartner, 2015). This was suspected due to the strong layer of circular muscles in the wall next to the lining epidermis (Lillie, 1940). It has previously been suggested that the wall of the follicle may function by muscular contraction in regulating the amount of blood in the feather pulp (Lillie 1940). When reviewing the roles of both blood supply and follicular muscles, it could be suggested that stress during feather growth may result in abnormal muscular constriction around the follicle, leading to a restriction in blood supply to the growing feather. This may then dismiss the suggestion that calcium is limited in the diet. However, in order to understand the relationship between nutrient supply in the blood and muscle activity in relation to fault bar formation, further research is required.

5.6.2.2 Endoparasite burden

Individuals with a high number of endoparasites were expected to have wide fault bars. This was predicted due to the withdrawal of energy by parasites and the trigger of costly immune responses (Hudson *et al.*, 1998; Møller, 1997; Reed *et al.*, 2012; Sheldon & Verhulst, 1996). For example, low breeding plumage quality was found to be associated with the presence of cestodes in female bar-tailed godwits (*Limosa lapponica taymyrensis*) (Piersma *et al.*, 2001). Moreover, in house sparrows (*Passer domesticus*) there was a positive relationship between immune response and the number of fault bars on feathers (Møller *et al.*, 1996). Results did not support this hypothesis, finding low numbers of endoparasite species in individuals with wide fault bars in the wing feathers. This suggests that endoparasite burden is not a cause of fault bar production in carrion crows. This could be explained by the concept of fitness trade-offs, where costly immune defences may be facilitated at the cost of feather quality (Sheldon & Verhulst, 1996). However, it is important to note that there was a large variation in data, in addition to the majority of individuals having no parasites. Moreover, nestlings were excluded from this study, which are known to be particularly vulnerable to parasites due to their less efficient immune system and exposure to nest-dwelling parasites (Reed *et al.*, 2012; Ros *et al.*, 2002; Szép & Møller, 1999). Therefore, further research is required to fully understand if there is a relationship between feather quality and endoparasite burden, with the addition of nestlings and larger sample sizes.

5.6.2.3 Sex and Age

The causes of poor feather quality were not predicted to vary between sexes, as carrion crows are monomorphic and non-migratory (Dawson *et al.*, 2001; Slagsvold, 1982b). Results supported this hypothesis, finding no significant association between sex and the average width of fault bars across all feather types. In addition, due to their susceptibility to fault bar production, young individuals were expected to have feathers with the widest fault bars (Hawfield, 1986; Serrano & Jovani, 2005). Results supported this hypothesis finding younger individuals to have wider fault bars in their tail feathers than adults. Due to the previously established relationship between fault bar width and the number of fault bars, this implies that younger individuals had a higher number of fault bars in the tail feathers than older individuals. However, no such relationship was found in the wing feathers. This may be due to the large majority of fault bars occurring in the tail, highlighting this relationship. The results discussed here are supported by a number of other studies, finding lower number of fault bars in adult birds in comparison to young birds (Hawfield, 1986; Jovani & Blas, 2004; Serrano & Jovani, 2005; Slagsvold, 1982a). In relation to young individuals, a study by Machmer *et al.* (1992) found that the smallest osprey (*Pandion haliaetus*) nestling had the highest number of fault bars. Interestingly, this study also found that fault bar production occurred less as the nestlings became older (Machmer *et al.*, 1992). This is also supported by Jovani and Tella (2004), who found a significant decrease in fault bar production throughout the growth period of nestlings. They suggested that this could also be due to an increase in resilience to adverse weather conditions (Jovani & Tella, 2004). In addition, a study conducted on the upland buzzard (*Buteo hemilasius*), found that sibling competition was a greater source of stress than variations in relative nutritional condition (Yosef *et al.*, 2013). This suggests that the pressures of nestling conflicts are also a factor in their vulnerability to fault bar production.

5.7 Conclusion

The first aim of this study was to explore the chemical composition of feathers in different levels of damage. Results found that chemical composition varied with feather position, raising questions about the structural roles of these chemicals. For instance, high levels of carbon were found at the rigid feather base, in contrast to high levels of calcium and sulphur at the flexible feather tip. The second aim of this study was to determine the causes of fault bar production in relation to nutritional deficiency, endoparasite burden, sex and age. Results found low levels of calcium in more severe fault bars, providing new information on the potential link between nutrient supply and feather quality. This study also found a potential trade-off between costly immune defences facilitated at the cost of feather quality, where low numbers of endoparasite species associated with wide fault bars in the wing feathers. However, certain sample size biases must be acknowledged in this conclusion. The causes of poor feather quality were not predicted to vary between sexes, as carrion crows are monomorphic and non-migratory. Results supported this hypothesis, finding no significant association between sex and the average width of fault bars across all feather types. Lastly, in the tail feathers, results found younger individuals to have the widest fault bars. This supports many other studies in highlighting the vulnerability of juveniles during the feather growth period. In order to further contributing factors of poor feather condition, the subsequent chapter used the variables outlined in the second study aim to identify the consequences of this occurrence.

Chapter 6: The consequences of poor feather condition (strength) in relation to the chemical profile of feathers, endoparasite burden, sex and age (Aim 3)

6.1 Introduction

Flight is one of most complex modes of locomotion, aiding foraging, predator avoidance and large-scale movements in most avian populations (Sullivan *et al*., 2017; Videler, 2007). Feathers must withstand large amounts of stress from air pressure during flight, requiring a crucial balance between stiffness and flexibility to avoid breakage (Bachmann *et al*., 2012; DesRochers *et al.,* 2009). Consequently, the term ‘stress tolerance’ will be used to describe the strength of feathers in the coming results (Bachmann *et al*., 2012; DesRochers *et al.,* 2009). A high tolerance to stress is associated with higher flexibility/stiffness. In this, flexible feathers generate less lift and bend when pressure becomes too strong (Sullivan *et al*., 2017). In contrast, materials that have low tolerance to stress are stiffer. Stiffer feathers are important to generate lift; however, when pressure becomes too strong, they break (Zhao *et al.,* 2020). This is an important factor to consider in relation to how feathers are attached to the wing. In this, primary feathers are rigidly attached to the wing bone, allowing them no freedom of movement (Sullivan *et al*., 2017; Pennycuick, 2008). In contrast, secondary feathers have a flexible attachment to the ulna, having the ability to hinge up and down (Sullivan *et al*., 2017; Pennycuick, 2008). This, therefore, impacts the feather’s ability to withstand air pressure in accordance to the position on the wing.

6.1.1 Feather strength as a proxy for feather quality

In Chapter 4, the lack of relationships seen between feather strength and other feather quality measures such as fault bar severity, iridescence and the occurrence of snapped and white feathers indicated that feather strength offers different information about feather quality than the other included measures. Strength in terms of rachis stiffness has been used as a proxy for feather quality in a variety of previous studies (Dawson *et al*., 2000; DesRochers *et al.,* 2009; Pap *et al.,* 2013). Therefore, feather shaft/rachis strength is investigated in this chapter to investigate the consequences of poor feather condition in relation to a range of factors: chemical profile of feathers, endoparasite burden, sex and age.

Feather quality often reflects fitness and is an honest signal to conspecifics (Griffith, Parker & Olson, 2006; McGraw, 2003; Pacyna *et al*., 2018) as high feather quality is costly to produce.

For example, it requires sequestration of certain minerals acquired through the diet to stimulate the production of colouration pigments (Griffith, Parker & Olson, 2006; McGraw, 2003; Pacyna *et al*., 2018). Specifically, a study on white-tailed eagles *Haliaeetus albicilla* and barn owls *Tyto alba* found a high concentration of calcium and zinc in the black, melanin-pigmented feathers compared to unpigmented white feathers (Neicke, Heide & Kruger, 1999). Melanic feather keratin is more resistant to stress than non-melanic keratin, owing to its increased thickness (Bonser, 1995). Therefore, due to calcium playing a role in the aggregation of melanin, this finding suggests that calcium is involved in feather stability (Bonser, 1995; Niecke *et al*., 1999; Okazaki *et al*., 1985). Similarly, Chapter 5 of this thesis found higher concentrations of calcium at the feather tip, potentially stabilising this highly flexible part of the feather (Butler & Johnson, 2004; Sullivan *et al*., 2017). Moreover, fault bars had a lower calcium concentration than areas without fault bars. In light of this, the consequences of poor feather condition in terms of feather strength will be assessed in relation to the chemical composition of feathers. This aims to identify if there is a connection between poor feather condition (low feather strength) and nutrient supply. For example, does chemical composition weaken specific regions of the feather (e.g. those with fault bars), as fault bars have been found to result in feather breakage at the site of occurrence (Dawson *et al*., 2001; Kose *et al*., 1999)? This will be discussed in relation to the chapter 3, where evidence of feather shaft damage was provided at the site of fault bars (see section 3.3.3). Alternatively, if these feather sections are not found to be structurally weaker, this leads to queries around the overall weakness of the feather. In addition, it could also be speculated that the presence of fault bars may still have negative effects on flight performance due to 'holes' that may reduce lift. Previous research has outlined evidence for the locomotion costs of poor feather quality (Swaddle *et al*. 1996; Chai 1997; Swaddle and Witter 1997; Chai and Dudley 1999; Lind 2001; Lind and Jakobsson 2001). For instance, studies on feather holes found impairments in the ability of the wing to maintain pressure differences between the air above and below the wing (Matyjasiak *et al.*, 2018; Videler 2005). This can, therefore, be detrimental to flight performance, as this is necessary for the generation of aerodynamic forces through wings (Matyjasiak *et al.*, 2018; Videler 2005).

As previously mentioned, references to feather strength in relation to parasite burden can be seen in previous research (Pap *et al.,* 2013), leading to the inclusion of this factor in this study. Results of chapter 4 found younger individuals to have wider fault bars in the tail feathers than adults. Higher numbers of fault bars in young birds in comparison to adult birds have been found in a range of other species (Hawfield, 1986; Jovani & Blas, 2004; Serrano & Jovani, 2005; Slagsvold, 1982a). This highlights the vulnerability of juveniles during the feather growth period. Therefore, consequences of poor feather condition were assessed in relation to age. The causes of poor feather quality were not affected by sex. Likewise, sex was not expected to affect the strength of feathers as carrion crows are monomorphic, of similar size and resident throughout the year (Dawson *et al*., 2001; Slagsvold, 1982b). However, sex was considered in the strength analysis for comparability with the other analyses.

6.1.2 Study aims and hypotheses

Previous studies on the flexural stiffness of feathers found measurements to vary significantly across a range of factors e.g. along the length of the feather, between feathers of different positions and between species investigated (Bachmann *et al.,* 2012; Bonser & Purslow, 1995). Therefore, some of these factors were first assessed in this study to establish a baseline understanding of variations in feather strength within the study samples. In addition, fault bars are a common feature in feathers of poor feather quality, with unknown consequences on feather strength. This was therefore explored in this study in reference to impacts on flight efficiency. Lastly, feather strength was assessed in relation to nutrient supply, endoparasite burden, sex and age.

6.1.2.1 Aim 1: To investigate differences in feather strength along the rachis and in relation to feather type and fault bar occurrence

Hypothesis 1: Feathers were expected to be harder/less tolerant of stress at the feather base, becoming gradually more flexible and resistant to stress towards the distal end of the feather (Butler & Johnson, 2004; Sullivan *et al*., 2017).

Hypothesis 2: Feather strength was expected to reflect the differences in function between feather types (Ginn & Melville, 1983; Videler, 2007). Tail feathers were significantly stronger than primary and secondary feathers (see chapter 4) and predicted to be less tolerant of stress. As primary feathers are larger and further away from the body, they have a higher exposure to wing forces and damage (Ginn & Melville, 1983; Videler, 2007), requiring stiffer feathers. Unlike secondaries, they also have a rigid attachment to the wing bone, making them more susceptible to breakage (Sullivan *et al.*, 2017; Pennycuick, 2008). Therefore, primary feathers were expected to be more stress tolerant than secondary flight feathers.

Hypothesis 3: In Chapter 5, lower proportions of calcium were found in feather vane sections with heavier fault bars. Due to calcium playing a role in feather stability by influencing the durability and resistance to mechanical stress (Bonser, 1995; Niecke *et al.*, 1999; Okazaki *et al.*, 1985; Pacyna *et al.*, 2018), feather shaft sections with the presence of fault bars were expected be less resistant to stress. Moreover, this was predicted due to the current understanding that fault bars often lead to feather breakage due to a lack of keratin (Dawson *et al.*, 2001; Kose *et al.*, 1999).

6.1.2.2 Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

Hypothesis 4: Due to the discussion of nutrient supply in the role of feather quality (Bonser, 1995; Niecke *et al.*, 1999; Okazaki *et al.*, 1985; Riddle, 1908;), it was expected that feathers with stress tolerant shaft bases have a different chemical composition than feathers with less stress tolerance.

Hypothesis 5: In line with results of chapter 5 (fewer endoparasite species = more fault bars in the wing feathers), a trade-off between feather quality and endoparasite burden was predicted. Therefore, due to the trigger of costly immune responses caused by endoparasites (cestodes, flukes and acanthocephalan), individuals with low numbers of endoparasite species were expected to have feathers with lower strength/stress resistance (Freed *et al.*, 2005; Jovani *et al.*, 2014; Møller *et al.*, 1996).

Hypothesis 6: Due to their susceptibility to fault bar production, young individuals were expected to have feathers with the lowest base strength (Hawfield, 1986; Serrano & Jovani, 2005). Due to carrion crows being monomorphic and non-migratory, feather base strength was not predicted to vary between sexes (Dawson *et al.*, 2001; Slagsvold, 1982b).

6.2 Study Methodology

6.2.1 Feather strength method

Feather strength measurements were gained using a three-point flexural test, providing information on their ultimate flexural load, which is the maximum stress that the sample can tolerate without permanent damage (Jayakrishna, Rajiyalakshmi & Deepa, 2018). Please refer to chapter 2 for methodology details (section 2.2.2.5). This type of measurement aimed to provide information on variations in feather robustness, as it is important for feathers to be strong and flexible to avoid breakage during the different levels of air pressure at each stage of flight (Corning & Biewener, 1998). This variable was not found to correlate significantly with any other variable in chapter 4, offering different information on feather quality. Feather strength was found to differ across all feather types (primary, secondary and tail) in chapter 4, resulting in the use of separate measurements for each feather type. Three feathers for each feather type were selected for measurement (see section 2.2.2.1; Fig 2.4). Aims 1 and 2 use different measurement points, to be described below.

6.2.2 Study method Aim 1: To investigate differences in feather strength

Using 38 individuals, three feathers each were selected from the following feather groups: primary wing feathers, secondary wing feathers and tail feathers. This consisted of primaries 1, 3 and 5, secondaries 1, 3 and 5, in addition to three randomly selected tail feathers (see section 2.2.2.1 for full details). In each individual feather, feather strength was recorded at 2cm intervals across the feather length, starting from the feather base/superior umbilicus (please see section 2.2.4 for more information; Fig.2.14). Presence/absence of a fault bar was recorded at each of these measurement points. Four variables were gained for this study: feather strength (N/mm^2) as the dependent variable, feather position (primaries 1, 3 and 5, secondaries 1, 3 and 5, tail 1, 2 and 3), distance from the feather base (cm) and presence of fault bar (absent/present) as independent variables.

6.2.3 Study method Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

For this analysis only one feather of each feather type was selected to reduce data collection time – primary 3, secondary 3 and tail feather 3. Feathers came from the same 38 individuals as in aim 1. Feather strength measurements were recorded only at the base/superior umbilicus of each feather for this study aim. This was conducted in order to allow for the use of broken feathers.

Feather strength was tested against independent variables. To explore nutrient supply in feathers, the chemical composition of feather samples (P3, S3 and T3 shaft bases) was gained using Scanning Electron Microscope Energy-Dispersive X-ray spectroscopy (SEM-EDS) (please refer to section 2.2.3.2.1 for more details). Chemical elements measured were as follows - Carbon C, Calcium Ca, Chlorine Cl, Oxygen O and Sulphur S. Endoparasite burden was measured using the number of endoparasite species found in the gut and trachea of each bird (see section 2.2.3.2.2; Fig.2.12). Age was categorised with reference to inner mouth colouration and descriptions of feather characteristics, using three age categories (first year juvenile, second year juvenile and adult; Svensson, 1992) (section 2.2.3.2.3; Tab.2.2; Fig.2.13). It is important to note that nestlings were not included in this study due to the presence of pin feathers, which were unfeasible for feather measurements. Sex (female/male) was identified during post-mortem, identifying urogenital structures for each sex (section 2.2.3.2.3; Fig.2.14; Panto, 2017).

6.2.4 Statistical Methodology

6.2.4.1 Aim 1: To investigate differences in feather strength (N=38)

6.2.4.1.1 Across the length of the feather

The dependant variable for this study was feather strength (N/mm^2). This variable was found to be non-normal, therefore, it was transformed using Log10. The independent variable was distance from the feather base (cm). A linear regression was then conducted between feather strength and feather position, saving the standardised residuals for the subsequent analyses. The standardised residuals were gained to provide positive and negative deviation of strength from the average, considering the location of each sample on the feather (Field, 2013). Outliers were excluded using the identification of high z-scores (van den Berg, 2022). In this, $|z| \geq 3.29$ indicates an outlier (van den Berg, 2022). When outliers were removed for analysis, this was clearly stated in the relevant result sections.

6.2.4.1.2. Between different feathers

Feather base strength measurements (0cm) were used for this study to maintain consistency and account for broken feathers. Due to non-parametric data, Friedman testing was used to determine differences in strength between individual feathers (P1, P3, P5, S1, S3, S5, T1, T2, T3). Wilcoxon signed-rank test was then used to further analyse these differences (Field, 2013).

6.2.4.1.3 Feather regions with and without fault bar occurrence:

Comparisons between regions with and without fault bars were tested in feathers using the previously gained residuals (section 6.2.4.1.1) to account for feather position. Results of the previous section (6.2.4.1.2) were used to group feathers of comparable strength, to increase the sample size of fault bar occurrence locations. In this, feathers with non-significant differences in strength were combined. Due to non-parametric data, a Wilcoxon test was used to test for differences in feather strength in relation to fault bar presence.

6.2.4.2 Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

This study used measurements of strength from the bases of 3 feathers (P3, S3 and T3) across 38 individuals, resulting in the following dependant variables for this study: feather base strength of primary, secondary and tail feathers. These variables were used to determine the relationship between feather strength and nutrient supply, endoparasite burden, sex and age. Shapiro-Wilk testing identified all dependant variables to be non-parametric; therefore, data was transformed using Log10. Linear regressions were conducted in steps to account for sample size differences in the independent variables. For instance, the sample size for chemical data was n=38, in comparison to parasite burden (n=30), sex (n=33) and age (n=22). Therefore, the first linear regression excluded age to take advantage of the larger sample size available in the other variables (n=30). The second linear regression incorporated age in addition to any variables that had a significant effect in the first analysis (n=22). Stepwise method was chosen for this analysis, to evaluate the order of importance of variables and select useful subsets of variables (Huberty, 1989; Lewis, 2007; Thompson, 1995). This method develops a sequence of linear models that, at each step, considers the removal of each entered predictor (Snyder, 1991; Lewis, 2007; Thompson, 1989). Stepping method criteria threshold was increased to p=0.099 to capture a wider range of variables.

6.3 Results

6.3.1 Aim 1: To investigate differences in feather strength

6.3.1.1 Across the length of the feather

A linear regression was conducted for across all sampled feather in order to determine how feather strength changes across the length of the feather. A highly significant, positive relationship was found between feather strength and distance from the feather base in all feathers, explaining 29.5% of the variance (F1,2127=888.486, p<0.001; Fig.6.1). This shows that stress tolerance increases towards the tip of the feather in all tested feathers.

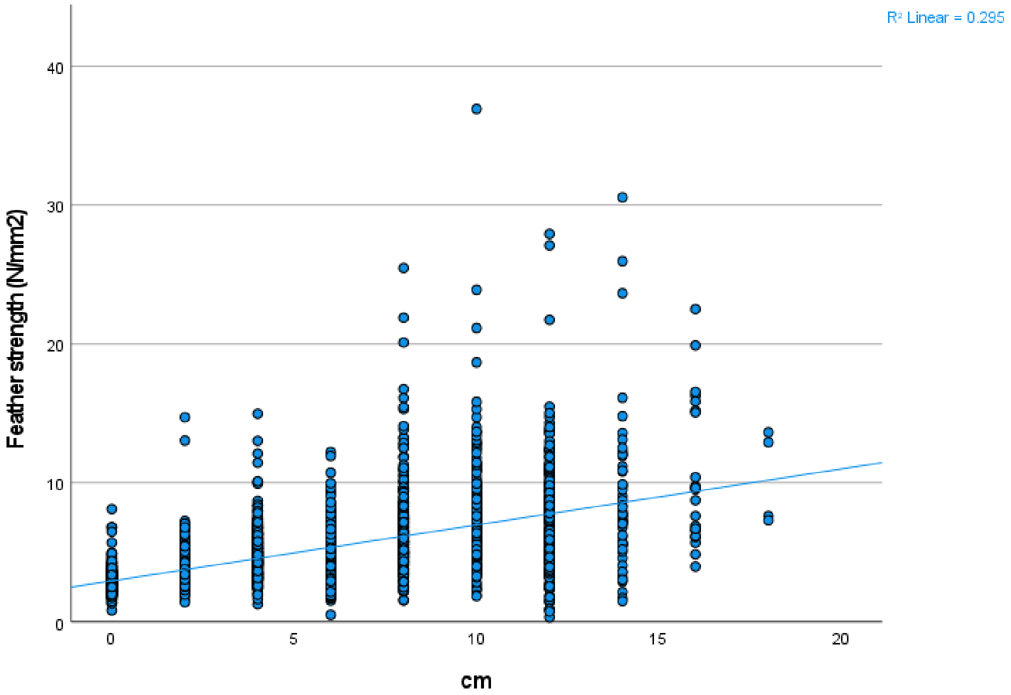


Fig.6.1 Feather strength (N/mm^2) in relation to position across the length of the feather (cm) (n=2128).

6.3.1.2 To investigate differences in feather strength between different feathers

Friedman testing found a significant difference in the feather shaft base strength across different feather types ($\chi^2(8)=52.086$, p=<0.001). Wilcoxon signed-rank testing was then conducted to determine these differences. Primary 5 (P5) was found to be the most stress tolerant in comparison to all other wing feathers (Tab.6.3; Fig.6.4). P5 is the largest wing feather to be tested and is located on the most distal part of the wing. P5 was found to be of comparable strength to the tail feathers.

Aside from P5, all other wing feathers were found to be of comparable strength, with one exception. P3 had a significantly higher stress tolerance than secondary 1 (S1). Tail feathers were found to have statistically equivalent strengths. Tail feathers had significantly higher stress tolerance than all wing feathers except P5 (stated above) and S5. S5 was found to be of comparable strength to tail feathers 1 and 2 (T1 and T2).

Tab.6.1 Wilcoxon signed rank results for differences in strength between feathers (N=38 individuals). Flight feathers are ordered in accordance with position on the wing (P5 most distal to S5 most proximal). Test statistic Z unemboldened with P value emboldened. Significant values highlighted in yellow.

	P5	**P3**	**P1**	**S1**	**S3**	**S5**	**T1**	**T2**	**T3**
P5		-3.069	-4.783	-4.933c	-3.103	-2.451	-0.346	-0.503	-0.943
		0.002	**<0.001**	**<0.001**	**0.001**	**0.013**	**0.739**	**0.625**	**0.354**
P3			-0.915	-2.231	-1.547	-0.487	-2.152	-2.137	-2.922
			0.370	**0.025**	**0.125**	**0.636**	**0.031**	**0.032**	**0.003**
P1				-0.880	-0.026	-1.807	-3.205	-3.378	-3.802
				0.388	**0.987**	**0.072**	**0.001**	**<0.001**	**<0.001**
S1					-1.021	-2.429	-3.879	-4.097	-4.387
					0.315	**0.014**	**<0.001**	**<0.001**	**<0.001**
S3						-1.068	-2.435	-2.671	-3.551
						0.293	**0.014**	**0.007**	**<0.001**
S5							-1.907	-1.501	-2.908
							0.057	**0.136**	**0.003**
T1								-0.109	-0.109
								0.920	**0.920**
T2									-0.761
									0.455
T3									
P = Primary, S = Secondary, T = Tail.									

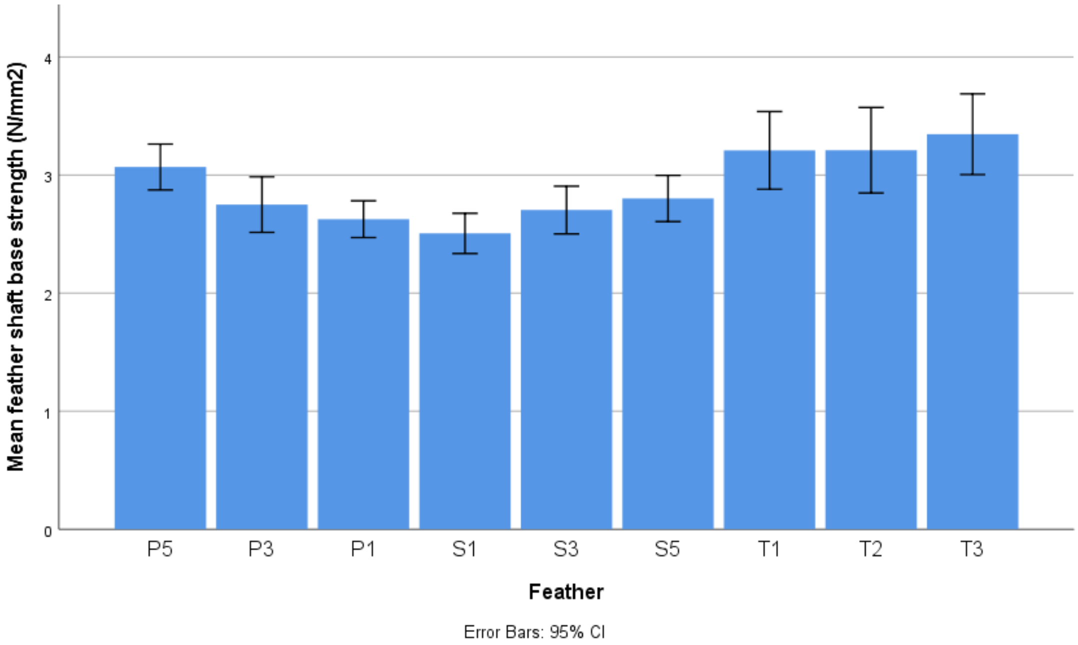


Fig.6.2 Mean feather strength (N/mm^2) in relation to feather: P=Primary, S=Secondary, T=Tail (N=38 individuals). Flight feathers are ordered in accordance with position on the wing (P5 most distal to S5 most proximal). Low strength values represent low stress tolerance, whereas high strength values represent high stress tolerance.

6.3.1.3 To investigate differences in feather strength in feather regions with and without fault bar occurrence

Wilcoxon testing then conducted to test for differences in strength between fault bar presence and absence within the same feather. Due to the results of section 6.3.1.2, feathers were grouped into two tests according to their comparable strength. Test 1 consisted of P1, S1, S3 and S5. P3 was excluded from analysis due to the absence of fault bars in all feathers, in addition to its significant difference in strength to S3. Test 2 consisted of P5, T1, T2, and T3. Results found no significant difference in strength in relation to presence and absence of fault bars in both tests (Test 1: Z=-1.543, p=0.125; Test 2: Z=-0.168, p=0.877).

6.3.2 Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

6.3.2.1 Primary feathers

Stepwise linear regression analysis was conducted to assess the relationship between primary feather base strength (N/mm^2) and the following factors: chemical composition, endoparasite burden and sex (n=30). Proportion of chlorine was retained in the model, explaining 14.8% of the variance ($F_{1,26}$=4.522, p=0.043; Tab.6.2). No other variables were included in the model (see Tab.6.4 for outputs). Fig.6.3 shows primary feather bases with the highest proportion of chlorine to be significantly less tolerant of stress.

Tab.6.2 Linear regression output for excluded variables, assessing the relationship between average feather base strength (N/mm^2) in primary feathers and the following factors: chemical composition (proportion of carbon, calcium, chlorine, oxygen and sulphur), endoparasite burden (number of parasite species) and sex (n=30).

	t	Sig.
Carbon	-0.360	0.722
Calcium	-0.491	0.628
Oxygen	0.661	0.514
Sulphur	-0.922	0.365
Parasites	0.831	0.414
Sex	1.490	0.149

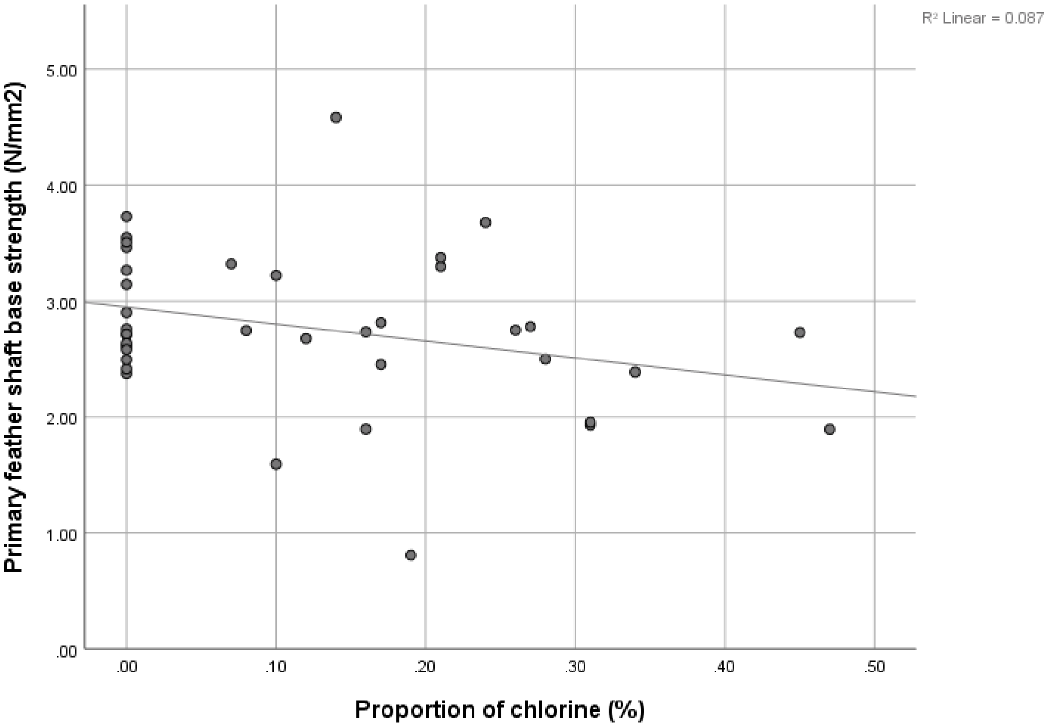


Fig.6.3 Primary feather base strength (N/mm^2) in relation to proportion of chlorine in feathers (N=30). Low strength values represent more rigid feathers, whereas high strength values represent flexible feathers.

A second stepwise linear regression analysis was conducted to assess the relationship between primary feather base strength (N/mm^2) and the following factors: proportion of chlorine and age (n=22). There was a strong trend for age to be related to feather strength explaining 17.5% of the variance ($F_{1,19}$=4.035, p=0.059). Primary feathers of adult birds tended to be more resistant to stress than juveniles. Proportion of chlorine was excluded from the model due to non-significance (t=-0.663, p=0.516).

6.3.2.3 Secondary feathers

Stepwise linear regression analysis was conducted to assess the relationship between average base strength (N/mm^2) of secondary feathers and the following factors: chemical composition, endoparasite burden and sex (n=30). There was a trend for a relationship between the proportion of carbon and feather strength, explaining 12.4% of the variance ($F_{1,26}$=3.678, p=0.066; Tab.6.3). Primary feathers with higher stress tolerance tended to have a higher proportion of carbon. No other variables were retained in the model (see Tab.6.3 for outputs).

Tab.6.3 Linear regression output for excluded variables, assessing the relationship between average feather base strength (N/mm^2) in secondary feathers and the following factors: chemical composition (proportion of calcium, chlorine, oxygen and sulphur), endoparasite burden (number of parasite species) and sex (n=30).

	t	**Sig.**
Calcium	0.924	0.364
Chlorine	-0.224	0.825
Oxygen	0.292	0.773
Sulphur	-0.648	0.523
Parasites	1.453	0.159
Sex	1.603	0.122

A second stepwise linear regression analysis was conducted to assess the relationship between average base strength (N/mm^2) of secondary feathers and age (n=22). There was a trend for a relationship between age and feather strength explaining 14.6% of the variance ($F_{1,19}$=3.239, p=0.088). As with primary feathers, secondary feathers of adult birds tended to be more resistant to stress than juveniles.

6.3.2.4 Tail feathers

Linear regression analysis with stepwise method was conducted to assess the relationship between average base strength (N/mm^2) of tail feathers and the following factors: chemical composition, endoparasite burden and sex (n=30). No variables had a significant effect ($F_{7.20}$=1.144, p=0.376; see Tab.6.4 for outputs).

Tab.6.4 Linear regression output assessing the relationship between average feather base strength (N/mm^2) in tail feathers and the following factors: chemical composition (proportion of carbon, calcium, chlorine, oxygen and sulphur), endoparasite burden (number of parasite species) and sex (n=30).

	t	**Sig.**
(Constant)	-1.680	0.109
Carbon	1.633	0.118
Calcium	0.446	0.660
Chlorine	-0.994	0.332
Oxygen	1.858	0.078
T_S	1.432	0.168
Parasites	1.415	0.173
Sex	-1.549	0.137

A second linear regression analysis was conducted to assess the relationship between average base strength (N/mm^2) of tail feathers and age (n=22). No significant effect was found ($F_{1,19}$=1.070, p=0.314).

6.4 Summary of results

6.4.1 Aim 1: To investigate differences in feather strength

- In all feather types, feathers were more tolerant of stress towards the tip of the feather (more flexible).
- P5 was found to be the most stress tolerant wing feather, positioned towards the outermost part of the wing (most distal). P5 was found to be of comparable strength to the tail feathers.
- Aside from P5, all other wing feathers were found to be of comparable strength, with one exception. P3 was found be more stress tolerant than S1.

- Tail feathers were found to have statistically equivalent strengths. Tail feathers were more stress tolerant than the wing feathers except P5 (stated above) and S5. S5 was found to be of comparable strength to T1 and T2.
- Feather regions with fault bar occurrence did not differ in strength to those with fault bar absence.

6.4.2 Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

- Primary feather bases with the lowest proportion of chlorine were significantly more tolerant of stress. In addition, primary feathers of adult birds tended to be more resistant to stress than juveniles.
- Secondary feather base strength was not significantly associated with any independent variables. However, secondary feathers with higher stress tolerance tended to have a higher proportion of carbon. In addition, secondary feathers of adult birds tended to be more resistant to stress than juveniles.
- Tail feather base strength was not significantly associated with any independent variables.

6.5 Discussion

6.5.1 Aim 1: To investigate differences in feather strength

The first section of this study investigated differences in feather strength across the length of the feather. Feathers were expected to be less tolerant of stress (harder) at the feather base, becoming gradually more resistant to stress (flexible) towards the distal end of the feather. Results confirmed this, supported by current literature (Butler & Johnson, 2004; Sullivan *et al.*, 2017). This reiterates the importance of this structural change, with the flexible feather tip reducing pressure at the feather base by bending under air pressure.

Differences in feather base strength between individual feathers were then investigated. In this study, feather strength was expected to reflect the differences in function between feather types (Ginn & Melville, 1983; Videler, 2007). Results identified primary feather 5 (P5) to have a significantly higher stress tolerance at the base than the other flight feathers, positioned towards the outermost part of the wing. This is due to distal primary feathers being subjected to higher aerodynamic forces during the flapping flight wing stroke; therefore, they are required to be more stress tolerant to avoid damage (Ennos, Hickson & Roberts, 1995; Pap *et al.,* 2019). Higher stress tolerance/flexibility is also associated with greater lift production in flapping flight (Gopalakrishnan & Tafti, 2010). P5 was also found to have comparable strength to the tail feathers. This is due to the requirement of tail feathers to be tolerant of stress to avoid damage from contact with the ground (Fitzpatrick & Price, 1997; Thomas, 1997; Tubaro, 2003).

Aside from P5, all wing feathers were found to be of statistically comparable strength, with the exception of P3 and S1. In this P3 was more stress tolerant than S1. This could be explained by the fact that primary feathers are rigidly attached to the wing bone, allowing them no freedom of movement (Sullivan *et al*., 2017; Pennycuick, 2008). In contrast, secondary feathers have a flexible attachment to the ulna, having the ability to hinge up and down (Sullivan *et al*., 2017; Pennycuick, 2008). Primary feathers spread out when the elbow and wrist joint are fully extended, whereas the secondaries are pulled downward by the tightened patagial tendon to increase the camber of the wing (Sullivan *et al*., 2017; Pennycuick, 2008). Therefore, due to the restricted movement of P3, this feather is required to be more tolerant of stress to avoid breakage.

Differences in feather strength in feather regions with and without fault bar occurrence were then investigated. In this, fault bars were expected to weaken the location of feather where present. However, results did not support this hypothesis, finding no difference in strength between feather regions with fault bar occurrence and those with fault bar absence. This is interesting to discuss in relation to the findings of Chapter 3, where evidence of feather shaft damage was provided at the site of fault bars. Due to the feather sections with fault bar occurrence not being found to be structurally weaker, this implies that fault bars do not affect strength. This contrasts with earlier findings that lower calcium levels were also found in the sections of feather impacted by the fault bar in comparison to areas with fault bar absence. This is interesting as a low proportion of calcium at fault bars would be expected to correspond to lower stress tolerance. Due to the absence of this outcome, it could be speculated that the presence of fault bars may still have negative effects on flight performance due to ‘holes’ that may reduce lift. This relates to studies conducted on feather holes, where impairments were found in the ability of the wing to maintain pressure differences between the air above and below the wing (Matyjasiak *et al.*, 2018; Videler 2005). This can, therefore, be detrimental to flight performance, as this is necessary for the generation of aerodynamic forces through the wings (Matyjasiak *et al.*, 2018; Videler 2005). Similarities between fault bars and feather holes therefore lead to questions around the stability of air pressure maintenance due to fault bar occurrence, which may consequently lead to feather breakage. In order to clarify the relationship between fault bar occurrence and flight efficiency, further studies are required. Wind tunnel flight tests are recommended here, testing differences in air pressure across wings with and without fault bars.

6.5.2 Aim 2: To investigate feather strength in the shaft in relation to nutrient supply, endoparasite burden, sex and age

6.5.2.1 Feather strength in reference to nutrient supply

Due to the discussion of nutrient supply in the role of feather quality (Bonser, 1995; Niecke *et al*., 1999; Okazaki *et al*., 1985; Riddle, 1908;), it was expected that feathers with stress tolerant shaft bases have a different chemical composition than feathers with less stress tolerance. This hypothesis was partly supported, as stress tolerant feather bases were found to have significantly lower levels of chlorine in primary feathers. Little is known about the role of chlorine in feathers; however, this result may suggest that this element plays a structural role in feathers. Alternatively, low levels of chlorine in feathers may reflect other fitness factors within the individual. For example, a study by Lavers and Bond (2016) found increased levels of chlorine in feathers of individuals with high levels of ingested plastic, along with increased concentrations of lead, iron and rubidium. Due to the lack of knowledge regarding the role of chlorine in feathers, further research is required to understand its relevance to feather quality.

In addition, secondary feathers with the highest proportion of carbon tended to withstand a higher amount of stress. From results in chapter 5, it was suggested that carbon plays a structural role in thicker, more rigid sections of the feather. It was suggested by Bortolotti (2010) that some of the most commonly used isotopes in ecology, such as carbon, are important to feather physiology and are mass dependent. In light of this, high levels of carbon in secondary feather bases suggests that this element plays an important role in the stress tolerance of these wing feathers. It could be suggested that they are required to be more tolerant of stress to avoid breakage when providing lift (Ginn & Melville, 1983; Videler, 2007). This implies that carbon adds flexibility. This is interesting in relation to the physics of carbon tripods in engineering. In this, provide excellent strength in the vertical direction, being able to carry a high load at comparable low weight (Katagiri *et al*., 2021; Khani *et al*., 2017; Lopes, Gürdal & Camanho, 2010). However, they are relatively susceptible to breakage in the horizontal direction (e.g. bending the tripod leg) (Katagiri *et al*., 2021; Khani *et al*., 2017; Lopes *et al*., 2010). However, it should be again noted that due to carbon being a basic component of keratin and all other organic molecules, this element cannot be considered as an isolated individual molecule.

6.5.2.2 Feather strength in reference to endoparasite burden

In line with results of chapter 5 (fewer endoparasite species = more fault bars in the wing feathers), a trade-off between feather quality and endoparasite burden was predicted. Therefore, due to the trigger of costly immune responses caused by endoparasites (cestodes, flukes and acanthocephalan), individuals with low numbers of endoparasite species were expected to have feathers with lower strength/stress resistance (Freed *et al*., 2005; Jovani *et al*., 2014; Møller *et al*., 1996). Results did not support this hypothesis, finding no effect of endoparasite burden on feather strength.

6.5.2.3 Feather strength in reference to sex and age

Due to carrion crows being monomorphic and non-migratory, feather base strength was not predicted to vary between sexes (Dawson *et al*., 2001; Slagsvold, 1982b). Results supported this hypothesis, finding no significant association between sex and the average strength of feather bases in all feather types. In addition, due to their susceptibility poor feather condition, young individuals were expected to have the weakest feathers (Hawfield, 1986; Serrano & Jovani, 2005). Results found a trend in the wing feathers, with adults tending to have more stress tolerant feathers than first and second year juveniles. Due to flexible wings being associated with greater lift production in flapping flight, it could be suggested that younger carrion crows are negatively impacted by having feathers with lower stress tolerance (Gopalakrishnan & Tafti, 2010).

6.6 Conclusion

Study aim 1 looked at differences in feather strength across the length of the feather, confirming the hypothesis that feathers are less tolerant of stress (harder) at the feather base, becoming gradually more flexible and resistant to stress towards the distal end of the feather (Butler & Johnson, 2004; Sullivan *et al*., 2017). Differences in feather strength between individual feathers were then assessed, finding strength measured at the feather base to reflect differences position on the wing. Contrary to predictions, no difference in strength were found between feather regions with fault bar occurrence and those with fault bar absence. Aim 2 then determined the consequences of feather strength at the feather base in relation to nutrient supply, endoparasite burden, sex and age. Results found stress resistant bases to be associated with a high proportion of chlorine. In addition, stress resistant secondary feather bases were associated with a high proportion of carbon in secondary feathers. Links to parasite burden and sex were not identified; however, in line with the above findings, younger individuals were found to have low stress tolerance in the primary feathers compared to adults. This supports many other studies in highlighting the vulnerability of juveniles during the feather growth period.

Chapter 7: General discussion and recommendations for future research

7.1 Study overview

Poor feather quality, particularly in reference to fault bars, has been reported in many species (Murphy *et al*., 1989; Machmer *et al*., 1992; Bortolotti *et al*., 2002; Jovani & Blas, 2004; Serrano & Jovani, 2005&2006; Sarasola & Jovani, 2006; Pap et al., 2007; Murphy *et al*., 1988; Møller *et al.*, 2009; Strochlic & Romero, 2008; Jovani, Montavole & Sabate, 2014), warranting further investigation. Some of these species were listed in Chapter 4, Tab.4.1, spanning across both passerine and non-passerine species. Nonetheless, little is known about the causes and consequences of fault bar production, which was most recently reviewed by Jovani and Rowher (2016). This particular study focused on carrion crows, as a considerable proportion of admissions to RSPCA Stapeley Grange Wildlife Centre are euthanised due to the presence of poor quality feathers (RSPCA, 2013). The range of feather defects currently described vary across species and individuals; however, most are recognised as 'fault bars'. This occurrence is characterised by translucent bands that appear through the width of the feather vane, caused by a malformation of barbules (Erritzøe, 2006; Jovani & Rohwer, 2016). The structural damage to the feather is formed during feather growth, allowing no further changes to its structure once the feather has fully grown (Jaspers *et al*., 2004). The current study first investigated the visual and microscopic morphological features of fault bars before exploring potential causes and consequences of this growth defect.

7.1.1 Fault bar formation theories

7.1.1.1 Nutrient supply hypothesis

There are two existing hypotheses about potential fault bar production mechanisms. The first of which was outlined by Riddle (1908). In this, a lack of nutrient supply to the growing feather at specific points of formation was described (Riddle, 1908). In order to assess the role of nutrient supply in this study, the chemical composition of feathers was first explored in relation to distribution within feathers and potential functions. Location on the feather was an important measure in this, as studies have shown that the chemical composition of feathers is non-uniformly distributed throughout the length of the feather (Howell *et al*., 2017). This identified variations in the proportion of carbon, calcium and sulphur across the length of the feather. In this, a higher proportion of carbon was found at the feather base, with higher proportions of calcium and sulphur found towards the tip. This implied that carbon plays a structural role in thicker, more rigid section of the feather, in contrast to calcium and sulphur which may be more prominent in delicate, flexible parts of the feather.

When looking at the role of chemicals in relation to the consequences of poor feather quality, a trend in results found secondary feathers with higher stress tolerance (i.e lower likelihood of breaking) to have a higher proportion of carbon. This, again, reiterates the potential importance of carbon in the structural integrity of feathers. In contrast, chapter 5 found wide fault bars in the tail to be associated with a high proportion of carbon. If carbon were to aid in maintaining the integrity of the feather structure, this may occur to avoid damage in areas of severe fault bars. However, due to carbon being a basic component of keratin and all other organic molecules, this element cannot be considered as an isolated individual molecule. Therefore, it is challenging to apply the 'fault bar allocation hypothesis' principles to this finding.

Moreover, primary feather bases with the lowest proportion of chlorine were significantly less tolerant of stress. Little is known about the role of chlorine in feathers; however, this result may suggest that this element also plays a structural role in feathers. Alternatively, low levels of chlorine in feathers may reflect other fitness factors within the individual. For example, a study by Lavers and Bond (2016) found increased levels of chlorine in feathers of individuals with high levels of ingested plastic, along with increased concentrations of lead, iron and rubidium. Due to the lack of knowledge regarding the role of these chemical elements, further research is required to understand their relevance to feather quality.

Findings in chapter 5 found lower proportions of calcium in heavier fault bars in the wing feathers. This implies that feather malformation may be linked to a nutritional deficiency, much like those seen in human nails (Cashman & Sloan, 2010). This suggests a relationship between nutrient allocation during feather growth leading to detrimental structural damage in times of stress. This idea will be discussed further in section 7.1.2.1. An important link to Riddle's (1908) nutrient supply hypothesis is a more recent concept outlined by Jovani & Blas (2004). In this, the 'fault bar allocation hypothesis' was described, stating that fault bars occur on feathers that are least important for flight (Jovani & Blas, 2004). This is an evolutionary adaptation to minimise negative fitness costs of fault bars on flight performance (Jovani &

Blas, 2004; Jovani *et al*., 2010). Consequently, this results in the majority of fault bars being located on the tail feathers, with the lowest numbers in the primary feathers (Jovani & Blas, 2004). Results of chapter 4 supported this hypothesis, finding higher numbers of fault bars in the tail feathers than the wing feathers. Jovani & Blas' (2004) hypothesis can also be discussed in relation to the results of chapter 6, where feather strength was not altered between areas with and without fault bar presence. From this, it could be speculated that the spread of lower feather strength may be a better strategy than having one area of weakness that may lead to breakage. Therefore, in line with the 'fault bar allocation hypothesis', nutrients are allocated to the feather in a way that best avoids overall damage.

7.1.1.2 Muscular constriction hypothesis

Soon after Riddle (1908) outlined his formation theory, Duerden (1909) put forward a new hypothesis, suggesting that barbule malformation could also result from mechanical damage rather than just a failure of nutrient delivery. This was also supported in more recent years by Murphy *et al*. (1989) who suggested that unusual muscle contractions around the soft feather shaft during feather growth result in barbule damage. Results of chapter 3 (section 3.3.2.1) identified feather shaft damage at the site of heavy fault bars, suggesting that this could portray evidence of muscular constriction, revealing the extent of damage caused to the overall feather structure. This occurs predominantly in heavy fault bars; however, slight indentations on the feather shaft can also be seen at the location of less severe fault bars. The occurrence of shaft damage in light fault bars may be the result of a weaker muscular constriction, in comparison to more severe constriction which may lead to shaft damage at the site of heavier fault bars. Differences in feather strength in feather regions with and without fault bar occurrence were investigated in chapter 6 to further explore consequences of this damage. Fault bars were expected to weaken the location of feather where present. However, results did not support this hypothesis, finding no difference in strength between feather regions with fault bar occurrence and those with fault bar absence. This reiterates the concept of distributed weakness to avoid breakage, in line with Jovani and Blas' 'fault bar allocation hypothesis' (2004), stated previously.

In addition, findings of chapter 3 revealed that the barbules within fault bars were found to be constricted. This is characteristically different to the commonly used descriptions offered by Riddle (1908) and Murphy *et al*. (1989), where areas of missing sections are displayed. This, again, highlights the possibility of muscular constriction in the role of fault bar formation, with stronger muscular constrictions resulting in barbules being pressed more towards the shaft. Consequently, this would result in fault bars with a broader visual appearance. The concept of mechanical damage was described as an "autonomic reaction powerful enough to over-ride a regional inhibition of feather muscles", as a result of many types of stress (Murphy *et al*., 1989). Jovani & Diaz-Real (2012) found that fault bars were not the result of a chronic (long-term) stress, but that of an acute (short-term) stressor. From this, they suggested that fault bar strength/width is not related to the duration of the stress, but to the intensity of the stress (Jovani & Diaz-Real, 2012). Results of chapter 3 indicated that in most cases, two scenarios occur in carrion crows, leading to fault bar formation: 1) tight muscle constriction around the growing feather/severe stress, resulting in heavy fault bars 2) loose muscle constriction around the growing feather/minor stress, resulting in light fault bars. However, the concept of blood and nutrient supply was not ruled out. In fact, multiple factors may be occurring together, which result in the formation of a fault bar.

In addition to the above morphological findings, evidence of the feather being impacted during feather growth in pin formation has been provided, displayed as dark lines of varying thickness around the circumference of the pin (see section 3.3.2.2). Fault bars were present on the emerging feather vane of Fig.3.18, indicating the occurrence of faults on this feather. Adjacent feather pins also shared these traits and was seen in a number of carrion crows during the study. A pin feather displaying the same characteristics showed a fault bar at the lines depicted on the feather pin. This occurrence has not been documented in prior research, suggesting a novel understanding of their presentation during feather growth. This visual display is potentially easier to depict in darker feathers; however, this conclusion cannot be drawn without comparison. Moreover, the visible fault bar line around the circumference of the pin feather may support the concept of constriction during feather growth (Murphy *et al*., 1989).

7.1.1.3 Stress events and individual fitness

An interesting perspective on stress in carrion crows was also discussed in chapter 3, where feathers with higher numbers of fault bars were found to have wider fault bars on average. This showed that both variables were equal in their representation of fault bars, whilst demonstrating that the number of fault bars also means on average heavier fault bars. This raises questions as to what this could this mean from a causal point of view, i.e. in relation to the different formation hypotheses. This suggests that carrion crows experience either infrequent minor stress events or a high occurrence of severe stress events. This is an interesting finding as it provides new information about individual fitness and natural selection in carrion crows. Previous studies have found that a high number of fault bars occurred in individuals of lower fitness (Bortolotti *et al.*, 2002; Machmer *et al.*, 1992; Blanco & de la Puente, 2002; Jovani & Rohwer, 2016; Møller, 1989). It has been argued that in some circumstances, fault bars may merely be an identifier of individuals in poor condition with low survival chances due to factors unrelated to feather malformation (Jovani & Rohwer, 2016). This result points towards the idea that individuals that suffer from a large amount of stress may be individuals of low fitness.

It is interesting to reflect here on the findings of chapter 4, where a strong relationship between fault bar occurrence/severity/width and feather iridescence was found. In this, dull feathers were associated with high fault bar occurrence/severity/width. This links back to comments around individual fitness, as iridescent feathers are thought to be an honest signal of individual quality in sexual displays due to their costly nature (Griffith, Parker & Olson, 2006; Pacyna *et al.*, 2018). Therefore, a relationship between fault bars and iridescence implies fault bars reflect individuals in poor condition.

Another commonly associated visual characteristic of low feather quality is aberrant white feathers. Feathers with white colouration are more susceptible to damage than those of normal melanin deposition (Bonser, 1994; RSPCA, 2013). Due to a lack of melanin, white feathers in general become more susceptible to UV damage and, therefore, deteriorate at a faster rate than dark feathers (Kaiser, 2008). Consequently, the aberrant white feathers may compromise the wing, as they are suggested to be a weak area in the overall wing structure. This occurrence is generally thought to be associated with genetic factors (Sage, 1962);

however, it has now been found to link more commonly with the physical condition and age of individuals and can also include environmental conditions e.g. food availability (van Grouw, 2018). The relationship between average fault bar width and aberrant white feathers was not found in chapter 4, suggesting that there may be different causes affecting fault bars and white feathers. This also leads to questions around the role of aberrant white feathers in mate selection of carrion crows and the role of this occurrence in the 'fault bar allocation' hypothesis (Jovani & Blas, 2004). Wing feathers were found to be more susceptible to lack of pigmentation; therefore, fault bars and white feathers appear to be 'allocated' to different feathers. This, again, gives the impression that white feathers are not detrimental the quality of carrion crow feathers. Moreover, a positive relationship between average feather strength and the number of white feathers was close to significant in all feather types combined, again, potentially suggesting a causal hereditary condition (Harrison, 1957a,b; Sage, 1954; Sage, 1962; van Grouw & Hume, 2016) in carrion crows. In order to understand this occurrence fully, further research is needed. Unfortunately, this project was not able to fully explore the intriguing topic of aberrant white feathers, as it requires a separate focus.

7.1.2 Causes and consequences of poor feather quality

It has been suggested that the main cause of fault bar production is stress caused by nutritional deficiency (Stocker, 2005). However, a variety of other possible causes have also been researched, including age and sex, heredity traits, human handling, environmental factors, parasite burden and disease (Erritzøe, 2006). In response to this, a variety of factors were assessed in relation to feather quality.

7.1.2.1 Chemical composition of feathers

Initially, the chemical composition of feathers was analysed with the intent to identify potential environmental contamination. This was assessed using the identification of high levels of heavy metals. However, no heavy metals were detected in the samples of this study; therefore, this 'possible cause' of poor feather condition was ruled out, focussing solely on nutritional elements. However, reflecting on the reference to calcium above, acid rain is known to limit calcium-rich prey whilst increasing dietary exposure to toxic metals such as lead and aluminium (Scheuhammer, 1991). The presence of such elements were expected in individuals with low calcium levels. However, it is important to note that elements may not be necessarily absent, but the value was below the limit of detection (CDL). Therefore, the possible influence of environmental contamination on poor feather condition cannot be fully ruled out.

Low levels of calcium in more severe fault bars implies a potential link to nutritional deficiency. This novel finding is extremely valuable and provides new insight into fault bar production and feather quality. A lack of calcium in regions of faults bars in these individuals leads to questions about the potential lack of availability of this nutrient in their diet. Wild birds typically gain calcium from food sources such as eggs, snails and mineral deposits in the soil (Mänd *et al*., 2000). However, even in calcium-rich areas, obtaining sufficient levels of calcium can be challenging and time-consuming (Graveland, 1998; Mänd *et al*., 2000). A study on the gizzard contents of carrion crows revealed a low consumption level of snails and eggs (Holyoak, 1968). This provides one explanation for this potential nutritional imbalance, as these food sources are very high in calcium. However, this species may potentially gain calcium from other sources such as soil. Moreover, changes in habitat may also be factor to consider in the quality of diet, as carrion crows have colonised new urban and suburban habitats, regularly visiting rubbish tips (Holden & Cleeves, 2010). Nevertheless, it should again be noted that the individuals used in this study are not representative of the entire population as they were admitted to a rehabilitation centre for a range of reasons. Therefore, this study needs to be expanded to account for this.

The nutritional deficiency hypothesis was supported by Slagsvold (1982a), finding higher numbers of fault bars and white feather colouration in undernourished juvenile hooded crows Corvus (*corone cornix*). Similarly, Newton (1986) found a greater number of fault bars in juvenile sparrow hawks (*Accipiter nisus*) when they had received less food during rainy days. In contrast, Jovani and Rowher (2016) examined 74 scientific papers in order to determine the reliability of Riddle's hypothesis. From this, an overarching conclusion was given that malnutrition does not strongly impact the production of fault bars (Jovani & Rohwer, 2016). Further studies have also come to this conclusion. For instance, a study conducted on white-crowned sparrows (*Zonotrichia leucophrys gambelii*) found that fault bars are an unreliable index of a nutritional status due to a lack of correlation with starvation days (Murphy *et al*., 1989). Furthermore, a study also found a correlation with human handling and fault bars rather than malnutrition (Negro *et al*., 1994). This contrast of findings is interesting and highlights the distinction between malnutrition in the form of starvation and the lack of important minerals in the diet. Nevertheless, as discussed in chapter 5, the combination of nutrient supply and other stressors must be considered together. In this, it could be suggested muscular squeezing from stress may damage blood vessels which deliver nutrients to the feather. As a consequence, the growing feather regions may be compromised as found in this study on sections of feather vane with fault bar occurrence. In order to understand the relationship between nutrient supply in the blood and muscle activity in relation to fault bar formation, further research is required.

7.1.2.2 Endoparasite burden

Individuals with a high number of endoparasites (cestodes, flukes and acanthocephalan), were expected to display poor feather condition. This was predicted due to the withdrawal of energy by parasites and the trigger of costly immune responses (Hudson *et al*., 1998; Møller, 1997; Reed *et al*., 2012; Sheldon & Verhulst, 1996). Results did not support this hypothesis, finding low numbers of endoparasite species in individuals with wide fault bars in the wing feathers. In addition, no effect of endoparasite burden was found on feather strength. This could be explained by the concept of fitness trade-offs, where costly immune defences may be facilitated at the cost of feather quality (Sheldon & Verhulst, 1996). However, it is important to note that nestlings were excluded from this study, which are known to be particularly vulnerable to parasites due to their less efficient immune system and exposure to nest-dwelling parasites (Reed *et al*., 2012; Ros *et al*., 2002; Szép & Møller, 1999). It remains to be tested whether this trade-off can also be observed in nestlings.

Nematodes were excluded from this study due to the difficultly in identification. The exclusion of nematodes may not have impacted the results of this study as they occurred seldom. However, further research into their presence may strengthen the understanding of how endoparasites may impact the production of poor quality feathers. In order to achieve this, DNA extraction methods are required to identify species. This method would also provide information on the exact species of *acanthocephalan* and *platyhelminth* species found in this study. This was attempted; however, due to storing cadavers in the freezer, the quality of

DNA within the parasites were too degraded for analysis. Future studies therefore require rapid extraction of endoparasites without the use of freezer storage.

7.1.2.3 Sex and age

Differences in feather condition between sex was not expected due to carrion crows being monomorphic and non-migratory (Dawson *et al*., 2001; Slagsvold, 1982b). Results supported this hypothesis, finding no significant association between sex and fault bar occurrence or feather strength. Due to their susceptibility of young individuals to fault bar production (Hawfield, 1986; Serrano & Jovani, 2005), this factor was investigated in first year juveniles, second year juveniles and adults in relation to fault bar occurrence and feather strength. Results supported the higher susceptibility in young birds, finding younger individuals to have wider fault bars in their tail feathers. Due to the previously established relationship between fault bar width and the number of fault bars, this implies that younger individuals had a higher number of fault bars in the tail feathers than older individuals. However, no such relationship was found in the wing feathers. This may be due to the large majority of fault bars occurring in the tail. The results discussed here are supported by a number of other studies, finding similar differences in feather quality between adult and young birds (Hawfield, 19867; Jovani & Blas, 2004; Jovani & Tella, 2004; Machmer *et al.,* 1992; Serrano & Jovani, 2005; Slagsvold, 1982a; Yosef *et al.*, 2013). In addition, due to their susceptibility to poor feather condition, young individuals were expected to have the weakest feathers (Hawfield, 1986; Serrano & Jovani, 2005). Results found a trend in the wing feathers, with adults tending to have more stress tolerant feathers than first and second year juveniles. It should be noted that carrion crows perform a partial post-juvenile moult, meaning that they have the same flight feathers from when they are nestlings to when they moult at the end of their second year (Ginn & Melville, 1983). As the feathers become worn and abraded in the second year, they become less tolerant to stress. This result reflects the age of the feathers, impacting individuals as they progress through different stages of life. The results of this study therefore lead to questions around the consequences of these strength differences across the age groups. Due to flexible wings being associated with greater lift production in flapping flight, it could be suggested that younger carrion crows are negatively impacted by having feathers with lower stress tolerance (Gopalakrishnan & Tafti, 2010). In regard to feather quality in terms of strength differences across different age groups, few studies have been conducted.

7.2 Recommendations for future research

7.2.1 Extending studies to different species and age groups

As stated previously, fault bars have been documented in a wide range of avian species, emphasising the importance and application of this research. In order to follow up the findings of this study, the presentation of fault bars should be assessed for potential similarities and differences across species. It should be noted that previous studies such as those seen in Murphy *et al*. (1989) display images using outdated scanning electron microscopy (SEM), which can now be re-assessed using updated equipment. Moreover, the findings in this study appear to be very similar to an illustration provided at the end of Riddle's 1908 publication (seen Fig.1.6 of the introductory chapter), which, again, lacked the benefits of modern scientific equipment.

Moreover, the inclusion of nestlings in future research would be useful in assessing this age group in relation to feather condition. However, the inclusion of this age group in this study would require nestlings to develop their feathers fully for analysis. Unfortunately, this highlights the limitations of using samples from a wildlife rehabilitation centre, as this research would require deviations from normal protocol. In response to this, feathers could be studied whilst in pin formation, as fault bars can still be seen during this stage of growth. Although studies such as feather strength and iridescence would not be viable, feather pins could be analysed for calcium deficiency using Scanning Electron Microscope Energy-Dispersive X-ray spectroscopy (SEM-EDS).

7.2.2 Development of methods

The research methods provided in this study would be greatly advanced by the use of high-end scientific instruments. For example, the use of the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipment would provide advanced accuracy of detecting chemical elements in feathers (Wilschefski & Baxter, 2019). This may then detect traces of elements associated with environmental pollution in addition to potential detrimental accumulations of these chemicals (Scheuhammer, 1991). Moreover, the use of stable isotope ratio analysis may offer valuable information physiological stress related to feather quality (Ross *et al.,* 2015). For instance, a study on juvenile grasshopper sparrows (*Ammodrammus savannarum*) found the occurrence of pallid bands to be associated with a diet consumed during a historic

4.2 km-wide tornado (Ross *et al*. 2015). Moreover, stable isotope analysis also offers information on geographic origins, with the relevance of this factor to be discussed in the subsequent section (Hobson, 2005).

7.2.3 Additional factors to consider

7.2.3.1. Agricultural versus urban impacts

Organochlorines may also play a role in altering the chemical composition of feathers. For example, agricultural fertilisers such as Polychlorinated biphenyl (PCB) have been found to impact calcium metabolism in bones (Andrews, 1989; Lind *et al*., 1999; Lind *et al*., 2004; Rodriguez-Navarro *et al*., 2006; Sonne *et al.,* 2004), suggested that feathers may also be impacted due their keratinous structure (Tubaro, 2003). Although PCBs were banned in the UK in 1986 (Environment Agency, 2000), they may still persist in the environment due to their ability to bind strongly to soil and sediments (SEPA, 2019). Feathers have also been used as bioindicators of PCB; for example, clapper rails (*Rallus longirostris*) have been used to detect environmental damage for the LCP (Liquid crystal polymers) superfund site located in Georgia, USA, which is contaminated with the PCB Aroclor 1268 (Summers *et al*., 2010). Therefore, this highlights the ability to assess the relationship between organochlorines and feather composition, which could provide further insight into contaminants which may hinder feather quality. This factor was not assessed in this study due to financial limitations.

In line with the suggestions made about organochlorides, assessing habitat data for relationships between land types and feather condition may be beneficial. It would be interesting to determine differences seen between feather quality in individuals in urban and agricultural home territories. This would also offer information around differences in food sources and potential sources of nutritional deficiencies. This was attempted at the start of this study using geographical information system (GIS); however, the location data gained from the RSPCA database was not sufficient. This could be improved by making small changes to RSPCA data collection methods. For example, specific finder location data was often missing, which would provide an insight into habitat use. This information would then offer useful information into differences in diet and the level of anthropogenic impact. Simple improvements to this data collection would hugely benefit wildlife research in general, highlighting the need to change current practices within the RSPCA. This also applies to veterinary surgeries that transfer animals to RSPCA wildlife rehabilitation centres after receiving them from the general public. The implementation of a simple record sheet for members of the public to fill in at veterinary surgeries for wildlife admissions would be an efficient method for this improvement. Moreover, transfer of admissions between RSPCA inspectors and collection officers often results in loss of important information. This can also be improved with amended communication methods.

7.2.4 Other feather defects

Feather quality assessment should also consider feather defects other than fault bars. For example, in recent years, a number of collared doves (*Streptopelia decaocto*) were admitted to Stapeley Grange with defective tail feathers. The observed feather defects in this example were comparable to Riddle's (1908) second type of feather defect, outlined in Chapter 1 (section 1.1.2; illustrated in Fig.1.8). This particular malformation consisted of an area of feather which entirely lacked barbs and barbules (Riddle, 1908). This also occurred in combination with a defective sheath, which failed to break down naturally within a normal time frame. A more extreme feather defect example can be seen in cases of 'angel wing', commonly seen in waterfowl species. Angel wing is a musculoskeletal disorder which causes the remiges to appear stripped of barbs, twisting and protruding away from the body (Kear, 1973; Zsivanovits, Monks & Forbes, 2006). This defect is thought to occur as a result of a high protein diet, associated with the provision of an artificial diet (Kear, 1973; Zsivanovits *et al.*, 2006).

The topic of aberrant white feathers was lightly covered in this study, finding no evidence of this occurrence being detrimental to the quality of carrion crow feathers. However, this is a complex colouration occurrence, requiring more extensive investigation. An early study by Harrison (1963) stated that carrion crows were particularly susceptible to aberrant white feathers in urban or suburban areas, due to an abnormal or deficient diet. Therefore, this factor requires further analysis, especially in relation to its connection to feather quality and growth defects.

8. References

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