Seasonal and lateral differences of the feathers of the Southern Masked-Weaver *Ploceus velatus*

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List of acronyms and abbreviations

A	Adult
AS	Antisymmetry
BMR	Basal metabolic rate
CORT	Corticosterone
CV	Coefficient of variation
DEE	Daily energy expenditure
DDE	Dichloro diphenyldichloro ethylene
E S	Early season
DA	Directional asymmetry
F	Female
FA	Fluctuating asymmetry
L	Left
LADPFS	Laterally-associated differences in primary feather structure
LvR	Left versus right
M	Male
OCs	Organochlorines
OCPs	Organochlorine pesticides
P8	Primary 8
PBS	Prozesky bird sanctuary
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyl
POPs	Persistent organic pollutants
R	Right
L S	Late season
SD	Standard deviation
S	Season
SADPFS	Seasonally-associated differences in primary feather structure
SMW	Southern Masked Weavers
Y	Young

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Abstract

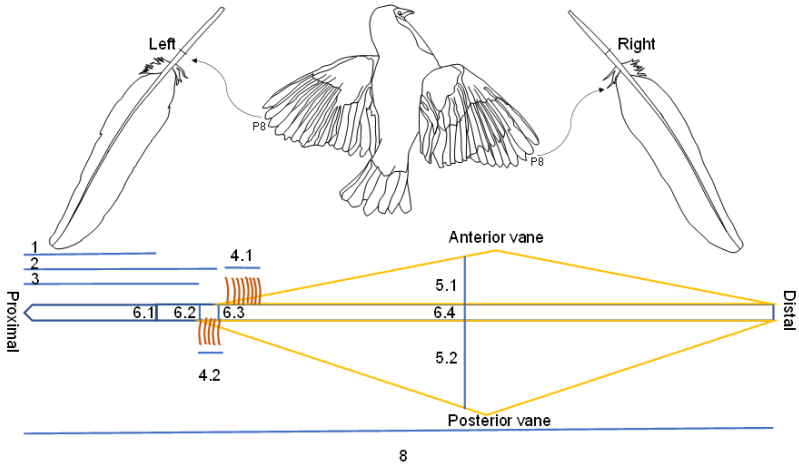
Are the left and right flight feathers of a bird the same?

Are the structures and dimensions the same regardless of individual age, feather age, and sex?

Structural flight feather asymmetry has not been explored beyond measurement of feather lengths. Differences in expression of structural asymmetry between feather age, age of the bird, and sex have also been neglected. This study investigated both aspects measuring 12 feather dimensions (illustrated below) of left and right primary 8 (P8) feathers of 248 Southern Masked-Weavers *Ploceus velatus*. Samples were divided into eight groups to distinguish between feather age, individual age, and sex. The left and right primaries of each group were compared for bilateral symmetry using two-tailed, paired t-tests. Significance was assumed at $p < 0.05$.

Differences were found between left and right primaries, suggesting laterally-associated differences in primary feather structure (LADPFS). The direction and expression of variables differed between groups. For females, I found significant differences in all groups, where the dimensions for Variables 1, 4.1, 4.2, 6.2, and 6.3, indicated structural differences between left and right primaries.

For males, I found significant differences in all groups, where the dimensions for Variables 1, 4.1,4.2, 5.1, 5.2, 6.2, 6.4, and 8, indicated structural differences between left and right primaries. As far as I am aware, and after many literature searches, this investigation is the first to show significant structural differences between left and right primaries. I also found differences in direction and expression of feathers between groups and I suggest avenues for further study.



Keywords: Asymmetry, Feathers, Primary feather asymmetry, Seasonal variation, Southern Masked-Weavers.

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Chapter 1: Introduction

1.1 Birds

Among vertebrates, the class Aves include more than 9700 species (Hickman *et al*., 2011). Birds are globally distributed - inhabiting deserts, forests, prairies, mountains, ice caps, and oceans (Hickman *et al*., 2011). Having feathers is the simplest feature to distinguish birds from any other animal. Along with feathers, all birds have hind limbs adapted for swimming, walking, or perching. All birds have forelimbs modified into wings (however, not always used exclusively for flight). All extant birds lack teeth in their keratinized beak, and all birds lay eggs (Hickman *et al*., 2011). Birds have significant functional and structural uniformity as birds are derived from the function of flight, restricting morphological diversity (Hickman *et al*., 2011).

1.2 Adaptations for flight

The ability to locomote by wing is a trait of birds that continue to fascinate scientists and the public. During migration, birds can travel thousands of kilometres without rest (Schmidt-Nielsen, 2007). To achieve this, birds have a number of adaptations to increase flight efficiency and decrease the energetic cost of flight.

- Due to the shape of a bird's wing, the air that travels over the surface of the wing (aerofoil) has to travel further than the air travelling below (Müller & Patone, 1998). Consequently, the air flowing over the upper surface has a greater velocity than the air flowing underneath (Müller & Patone, 1998). Keeping Bernoulli's equation in mind, an increase in velocity will cause a decrease in static energy to keep the sum of all energies constant (Müller & Patone, 1998). The result is that the lower surface of the wing provides more force than the upper surface, consequently providing the bird with lift (Kidson & van Niekerk, 2014; Müller & Patone, 1998). Due to this principle, birds in flight need relatively low energy to remain in the air - one of the factors that allow birds to travel long distances (Kidson & van Niekerk, 2014). Birds have air sacs distributed between the internal organs that extend into the bones. Because normal flight increases the oxygen consumption rate by eight to ten times, birds require a highly efficient respiratory system (Hickman *et al*., 2011; Schmidt-Nielsen, 2007). Air sacks allow effective distribution of oxygen and decrease the overall mass and density of the bird (Schmidt-Nielsen, 2007).

- Birds are equipped with a high-pressure circulatory system, an efficient respiratory system, and a high metabolic rate.
- Birds tend to have rapid digestive systems, and have energy-rich diets to meet the intense metabolic demands of flight (Hickman *et al*., 2011).
- Birds have a semi-hollow bone structure that is internally reinforced by a network of bony mesh. This enables the bird to have a relatively low body mass in comparison with most other animals (Kidson & van Niekerk, 2014).
- Birds need to have acute senses and a sensitive nervous system to handle high-velocity flight (Kidson & van Niekerk, 2014).
- A study conducted by Able (1973) found that birds display behavioural adaptations by waiting for favourable wind directions and synoptic weather conditions before initiating migration.
- The amount of resistance a flight feather experiences increases toward the distal side of the feather; exceptions include flightless birds such as the Ostrich (McKittrick *et al*., 2012).
- Finally, flight altitudes of soaring birds such as buzzards and swifts increase with development of thermal convection, increasing temperatures, decreasing relative humidity, decreasing cloud cover, and increasing atmospheric instability (Shamoun-Baranes *et al*., 2006). Birds that use thermal convection for flight (soaring) however are affected more by weather than those using powered flight (Shamoun-Baranes *et al*., 2006).

1.3 Feathers

Feathers are a defining trait of birds that provide a significant increase in surface area without considerably increasing body mass (Müller & Patone, 1998). Feathers provide many advantages, some of which are: thermal insulation, camouflage, skin protection, flight surface, external appearance, water repellency, and they play a fundamental part in lowering the specific gravity of flying birds (Brooke & Birkhead, 1991). Feathers are frequently covered with oils during preening for protection (Kidson & van Niekerk, 2014). All other vertebrates that exhibit some sort of flight (e.g. fish, mammals, amphibians, and various reptiles) accomplish the increase of surface area through thin layers of integument spread between extendable appendages (Müller & Patone, 1998). These skinfolds are naturally impervious to air, providing the individual with lift. The aerofoil of birds however, consists mainly of feathers, with the extremity itself (wing bones and muscles) having a much smaller role (Müller & Patone, 1998). In comparison with skin folds, the advantage of feathers lies in the ease in which minor damage can be repaired, regular replacement via moulting, light weight, and flexibility (Müller & Patone, 1998).

1.3.1 Feathers and moulting

Understanding feather moult is integral in understanding bird adaptations to variable environments (de Beer *et al*., 2001). Since feathers play such a major role in the overall health and fitness of an individual, feathers need to be kept in a good condition (de Beer *et al*., 2001). Birds achieve this by daily preening, dustbathing, and sunbathing. Despite constant upkeep, feathers deteriorate with time due to abrasion, ultraviolet light, and exposure. This deterioration coincides with a loss of sheen and colour. Deterioration leads to regular replacement of the feathers, known as moult (de Beer *et al*., 2001).

Most species replace their feathers once a year, but some species do so twice a year (de Beer *et al*., 2001). Larger species, such as birds of prey, moult once every two or three years, with feather growth continuing over the whole duration. Ducks and geese moult their primaries and secondaries simultaneously, resulting in flightlessness during moult.

1.4 Feather growth

The formation of a feather is initiated as a conical pinching of the epidermis (Price *et al*., 1991; Møller, 1996). Feather follicles are formed from an epidermal ring and a mesodermal core. Feather growth is the product of cell division in the collar (a ring of epidermal tissue at the base of pinching), which pushes out previously formed cells. After differentiation, these cells become keratinized to form the rachis and barbs or die off to form the spaces between the barbs. The tip is formed first, followed by later cells that differentiate into the subsequent barbs (Price *et al*., 1991; Møller, 1996).

1.5 Energetic cost of feather production

The energetic cost of feather production varies substantially between species (Lindström *et al*., 1993). Body mass and basal metabolic rate (BMR) is associated with the cost of feather production (Croxall, 1982; Lindström *et al*., 1993). Increased thermal conductance during moult results in increased thermoregulatory expenses to produce new feathers (Lindström *et al*., 1993). To some extent, it can be expected that increased energy expenditure during moult would be due to compensation for heat loss during increased peripheral blood flow and reduced insulation (Newton, 1968).

The rise in metabolic rate during feather production is not only attributable to the cost of the formation of keratin but also to the maintenance of tissues contributing to feather synthesis (Dietz *et al*., 1992; Lindström *et al*., 1993; Murphy and King, 1984). According to Dietz *et al.* (1992), the cost of the aforementioned maintenance is parallel with the BMR. Ultimately, this means that the cost of feather production in large birds (with comparatively lower BMR) would be substantially lower than the cost of feather production in small birds, especially

passerines that have a higher BMR (Lasiewski & Dawson 1967; Lindström *et al*., 1993). Notably, the mass of plumage per unit of surface area is less in smaller birds compared with larger birds (Kendeigh, 1970). Smaller passerines have a high BMR and less insulation per unit of surface area, resulting in high daily energy expenses (DEE) required for survival (Dietz *et al*., 1992; Kendeigh, 1970). This is especially true under cold conditions (Dolnik & Gavrilov, 1979; Kendeigh, 1970).

Therefore, feather growth can have tremendous implications on DEE of smaller birds, to such an extent that it can even impinge other energetically expensive activities such as fat deposition and locomotion (Lindström *et al*., 1993). The high cost of feather synthesis results in immense selection pressure to adapt to the energy requirements for moulting, especially for smaller passerines. Not much is known on how the feathers of species that moult their primaries twice a year differ structurally between summer and winter seasons. What we do know is that under environmental conditions that force birds to moult feathers rapidly, a compromise can be made in the quality of feathers (Graham *et al*., 2010; Griggio *et al*., 2009; Susanna & Hall, 2000; Rohwer & Rohwer, 2013, Møller, 1996). Optimal energy allocation, therefore, poses the question whether there would be structural differences between summer and winter feathers driven by natural selection for optimal energy allocation. This question, however, can only be answered by species that moult their flight feathers twice a year.

1.6 Feather structure

Since feathers vary in function and structure, it is useful to distinguish between contour feathers and flight feathers.

- Contour feathers occur on most parts of the body, excluding the feet and beak (Brooke & Birkhead, 1991). Contour feathers have more down at the base compared to flight feathers. Contour feathers provide the bird with insulation and pigmentation. Unlike flight feathers (primaries, secondaries, and tertiaries), they do not provide flight surface, but may serve to smooth the body contours for less resistance during flight.
- Flight feathers are morphologically longer and stiffer than contour feathers with reduced down at the base (Brooke & Birkhead, 1991). The interlocking mechanism of the barbules of flight feathers is more developed than for contour feathers, providing greater cohesiveness, ensuring the provision of lift during flight (Brooke & Birkhead, 1991).

Flight feathers need to withstand aerodynamic forces during flight. This demand is partially achieved by feathers being made of keratin, which is a strong and lightweight material (Lingham-Soliar, 2017). The structure of a feather (Fig. 1.6.1) is composed of:

- The main shaft (rachis), which is derived from the dorsal ridge as the feather matures.
- The lower part of the rachis is called the calamus and is embedded in the skin (Lingham-Soliar, 2015).
- The rachis is comprised of many branches called barbs.
- These barbs branch, in turn, into smaller barbules.
- Barbules are covered with tiny hooks (barbicels) which overlap with the barbicels of the anterior barbules.
- This forms an interlocking structure providing the various functions of feathers (Brooke & Birkhead, 1991).
- Barbicels ensure that the web can be reattached if there were any interruption in the .continuity of the web (Lingham-Soliar, 2015).

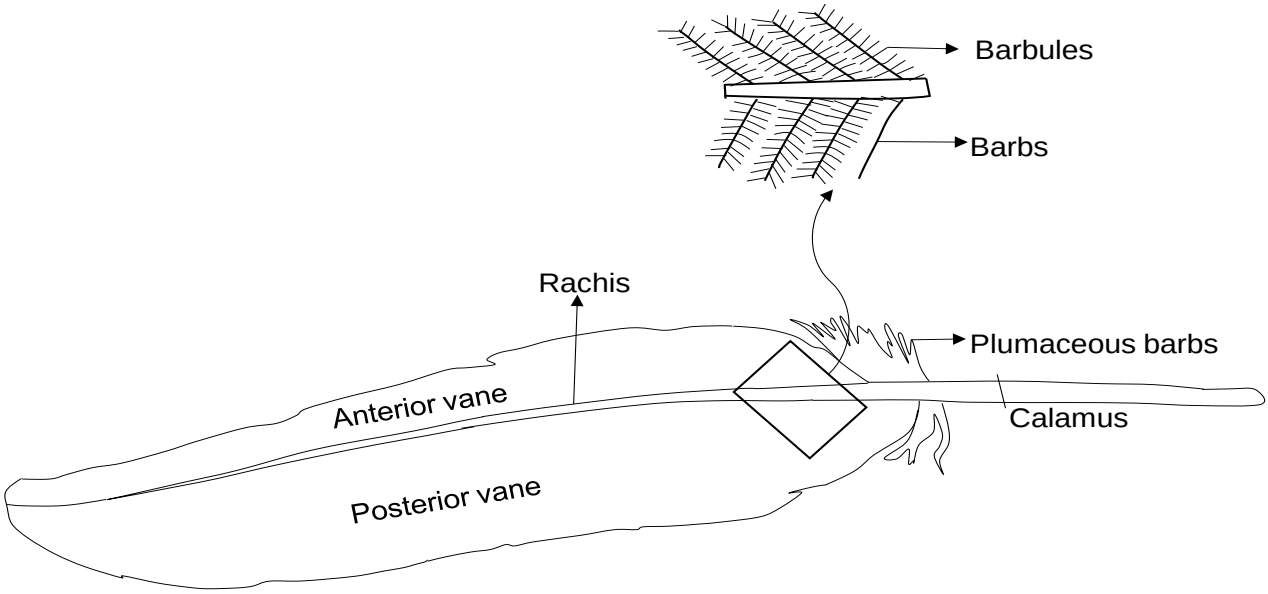

Fig 1.6.1: *A schematic representation of feather structure.*

1.7 Asymmetry

1.7.1 Antisymmetry, directional asymmetry, and fluctuating asymmetry

Morphological traits can occasionally deviate from perfect bilateral symmetry. This asymmetry can occur through antisymmetry (AS), directional asymmetry (DA), and fluctuating asymmetry (FA). Before continuing, I need to explain these three definitions in more detail.

- AS: Antisymmetry is the phenomenon where one side is larger than the other, but has an approximately equal frequency as to which side is larger (Palmer & Strobeck, 1986; Van Valen, 1962). Antisymmetry is reflected by a platykurtic or bimodal distribution (normally

two peaks) with a mean of zero and a negative kurtosis value (Palmer & Strobeck, 1992; Palmer, 1994).

- DA: Directional asymmetry is a consistent bias of a trait where there is greater development on one side of the body in comparison with the other side (Palmer & Strobeck, 1986; Van Valen 1962). Directional asymmetry is characterised by a normal distribution of which the mean is not zero.
- FA: Fluctuating asymmetry is defined as non-directional deviations from bilateral symmetry and is the left minus right deviation from zero. (Bustnes *et al*., 2002; Leung, 1998; Thomas 1993). Fluctuating asymmetry is reflected by a normal distribution around a mean of zero. Fluctuating asymmetry is associated with developmental noise and has no known functional importance.

FA can be measured in bilaterally symmetrical organisms as the difference between the traits on each side (Jenssen *et al*., 2010). In proportion to the dimension of the traits being measured, FA tends to be relatively small (Swaddle *et al*., 1994). Albeit small, FA still implies deviation from the ideal morphological symmetry. In the context of genetic, environmental, and developmental stress, the extent of FA is indicative of developmental stability because the same genome is responsible for the production of both sides of the bilateral trait (Bustnes *et al*., 2002; Thornhill, 1992b; Watson & Thornhill, 1994). Thus, a high level of FA is assumed to reflect reduced developmental stability (Aparicio & Bonal, 2002). Differences in traits are invariably explained by different levels of developmental stability. FA may vary per trait functionality, mode of selection, and stress associated during the developmental process (Aparicio & Bonal, 2002).

1.7.2 Possible causal factors of fluctuating asymmetry

Environmental or genetic stress is known to disrupt bilateral symmetry through two pathways – an increase in developmental noise, or a reduction in developmental stability. Stress in general places an organism at a disadvantage due to energy expenditure that ultimately threatens fitness and survival (Parsons, 1992). Since genetic predisposition for FA, and even maternal age can be causal (Knierim *et al*., 2007; Møller, 1996; Parsons, 1990; Watson & Thornhill, 1994), the list of other factors (often interrelated) that may be associated with FA is quite extensive and will be discussed below. More attention will be given to those factors relevant to feathers and wings of birds.

1.7.2.1 Genetic stress

Developmental homeostasis is assumed dependent on two factors – canalization and developmental stability (Graham *et al*., 2010). Canalization refers to stable development

under different environmental and genetic conditions, while developmental stability refers to stable development under constant environmental and genetic conditions (Graham *et al*., 2010). Genetic stress may include factors such as inbreeding, hybridization, mutation, (Møller, 1996; Palmer & Strobeck, 1986; Parsons, 1990). FA can be indicative of developmental stability and some species may have buffering capacities that resist FA (Palmer & Strobeck, 1986; Swaddle & Witter, 1994; Thornhill, 1992; Van Valen, 1962). As an example, a study done on European Starlings *Sturnus vulgaris* found an increase in asymmetry with increasing nutritional stress and low fat stores (Swaddle & Witter, 1994; see also Grieco, 2003). In the same study, spottiness of the chest is considered a measure of fitness as the starlings with more spots start their ovarian development earlier than those that have less spots (Swaddle & Witter, 1994). Individuals with a spottier chest displayed less signs of asymmetry in their primaries. This suggests an association between fitter individuals and resistance to FA (Swaddle & Witter, 1994). A critical assumption of studies on FA as an indicator of developmental stability is that the heritability of FA is low or zero (Palmer, 1994). However, some studies have shown associations between dominance in the alleles and that an epistatic effect may influence the expression of FA, especially in wild populations where individuals may be exposed to a variety of stressors (Leamy & Klingenberg, 2005).

There also seems to be a pattern where both heavy metals and persistent organic pollutants (POPs), which will be discussed later, are not necessarily collectively distributed between feather types or species (Abbasi, *et al*., 2016; Abbasi *et al*., 2017; Swaddle *et al*., 1994). Therefore, each bird will express exposure to pollutants differently. FA is not even expressed uniformly in the same feather (Møller, 1996). A study on Yellow-browed Warblers *Phylloscopus inornatus* that focussed on development of feather tips concluded that variance in the earlier formed parts (the feather tip) were higher than in the later formed parts (base) (Møller, 1996; Price *et al*., 1991; but also see Aparicio, 1998; and Grieco, 2003 for contrary arguments). Finally, traits that have many components per unit of length can show less FA, than simpler ones (Aparicio & Bonal, 2002). Aparicio & Bonal (2002) argue that asymmetrical allocation of resources caused by developmental instability would produce FA in that trait. However, the same magnitude in asymmetrical allocation will result in a higher asymmetry in those traits that need less structural components for a unit of length to be formed.

1.7.2.2 Developmental stress

FA can increase due to environmental and genetic stress because of a reduction in developmental homeostasis (Parsons, 1992). This can occur due to allocation of too much

energy to compensate for the stressors, resulting in less allocation of energy for developmental precision. Although the association between feather moult and growth is poorly understood, it seems that thyroid hormones are integral to shedding and replacement of feathers. For example, abnormal feathers are grown by birds that have been thyroidectomised (Jenssen *et al*., 2010). According to Jenssen *et al*. (2010), aquatic birds with high body burdens of POPs are associated with disruption of the thyroid hormone, and disruption of vitamin A and vitamin E homeostasis.

This disruption of the homeostasis of the endocrine hormones contributes to disruption in growth and development. Not surprisingly then, corticosterone (CORT) is one factor that can influence the structure of a feather. CORT plays a role in the maintenance of a homeostatic energy balance, but CORT can be released in high quantities under stressful situations (Jenni-Eiermann *et al*., 2015; Strochlic & Romero, 2008). This allows organization of behavioural and physiological responses to unexpected environmental conditions; allowing the individual to cope with such stressors. (Jenni-Eiermann *et al*., 2015). Romero *et al*. (2000) found that adverse weather patterns could stimulate the release of CORT into the bloodstream during moulting season. Higher levels of CORT in blood increases CORT levels in feathers, although not necessarily proportionally, and can alter feather structure. Benderlioglu (2010) points out that not only higher CORT levels, but also higher cortisol levels have negative effects on ideal growth patterns. Jenni-Eiermann *et al.* (2015), Møller (1996), and Watson & Thornhill (1994) also suggest that unusual temperature and adverse weather effects can promote FA. Furthermore, nutritional and energetic stress can be causal of FA as found in the primaries of European Starlings as a response to nutritional and energetic stress (Swaddle & Witter, 1994).

1.7.2.3 Pollution

A wide variety of pollutants such as heavy metals and POPs can affect bilateral symmetry in birds (Parsons, 1990). It should be noted that feathers are connected to the bloodstream during growth, but become physiologically inert after the feather has matured. Therefore, feathers can contain compounds proportional to the bloodstream at the time of growth (Abbasi *et al*., 2017; García-Fernández *et al*., 2013). Contaminants also seem to be retained once incorporated (García-Fernández *et al*., 2013), but feather burdens of POPs are not necessarily indicative of body burdens due to the lipophilic nature of POPs, external contamination, and the limited exposure time of feathers to blood (Abbasi *et al.*, 2017; Eulaers *et al*., 2014; García-Fernández *et al*., 2013).

Below I will first discuss some examples of heavy metal pollution associated with FA, followed by examples of POPs.

There is an association between increased FA and relative proximity of the individual to areas with heavy metal pollution or radioactivity (Bustnes *et al*., 2002; Eeva *et al*., 2000; Herring *et al*., 2017; Watson & Thornhill, 1994). Eeva *et al*. (2000) measured FA in Pied Flycatchers *Ficedula hypoleuca* and Great Tit *Parus major* nestlings along the pollution gradient of a copper smelter. They found that the FA in the length of the third primary and in tarsus length (the only two variables that were measured) of Great Tit nestlings increased closer to the pollution source. Herring *et al*. (2017) investigated mercury levels in blood and breast feathers to investigate the association with FA in four water-related bird species (American Avocets *Recurvirostra americana*, Black-necked Stilts *Himantopus mexicanus*, Caspian Terns *Hydroprogne caspia*, and Forster's Terns *Sterna forsteri*). Significantly, they only found a positive association between elevated mercury levels and FA in Forster's Terns, even though the remaining species also had elevated mercury levels.

Many previous studies investigated the associations between POPs and FA. Bustnes *et al.* (2002) found that feathers seem to be influenced by organochlorine compounds. Jenssen *et al.* (2010) exposed 21 chicks of the European Shag *Phalacrocorax aristotelis* to polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated biphenyls (PBDEs), and found an association between high loads of PCBs and FA in the length of the wing bone. Furthermore, Bustnes *et al*. (2002) found that FA in the length of the third primary of Glaucous Gulls *Larus hyperboreus* was positively associated with the presence of PCB-99, PCB-118, oxychlordane, dichlorodiphenyldichloroethylene (DDE), and hexachlorobenzene (HCB). A study done by Bustnes *et al*. (2007) on the relations between POPs and wing feather growth in Great Black-backed Gulls *L. marinus* saw a weak but significantly positive relationship between POPs and differing feather lengths on opposing wings. A separate analysis revealed that males with high residues of PCBs had an increased probability of deviation from bilateral symmetry of wing feathers than birds with lower residues of PCBs.

1.7.2.4. Other factors

Watson & Thornhill (1994) suggest that FA can in some cases be attributed to measurement error. Helm & Albrecht (2000) sought to determine the effect of handedness of the measurer and the consistency of the measurement. They took four measurers (two left-handed and two right-handed) and had them measure the tarsus, wing length, and the length of the eight primary of 111 live-caught European Stonechats *Saxicola rubicola*. Each measurement was repeated thrice. Handedness of the measurer affected the consistency of the measurements. Due to the change of hands during measurements on opposing wings, Helm

& Albrecht (2000) argue that measurements conducted on the side of the preferred hand results in greater extension of the wing during measurement.

FA can also be attributed to other environmental stressors such as parasites and pathogens (Bize *et al*., 2004; Brown & Brown, 2002; Møller & Pomiankowski, 1993; Møller, 1996; Parsons, 1990) and increased predation risk (Benderlioglu, 2010).

Moreover, it has been hypothesized that directional external cues in sessile organisms could promote asymmetry on one side of the soma (Kellner & Alford, 2003; van Dongen, 2006). Examples include light or currents affecting sessile plants on one side, while handedness (preference for left or right) could be another bias. Directional cues could lead to antisymmetry or FA at population level, depending on the strength and direction of the cues individuals are exposed to (Kellner & Alford, 2003). This could explain why Kellner & Alford (2003) found strong directional asymmetry of skeletal measures in the first few post hatching days of domestic fowl chicks, attributable to contortion in the egg during embryonic development. They found however, that asymmetry rapidly decreased as the chicks matured.

An association between both hatching order and clutch size between FA of tarsus length has been found in common tern *S. hirnundo* chicks (Palestis, 2009). Chicks that hatched first had lower levels of FA, as did chicks from larger clutches.

Finally, absolute trait size could also provide more opportunity for the trait to display FA. The costly production of larger traits may act as a stressor that promotes FA (Aparicio & Bonal, 2002; Møller & Pomiankowski, 1993). For instance, FA in ears (small) could be smaller than FA in legs (large) (Leung, 1998).

1.7.3 Asymmetry and statistics

The statistical analysis of asymmetry has received much attention, especially the use of FA as an indicator of developmental stability. As there are many indices for the analysis of FA as an indicator of developmental instability, there is a need for consistency in the experimental and statistical design of studies on FA. There have been many reviews on the statistical analysis of FA and I will highlight the key concepts. For full reviews, I refer to that of Graham *et al*., (2010); Lens, (2001); Palmer, (1994); Palmer & Strobeck (1986); and Palmer & Strobeck (1992).

1.7.3.1 AS and DA

The validity of FA as an indicator of developmental instability relies on the exclusion of AS and DA from the trait being measured as both have a genetic basis (Knierim *et al*., 2007; Palmer, 1994). Traits could exhibit mixtures of AS, DA, and FA that confound the interpretation thereof (Palmer & Strobeck, 1992; Lens *et al*., 2001). Mixtures of different forms of asymmetry have a leptokurtic distribution (Palmer & Strobeck, 1992). Failure to detect and describe mixtures of asymmetries will weaken presumed associations with developmental instability (Palmer & Strobeck, 1992; Lens *et al*., 2001). Because the presence of AS and DA indicates that the differences found does not reflect "pure" developmental noise, these traits should be excluded when drawing associations with developmental stability (Palmer & Strobeck, 1992). The presence of DA could be tested through factorial ANOVA, while AS is described by the skew and kurtosis values (with a cut-off value of -1) (Knierim *et al*., 2007; Palmer, 1994; Palmer & Strobeck, 1992). It should be noted that outliers due to measurement error would affect kurtosis values (Palmer & Strobeck, 2001). Furthermore, the presence of the aforementioned asymmetric mixtures could be described with mixture analysis (van Dongen, 2006). Studies on FA should not only report location and variance, but also include skew and kurtosis values (Palmer & Strobeck, 1992; Graham *et al.*, 2010) together with signed and unsigned asymmetries for future meta-analysis (Graham *et al*., 2010).

1.7.3.2 Statistical power

Statistical power of analyses that compare variance is limited compared with analyses that compare means (Knierim *et al*., 2007; Palmer & Strobeck, 2001). Because FA is determined by the distribution of variance around the mean of zero, inconsistencies such as measurement error further weakens the statistical power (Knierim *et al*., 2007; Palmer, 1994; Lens *et al*., 2001). Thus, measurement error should be accounted for through hypothetical repeatability measurements (Palmer & Strobeck, 2001). Researchers must ensure that measurement error does not outweigh the variance that is considered FA. Since FA only accounts for 1-2% relative to the size of the trait being measured, one can see why consistent measurements are fundamental (Palmer, 1994; Palmer, 1996; Lens *et al*., 2001). Measurement error in relation to asymmetry can be tested through a two-way ANOVA (Palmer, 1994; Palmer & Strobeck, 1986; Palmer & Strobeck, 2001). Furthermore, sample sizes must be as large as practically possible (Graham *et al*., 2010). Statistical power could also be improved by using composite indexes that compares standardised measures of asymmetry in different traits (Graham *et al*., 2010). The Levene's test can be used to compare composite asymmetries of different groups (Graham *et al*., 2010). Finally, when

comparing FA among populations a one-way ANOVA with a Levene's test is recommended (Graham *et al*., 2010).

1.7.3.3 Trait selection

Organism-wide asymmetry does not frequently occur; therefore, researchers should make informed decisions regarding trait selection and include multiple traits in the experimental design (Lens *et al*., 2001; Leung *et al*., 2000 Palmer & Strobeck, 2001). Traits that are susceptible to wear should be avoided as this could lead to false-positive results (Knierim *et al*., 2007; Palmer & Strobeck, 2001). Finally, larger traits and ornamental traits in some cases display larger asymmetries (Knierim *et al*., 2007; Møller & Hoglund, 1991; Palmer & Strobeck, 2001; but see also Klingenberg & Nijhout, 1999). When estimating FA in populations, researchers must control for individual size-dependence within the population, this can be determined with a Levene's test (Palmer & Strobeck, 2001). If size dependent variation is present, it could be corrected with log-transformations.

1.7.4 Effect of FA

It is known that FA is associated negatively with growth rate, fecundity, and survival (Thornhill, 1992b). FA is a measure of an individual's ability to control development even under stressful circumstances (Møller, 1996; Thomas, 1993; Leung, 1998; Zackharof 1992 as seen in Møller, 1996). This gives insight on the overall health of the bird (Møller & Pomiankowski, 1993). There seems therefore to be a buffering capacity against the expression of FA, working to the advantage of species and individuals displaying low levels of FA.

Some examples of detrimental effects include:

- Wing asymmetry in birds can result in increased energetic cost during flight (Bustnes *et al*., 2002; Bustnes *et al*., 2007; Thomas, 1993).
- Agility, manoeuvrability, and take-off is enhanced by a reduction in asymmetry (Swaddle & Witter, 1998)
- Furthermore, it is known that poor quality feathers and FA can be associated with poor health such as low immune-competence and endocrine disruption (Jenni-Eiermann *et al*., 2015; Parsons, 1990; Rohwer & Rowher, 1994; Swaddle & Witter, 1994; Watson & Thornhill, 1994).
- Moreover, FA can have detrimental effects on an individual's mating success. Thornhill (1992a) found that male scorpion flies *Panorpa japonica* with little FA displayed higher mating success than males with high FA. Thornhill (1992b) also

found that low FA had a positive relationship with competitive success between two species of Japanese scorpion flies *P. nipponensis* and *P. ochraceopennis*. Furthermore, Møller (1992; 1993) and Shykoff & Møller (1999) found that female Barn Swallows *Hirundo rustica* preferred males with the least asymmetry in their elongated tail feathers.

Based on the above, it is safe to assume an association between increased FA and reduced fitness. Natural selection therefore can act on the expression and extent of fluctuating asymmetry (Van Valen 1962). This can happen through directional selection (Aparicio & Bonal, 2002). Thus, FA may have a significant role in the organisation of communities, especially in the persistence and relative abundance of competing species that react differently to the same stressors and individual genetic quality, phenotypically expressed as FA (Thornhill, 1992b).

1.7.5 Limitations of studies on FA

Although studies on FA have intrinsic limitations, it can be a useful indicator of stress and an individual's ability to cope with said stressors. Population-level FA can ultimately be a useful indicator of environmental stress (Lens *et al*., 1999). However, the argument that FA may be a poor general predictor of either stress or fitness also features in the literature (Leung & Forbes, 1996). Below, I list some limitations on the viability of FA as a measure of stress and fitness.

- Allometry needs to be controlled for (Leung, 1998),
- The need for a control/reference population (Clarke, 1995),
- Inadequate sample size (Graham *et al*., 2010; Leung & Forbes, 1996),
- Irregular expression between feathers (Abbasi *et al.*, 2017) and trait type (Graham *et al*, 2010; Leung & Forbes, 1996),
- Irregular intra-species responses (Swaddle & Witter, 1994; Swaddle *et al*., 1994),
- Irregular inter-species responses (Abbasi *et al.*, 2017; Herring *et al*., 2017),
- Inconsistency during measurement (Helm & Albrecht, 2000; Leung & Forbes, 1996; Watson & Thornhill, 1994),
- Variable and low-effect sizes (Leung & Forbes, 1996),
- Small deviations that are statistically significant, but not necessarily biologically significant (Underhill, 1999).

The current consensus regarding FA as a welfare indicator is that FA is a useful non-invasive indicator of stress and an individual's ability to cope with stressors (Graham *et al*., 2010; Knierim *et al*., 2007; Palmer, 1996). FA is important because it is the only indicator of

developmental homeostasis (Graham *et al*., 2010). Furthermore, FA is one of few morphological phenomena for which the ideal is known (Knierim *et al*., 2007; Lens *et al*., 2001; Palmer, 1996). There remains a need for consistency regarding the statistical analysis of FA. Finally, only a few studies have shown associations with fitness while associations with stress are more frequent (Graham *et al*., 2010; Palmer, 1996).

1.7.6 Current hypotheses on the origin of FA

Asymmetry has been studied intensely but many questions on causality remain unanswered. Consequently, there are many arguments in the literature that seek to explain this phenomenon. The present study focussed on determining whether structural asymmetry is present in the P8 feathers of Southern Masked-Weavers (SMWs) *Ploceus velatus*, rather than determining causal factors. Hypotheses that could explain the origin of FA (and asymmetry in general) are described below.

- *The accumulation of accidents hypothesis* posits that the developmental program does not target perfect symmetry but rather aims for a range of left-minus-right values about prefect symmetry. As long as developmental stressors do not cause asymmetry to deviate outside these ranges, asymmetry will follow a random walk through time (Kellner & Alford, 2003).
- *The directional external cues hypothesis* posits that asymmetry can be induced by side-biased environmental influences (Kellner & Alford, 2003).
- *The persistent asymmetry hypothesis* posits that deviation from symmetry can be genetic or caused by environmental effects in the early ontogeny, and that the magnitude will persist over time (Kellner & Alford, 2003).
- *The compensatory growth hypothesis* posits that large deviations between left and right symmetry is not the norm - there would be feedback mechanisms. This can occur through increased growth on the lagging side, or halted growth on the larger side (Kellner & Alford, 2003).
- *The residual growth hypothesis* posits that asymmetry is influenced by compensatory mechanisms that counter developmental stressors. Therefore, asymmetry reflects only recent exposure to developmental stressors as individuals have the ability to correct the deviation (Kellner & Alford, 2003).
- *The coin-toss hypothesis* posits that structures grow through the accumulation of independent morphological subunits. Asymmetry is the result of differences between corresponding morphological subunits. These differences are determined by chance (Kellner & Alford, 2003).

- *The magnification of asymmetry hypothesis* posits that morphogenesis magnifies small variations in the initial growth conditions. This would ultimately lead to large asymmetries in larger traits (Kellner & Alford, 2003).

1.8 Feather adaptations and hypotheses

Over millions of years, natural selection of animals favoured effective locomotion distinctive to life history components (i.e. mating, development, learning, foraging, hunting/escaping, reproduction, homing, and migration). Morphological and locomotary characteristics are integrated with ecology and habitat toward enhanced fitness. Characteristics are integrated in such a way that the resultant combination favours the least amount of energy needed for survival while maximising fitness (Lingham-Soliar, 2015). Birds have adapted to their environments (seasons, climate, etc.), but over the past few centuries they are facing new and previously un-encountered challenges caused by the rapid growth in humanity, industry, fisheries, and agriculture (Clarke, 1995). Some of these challenges include pollution, climate change, habitat destruction, and invasive species (Clarke, 1995).

The complexity of nature makes it a daunting task to determine the long-term interactions between the fitness of a species and its surrounding environment. Therefore, understanding the bio-indicators such as the expression and causal factors of asymmetry has become integral to conservation.

My research was constructed to investigate the degree of laterally-associated differences in primary feather structure (LADPFS) in the P8 feathers of newly moulted (early season) and old feathers (late season) of SMWs. Young and adult males and females were compared separately (Fig 1.8.1) to determine any anomalies found between age and sex.

Fig 1.8.1: *A schematic representation of the seven comparisons in the present study.*

In this study, the P8 feathers of SMWs were compared between left and right wings, across old and newly moulted feathers, age, and sex. Since SMWs are sexually dimorphic, I did not compare the sexes.

- My first hypothesis is that there are laterally-associated differences in primary feather structure (LADPFS) between the left and right P8 feathers of SMWs for both males and females, and that the LADPFS patterns will differ between sex and age.
- My second hypothesis is that LADPFS will be expressed differently between early season (new) primaries and late season (old) primaries for both sexes and ages.

Chapter 2: Materials and methods

2.1 Species

This study was conducted on the P8 flight feathers of the plentiful Southern Masked-Weaver *Ploceus velatus*. The SMW is a resident breeding species in most parts of southern Africa and has an IUCN Red List category of Least Concern (IUCN, 2015). They do not have seasonal migration, and only show limited local movement (Chittenden *et al*., 2012; Sinclair *et al*., 2011), more so in arid regions (Herremans, 1994). Habitat includes arid, fynbos, Nama-Karoo, and moist savanna (Chittenden *et al*., 2012). The breeding season is normally from September to March in the summer rainfall region (Sinclair *et al*., 2011) where males build several nests (Walsh *et al*., 2011). Adults of the sexually dimorphic SMW have a partial pre-breeding (spring) moult and a complete post-breeding (autumn) moult.

Primaries of SMW are numbered from the carpal joint to the end of the extended wing (descendent numbering). SMWs start their moult at P1 and moult their feathers descendently (from P1 to P10). The moulting period can last between 74-80 days and is linked to rainfall (Hockey *et al*., 2005).

In the breeding season, males can be distinguished from females by their bright yellow colour and a black face mask. Females have a pale yellow colour and a brown eye, while males have red eyes. In some cases, females have a red eye, in which case one has to rely on other features (e.g. colour and size) to identify the sex in the non-breeding season.

2.2 Study site

OPM Prozesky Bird Sanctuary (PBS) is located south of Potchefstroom in the North-West Province, South Africa. PBS is a wetland with numerous ponds and many reed beds and is densely vegetated and has a notable abundance of birds. The site is surrounded by housing to the north and agriculture to the south, west, and east. Notably, it is also adjacent to a waste-water treatment plant. PBS boasts a high number and diversity of birds, and has hides overlooking the ponds, making it a beloved sanctuary for birders of the area.

2.3 Data collection

Late season feathers were collected in the summer (August to March) before they started their complete post-breeding moult. Early season feathers were collected shortly after the completion of their complete post-breeding moult (June to July). Birds were live-trapped using mist nets and ringed as per normal procedure for bird ringing. The nets were placed on a bank between thick reeds and a pond (Figs. 2.3.1 – 2.3.3). The nets were inspected and

emptied every 20 minutes until the daily quota of 30 individuals was reached. Trapping started between 05:00-06:00 am, depending on the time of sunrise for the particular season.

Fig 2.3.1: *Pond adjacent to mist net.*

Fig 2.3.2: *Mist net used to capture birds.*

Fig 2.3.3: *Reeds adjacent to mist net.*

Feathers were collected in collaboration with and supervised by an experienced local bird ringer. To ensure as little stress as possible and enough time for processing, the daily maximum of individuals sampled never exceeded 30. Birds showing signs of injury were excluded. Birds that had yet to replace fully their eighth primary for the early season were also excluded. One hundred-and-eighteen birds were trapped and sampled in the late season (73 females and 45 males). During the early season, 131 (83 females and 48 males) birds were trapped and sampled. More females than males were caught.

Captured individuals were placed individually in linen bags. Birds were individually processed before the next bird was processed. Sex was determined and all the birds were

Fig 2.3.4: *A photo was taken of the head of each individual; here is a female SMW.*

Fig 2.3.5: *A photo was taken of the stomach of each individual; here is a female SMW.*

Fig 2.3.6: *A photo was taken of the ring of each individual; here is a female SMW.*

scored for moult and age (young or adult). Each individual was measured to determine body length (head to tail), and left and right wing length (shoulder to primary tip), using a stopped metal ruler. Once measurements were completed, the bird was weighed by placing it in a small container with a tared mass. This information was kept as a reference and for use in possible future studies (see Section 5.3). Primary eight was plucked from both the left and right wing of each individual and stored in paper envelopes. Care was taken to ensure that newly moulted P8s in the early season had fully emerged (see de Beer *et al*., 2001). Fully emerged primaries are recognisable as they do not have a blood quill or a sheaved calamus. Photos of the head, body, and ring number were taken of each individual as a reference (Fig 2.3.4 - 2.3.6).

2.4 The measurement of feather Variables

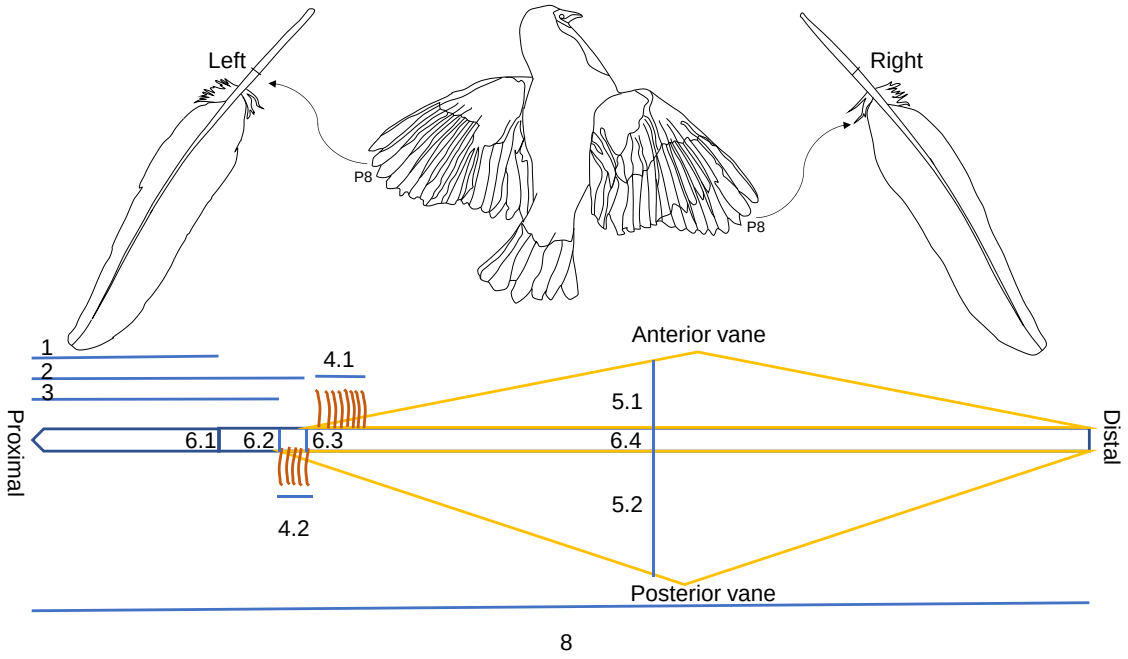

Fig 2.4.1: *A schematic representation of the numbered Variables measured of the left and right P8 feathers of each individual.*

The left and right P8 feathers were placed between two microscope slides and measured according to the prescribed numbered Variables (Fig 2.4.1). Variables were measured in micrometres (µm) on a Nikon AZ 100 microscope at appropriate magnifications. The measurement of each Variable is explained below using photographs.

2.4.1 Variable 1

Variable 1 was measured as the distance between the proximal tip of the rachis and the distal end of the calamus.

Fig 2.4.2: *Example of the measurement of Variable 1.*

2.4.2 Variable 2

Variable 2 was measured as the distance between the proximal tip of the calamus and the start of the plumaceous barbs of the anterior vane, where the first proximate barb emerges from the rachis.

Fig 2.4.3: *Example of the measurement of Variable 2.*

2.4.3 Variable 3

Variable 3 was measured as the distance between the proximal tip of the calamus and the start of the plumaceous barbs of the posterior vane, where the first proximate barb emerges from the rachis.

Fig 2.4.4: *Example of the measurement of Variable 3.*

2.4.4 Variable 4.1

Variable 4.1 was measured from the proximal edge of the first plumaceous barb of the anterior vane to the distal edge of the last plumaceous barb, where the respective barbs emerge from the rachis.

Fig 2.4.5: *Example of the measurement of Variable 4.1.*

2.4.5 Variable 4.2

Variable 4.2 was measured from the proximal edge of the first plumaceous barb of the posterior vane to the distal edge of the last plumaceous barb, where the respective barbs emerge from the rachis.

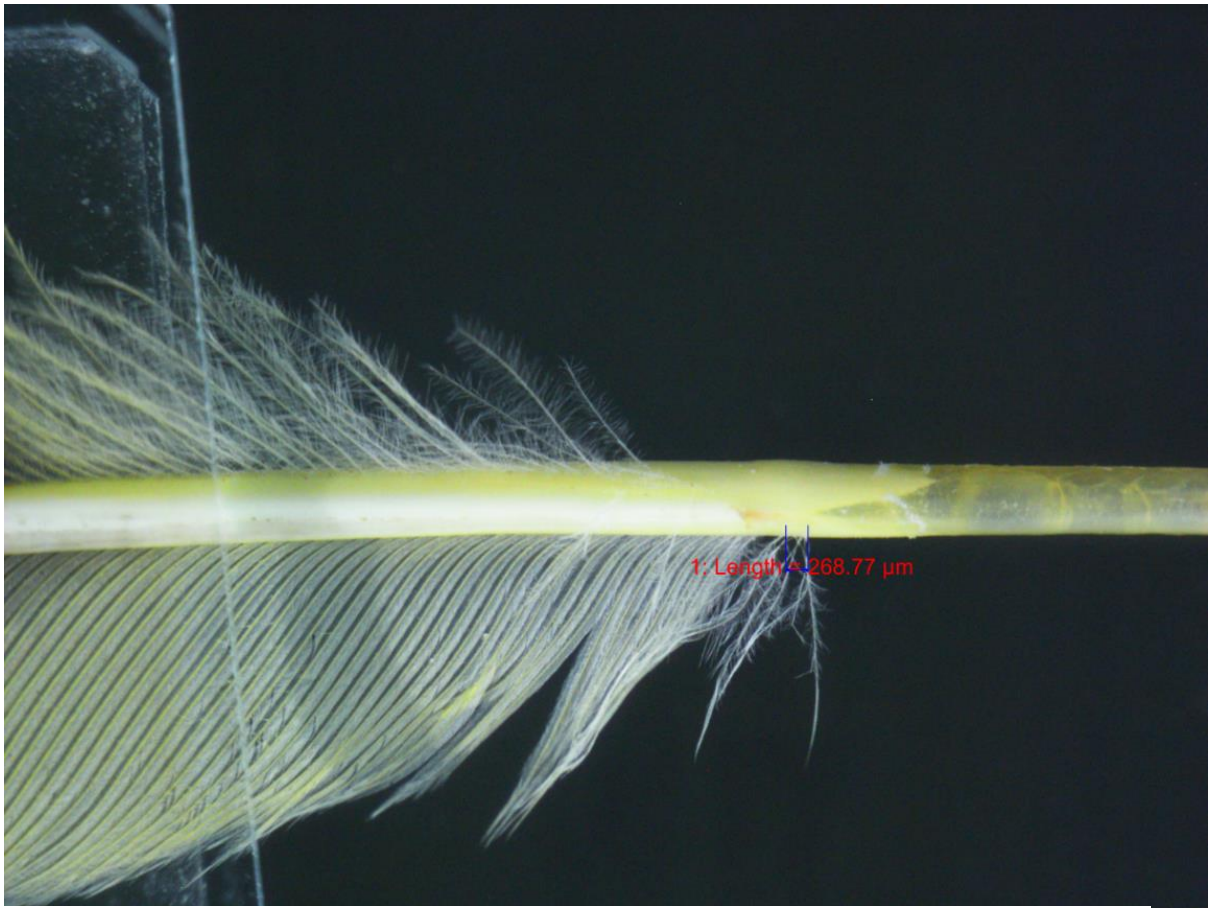

Fig 2.4.6: *Example of the measurement of Variable 4.2.*

2.4.6 Variable 5.1

To determine a comparable (between individual feathers that may have different lengths) proportional location for Variable 5.2, the length of Variable 3 (Fig 2.4.1 and Fig 2.2.4) was multiplied by 2.5. This calculated distance was measured from the proximal tip of the calamus. Variable 5.1 was measured at 90º from the anterior edge of the rachis until the edge of the anterior vane. Care was taken to not disturb or distort the vane.

Fig 2.4.7: *Example of the measurement of Variable 5.1.*

2.4.7 Variable 5.2

To determine a comparable (between individual feathers that may have different lengths) proportional location for Variable 5.2, the length of Variable 3 (Fig 2.4.1 and Fig 2.2.4) was multiplied by 2.5. This calculated distance was measured from the proximal tip of the calamus. Variable 5.2 was measured at 90º from the posterior edge of the rachis until the edge of the posterior vane. Care was taken not to disturb or distort the vane.

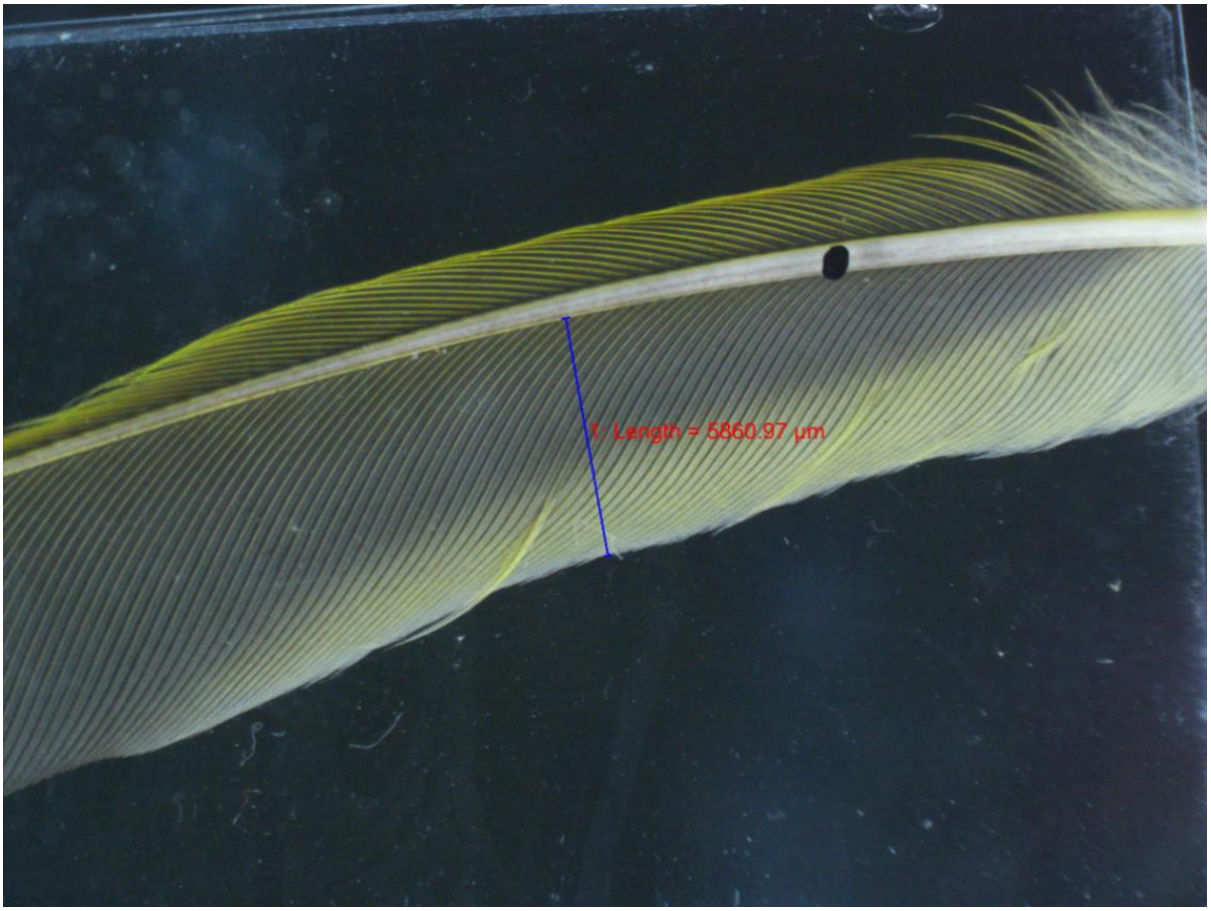

Fig 2.4.8: *Example of the measurement of Variable 5.2.*

2.4.8 Variable 6.1

Variable 6.1 was measured as the width of the rachis at the distal edge of the calamus.

Fig 2.4.9: *Example of the measurement of Variable 6.1.*

2.4.9 Variable 6.2

Variable 6.2 was measured as the width of the rachis at the start of the plumaceous barbs on the posterior vane.

Fig 2.4.10: *Example of the measurement of Variable 6.2.*

2.4.10 Variable 6.3

Variable 6.3 was measured as the width of the rachis at the start of the plumaceous barbs on the anterior vane.

Fig 2.4.11: *Example of the measurement of Variable 6.3.*

2.4.11 Variable 6.4

To determine a comparable (between individual feathers that may have different lengths) proportional location for the measurement of Variable 6.4, the length of Variable 3 (see Fig 2.4.1 and Fig 2.2.4) was multiplied by 2.5. This calculated distance was measured from the proximal tip of the calamus. Variable 6.4 was measured as the width of the rachis at this location.

Fig 2.4.12: *Example of the measurement of Variable 6.4.*

2.4.12 Variable 8

The entire P8 feather was too long to be viewed as a single frame under the microscope at its smallest magnification. Therefore, the feather was placed between two microscope slides that were marked with a permanent marker. These marks were positioned over the centre of the rachis, to ensure that accurate measurements could be taken along the natural curvature of the rachis. Measurements for Variable 8 were taken from the centre of the most proximal point of the calamus to the most distal centre of the fist mark. Hereafter, the image was adjusted by moving the objective table, without disturbing the feather or slide, and a second measurement was taken from the previous endpoint to the distal centre of the second mark. After another adjustment of the image, the last measurement was taken from the previous endpoint to the most distal point of the rachis. The sum of these three measurement was the total length of the rachis (Variable 8)

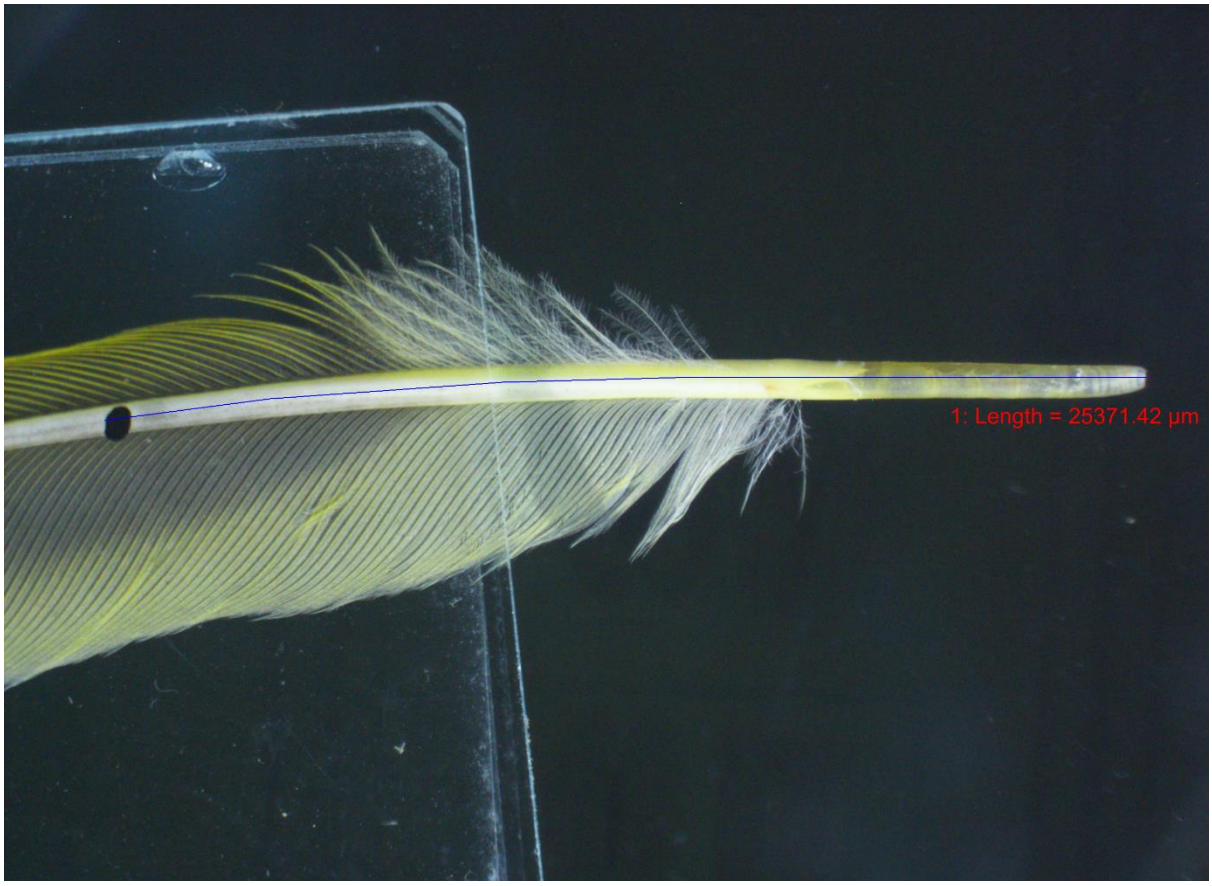

Fig 2.4.13 A: *Example of the first part of the measurement of Variable 8.*

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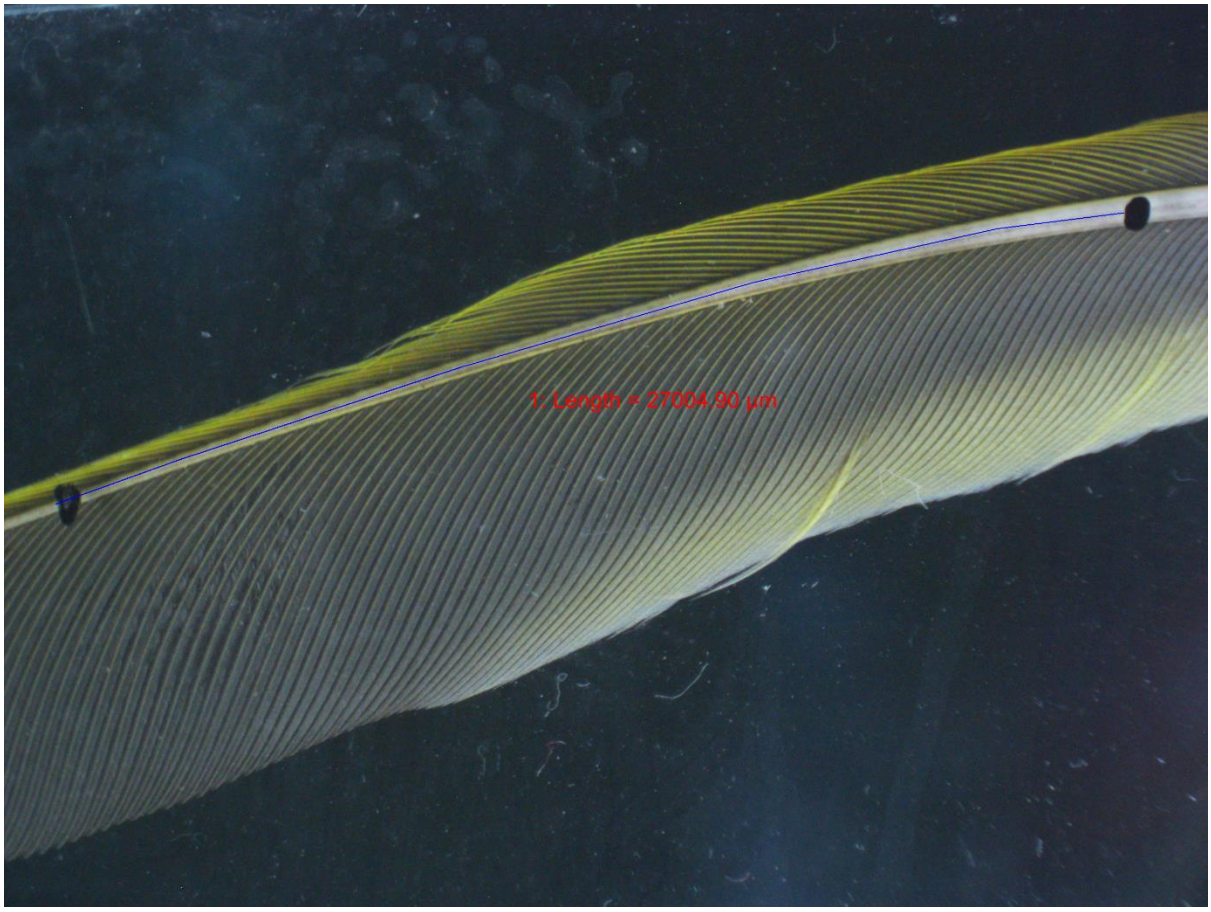

Fig 2.4.13 B: *Example of the second part of the measurement of Variable 8.*

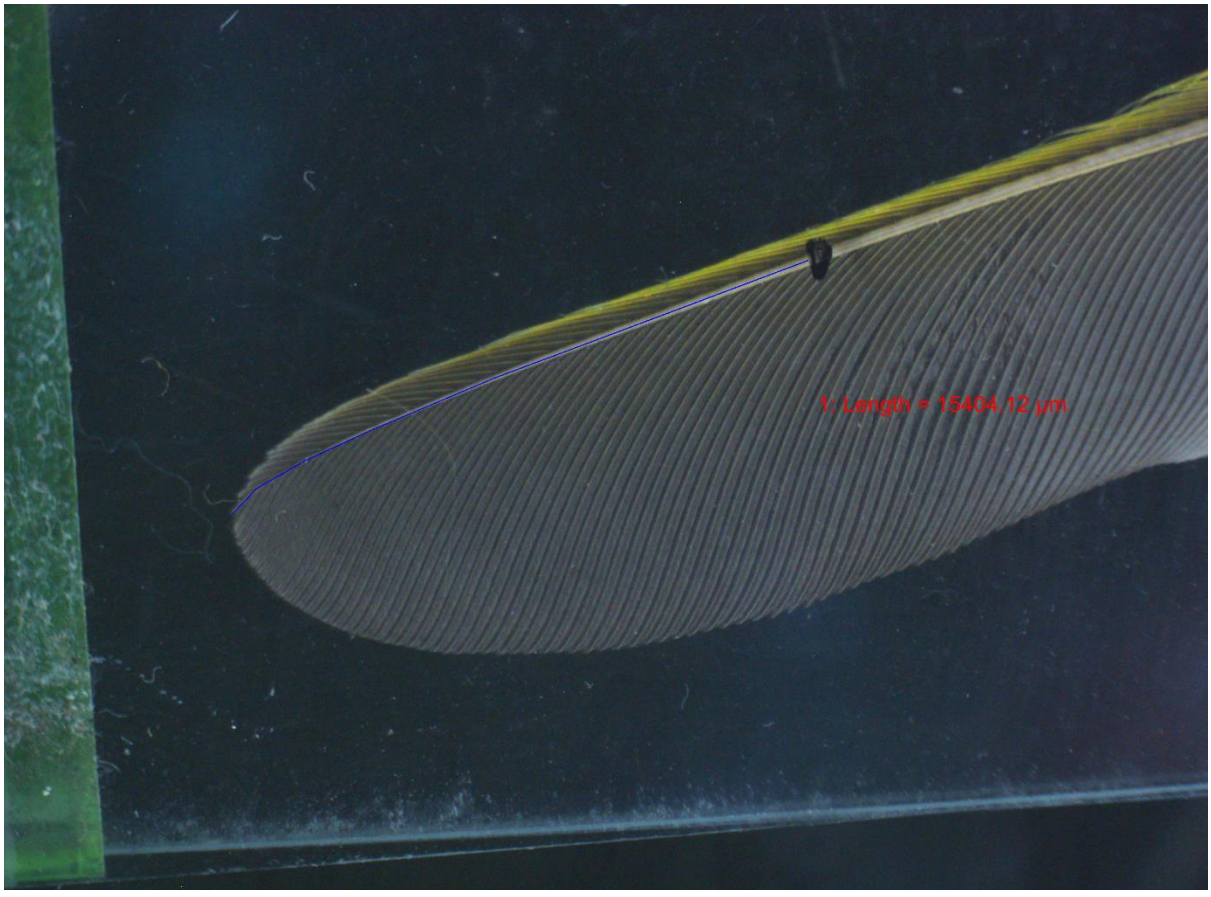

Fig 2.4.13 C: *Example of the final part of the measurement of Variable 8.*

2.5 Statistics

Statistics were calculated using GraphPad Prism 5.0 (www.graphpad.com). The D'Agostino & Pearson omnibus normality test was used to determine normality. Two tailed, paired t-tests were performed. Since data were not always normally distributed, t-tests (two-tailed) of untransformed (for normally distributed data) and log-transformed (for skewed) data were conducted. More details are presented in the following sections. Significance was assumed at $p < 0.05$.

2.5.1 Left vs right

Because asymmetries in the feather structure can be influenced by antisymmetry, directional symmetry, and fluctuating asymmetry, the aim of this study was to show that LADPFS are present and that it will be expressed differently between individual age, feather age, and sex; regardless of the causal factor (AS, DA, or FA). This study does not include left-minus-right comparisons or deviation from zero, as the causal factor behind LADPFS were not within the scope of this study. However, some inferences will be made. Therefore, two-tailed paired t-tests were used as a robust measure of structural variation between left and right primaries.

The individuals (n = 248) were grouped according to sex (male and female), age (young and adult), and the season they were sampled in (late season and early season). The seven groups were late season adult females (n = 53), late season young females (n = 20), late season adult males (n = 37), late season young males (n = 8), early season adult females (n = 45), early season young females (n = 38), and early season adult males (n = 43). Early season young males (n = 5) could not be compared as there were too few individuals for statistical significance. Left and right primaries were compared as paired data, with two-tailed t-tests for all Variables. Scatterplots for both untransformed and log-transformed data will be presented to display the absolute differences in µm.

Chapter 3: Results

This chapter is divided into two sections to present and characterise the differences observed. The results of left vs right (LvR) comparisons of female groups are presented first, followed by male groups.

Female and male left vs right (comparisons 1-7, see Fig 1.8.1)

I present the results of LvR primaries for all Variables with scatterplots, demonstrating means and standard deviations. Significant ($p < 0.05$), and insignificant differences will be shown for the seven groups (late season adult females, late season young females, early season adult females and early season young females, late season adult males, late season young males, and early season adult males). Scatterplots are followed by Table 3.1.1 (females) and Table 3.5.1 (males), which summarizes the means, t-test results (p-values), and normality of LvR comparisons, for each Variable of all groups. These values, along with the percentage coefficients of variation (%CVs) and standard deviations (SDs) can be found in Appendix A. This section concludes with a schematic representation of the results of adult females (Table 3.1.2), young females (Table 3.1.3), adult males (Table 3.5.2) and young males (Table 3.5.3) for early season and late season primaries.

3.1 Female left vs right (comparisons 1-4, Fig 1.8.1)

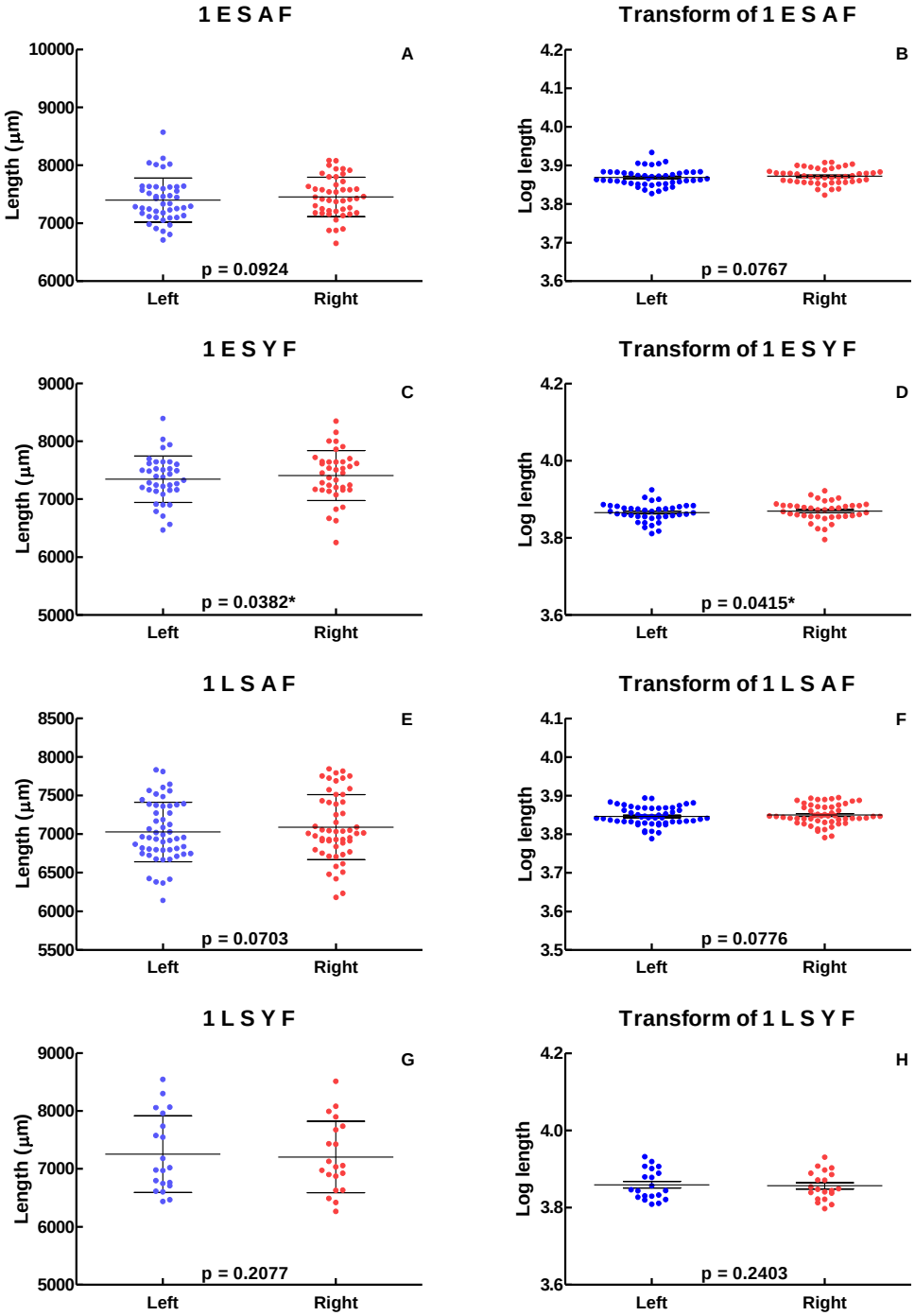

Fig 3.1.1: *Scatterplots of feather Variable 1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.1 Variable 1

Fig. 3.1.1 A and B: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data for early season adult females. There were no significant differences for log-transformed data.

Fig. 3.1.1 C and D: Data were normally distributed for non-transformed and log-transformed data for early season young females. Variable 1 (non-transformed) was significantly shorter for the left primaries in comparison with the right primaries.

Fig 3.1.1 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.1 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

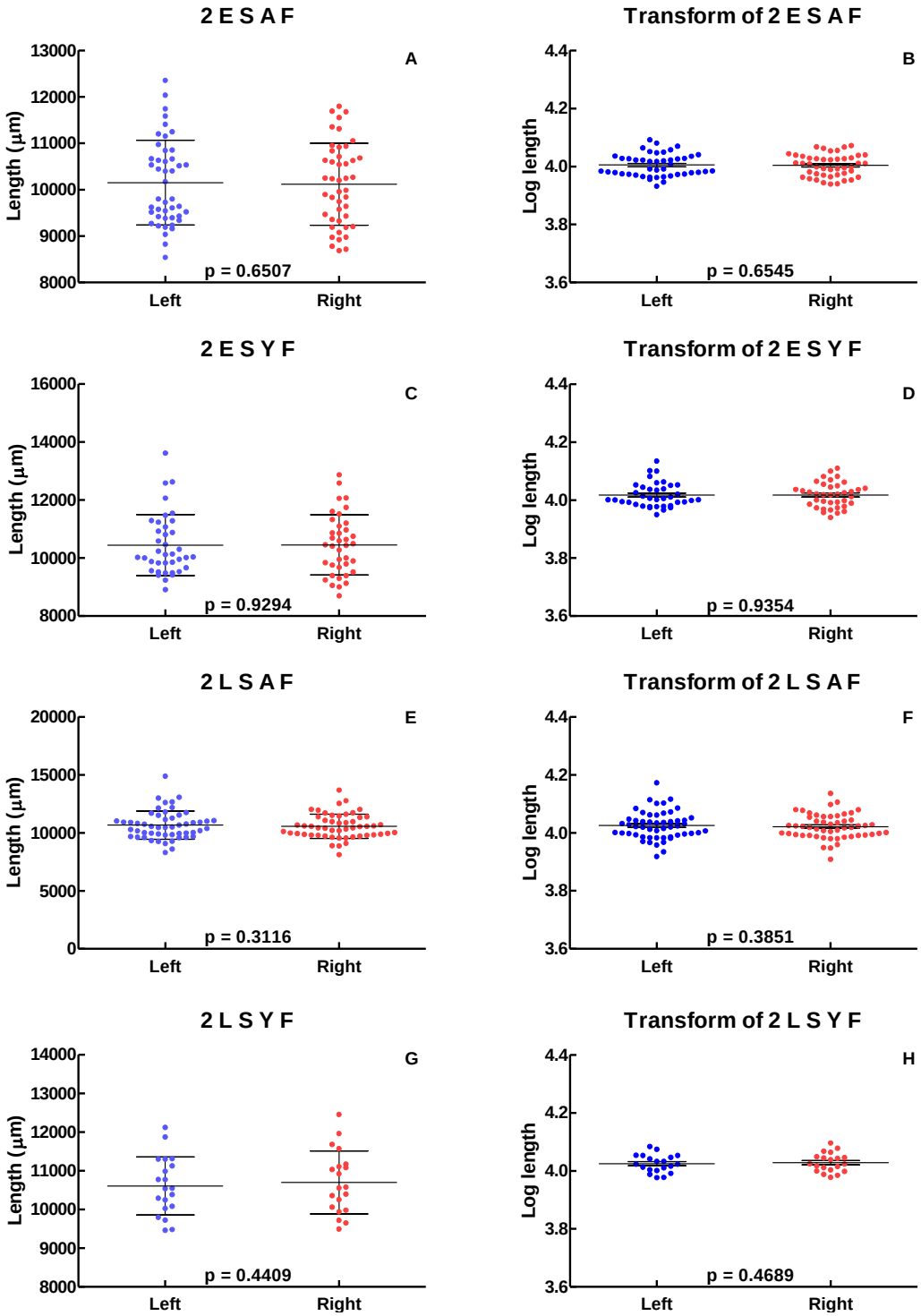

Fig 3.1.2: *Scatterplots of feather Variable 2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.2. Variable 2

Fig. 3.1.2 A and B: Data were normally distributed for non-transformed and log-transformed t-tests of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.2 C and D: Data were not normally distributed for non-transformed or log-transformed t-tests of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.2 E and F: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult females. There were no significant differences for log-transformed data.

Fig 3.1.2 G and H: Data were normally distributed for non-transformed and log-transformed t-tests of late season young females. There were no significant differences for non-transformed data.

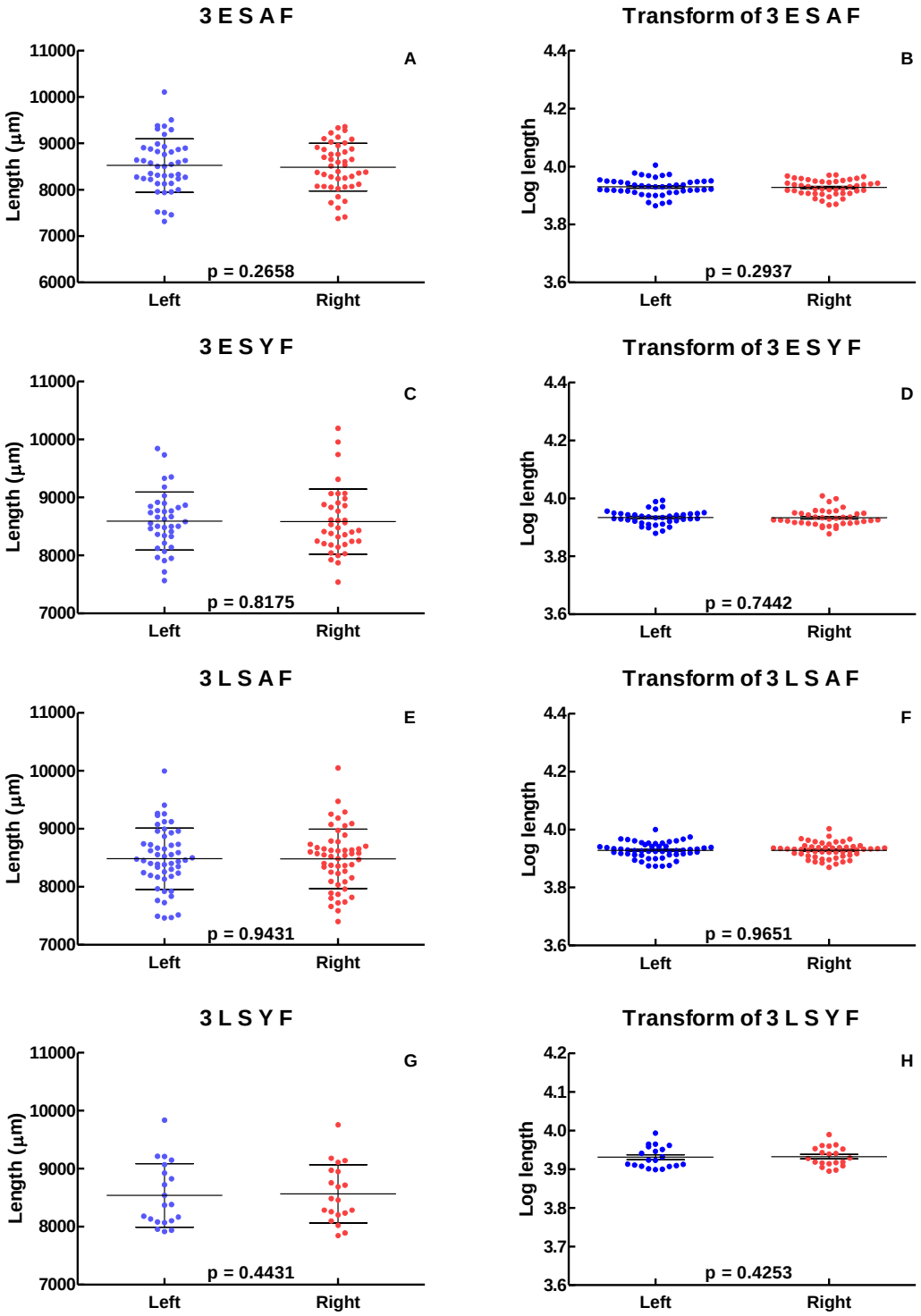

Fig 3.1.3: *Scatterplots of feather Variable 3 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.3. Variable 3

Fig. 3.1.3 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.3 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of early season young females. There were no significant differences for log-transformed data.

Fig 3.1.3 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.3 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

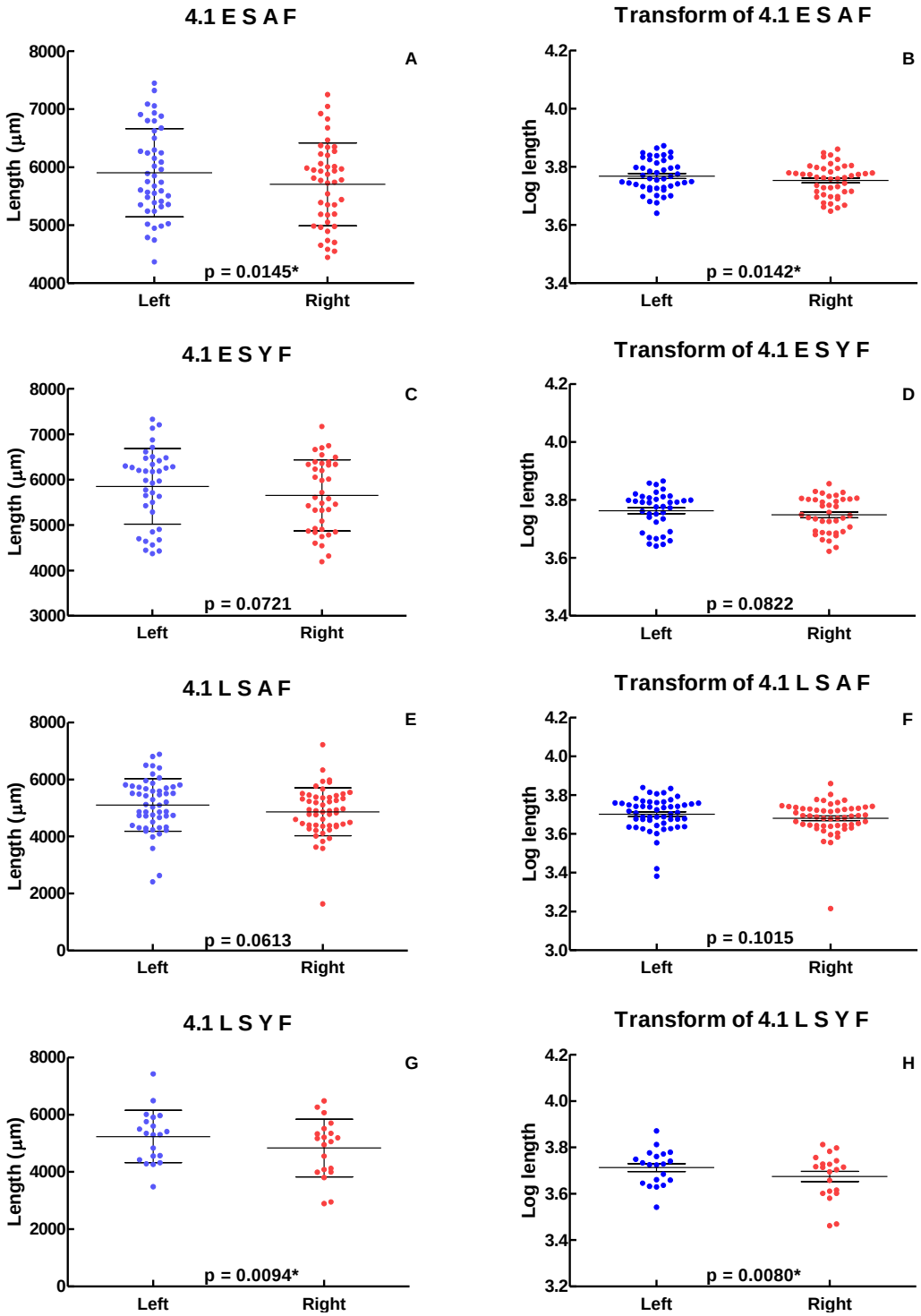

Fig 3.1.4: *Scatterplots of feather Variable 4.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.4. Variable 4.1.

Fig. 3.1.4 A and B: Data were normally distributed for non-transformed and log-transformed data for early season adult females. Variable 4.1 (non-transformed) was significantly longer for the left primaries in comparison with the right primaries.

Fig. 3.1.4 C and D: Data were normally distributed for non-transformed and log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.4 E and F: Data were not normally distributed for non-transformed or log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.4 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. Variable 4.1 (non-transformed) was significantly longer for the left primaries in comparison with the right primaries.

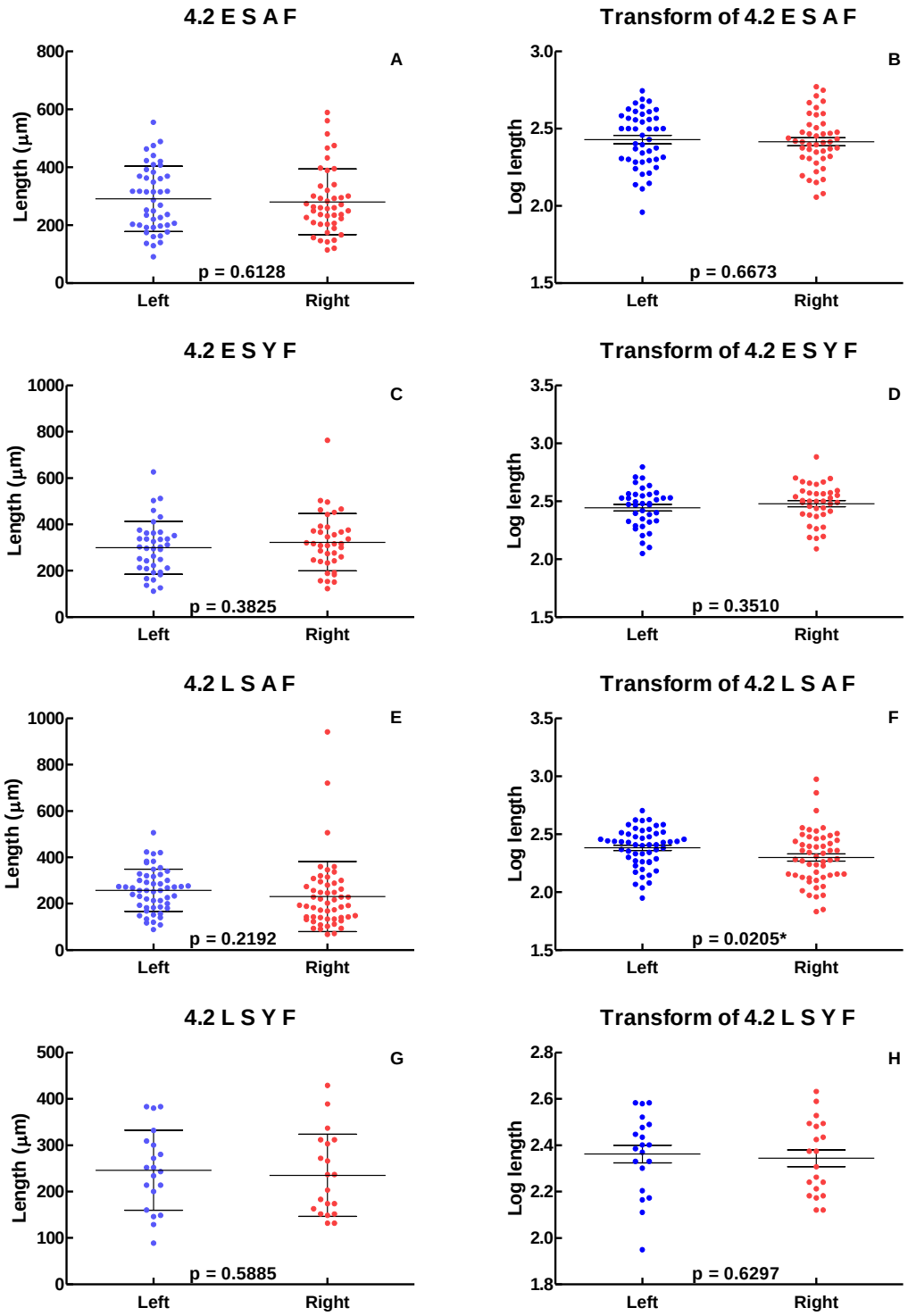

Fig 3.1.5: *Scatterplots of feather Variable 4.12(see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.5. Variable 4.2

Fig. 3.1.5 A and B: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of early season adult females. There were no significant differences for log-transformed data.

Fig. 3.1.5 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of early season young females. There were no significant differences for log-transformed data.

Fig 3.1.5 E and F: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult females. Variable 4.2 (log-transformed) was significantly longer for the left primaries in comparison with the right primaries.

Fig 3.1.5 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

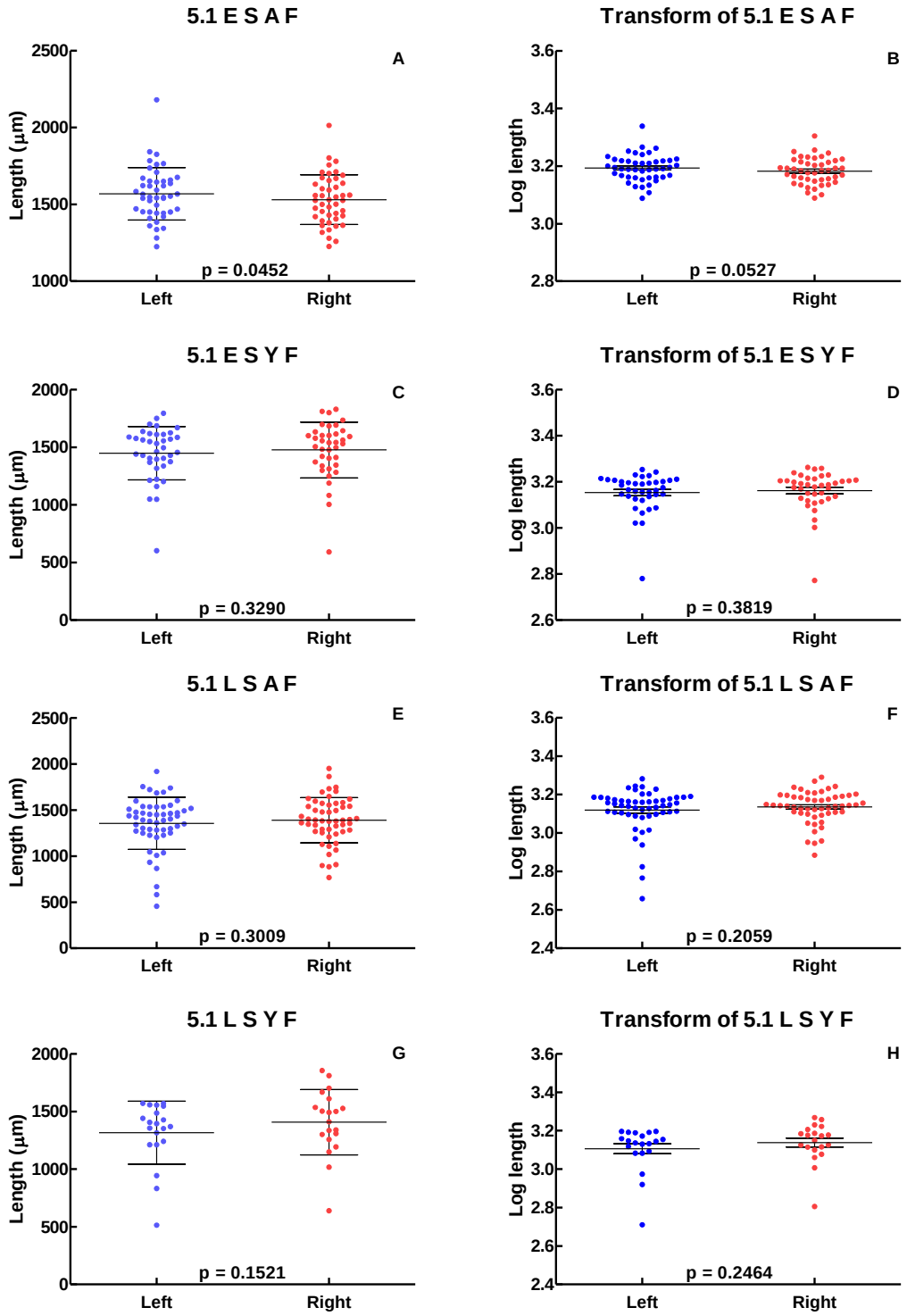

Fig 3.1.6: *Scatterplots of feather Variable 5.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.6. Variable 5.1

Fig. 3.1.6 A and B: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of early season adult females. Variable 5.1 (non-transformed was significantly wider for the left primaries in comparison with the right primaries, but lacked normal distribution.

Fig. 3.1.6 C and D: Data were not normally distributed for non-transformed or log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.6 E and F: Data were not normally distributed for non-transformed or log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.6 G and H: Data were not normally distributed for non-transformed or log-transformed data of late season young females. There were no significant differences for non-transformed data.

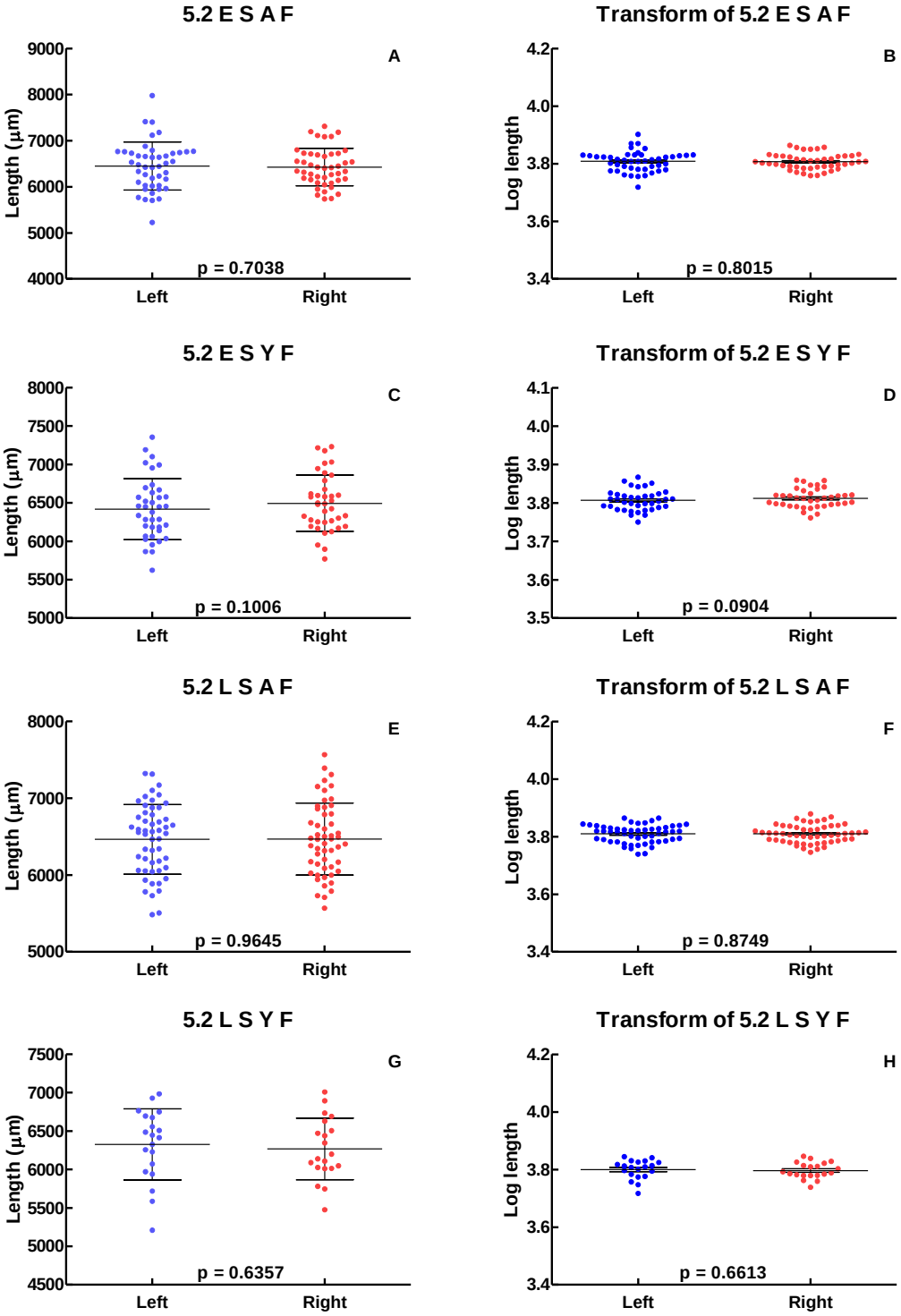

Fig 3.1.7: *Scatterplots of feather Variable 5.2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.7 Variable 5.2

Fig. 3.1.7 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.7 C and D: Data were normally distributed for non-transformed and log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.7 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.7 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

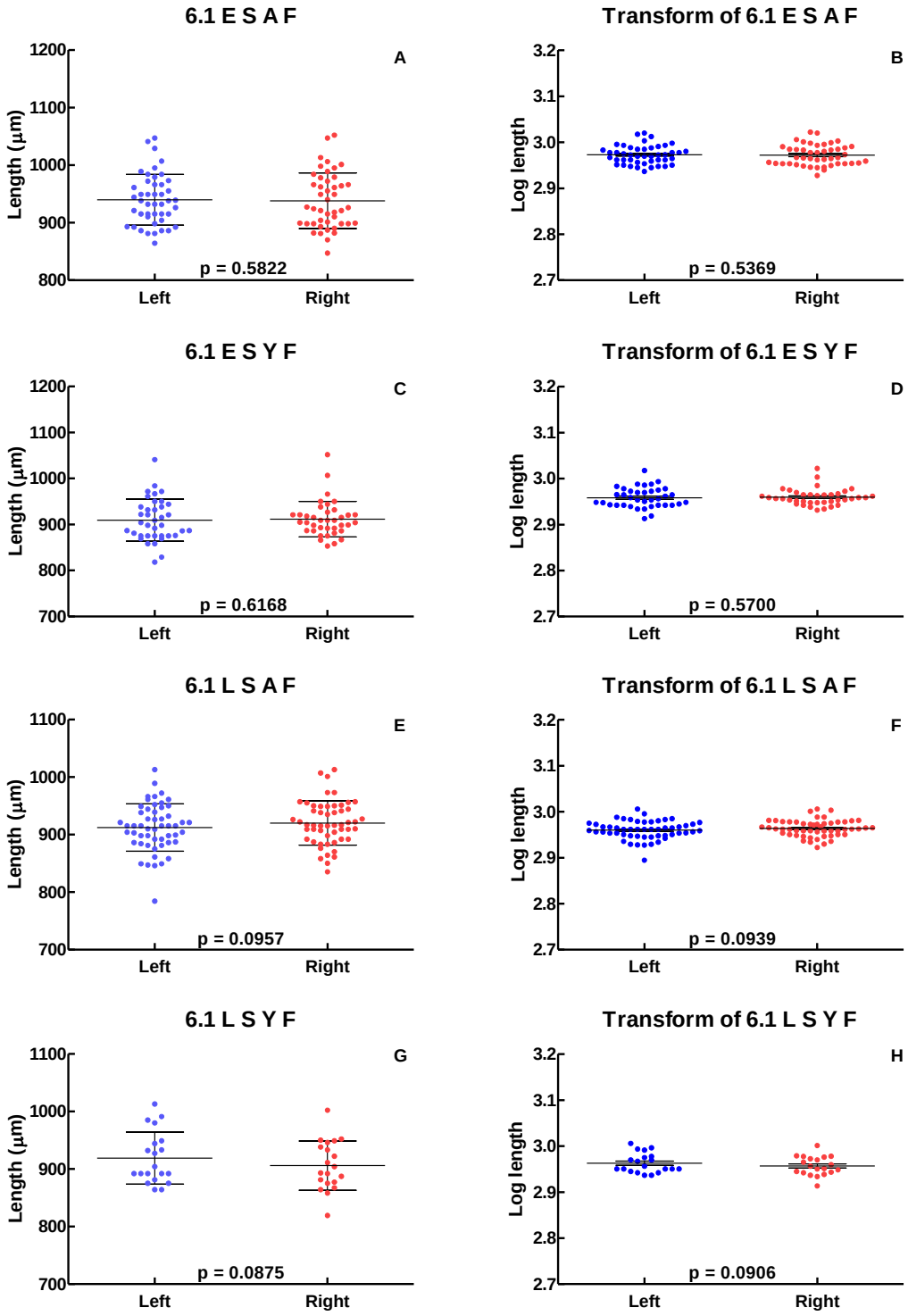

Fig 3.1.8: *Scatterplots of feather Variable 6.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.8 Variable 6.1

Fig. 3.1.8 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.8 C and D: Data were not normally distributed for non-transformed or log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.8 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.8 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

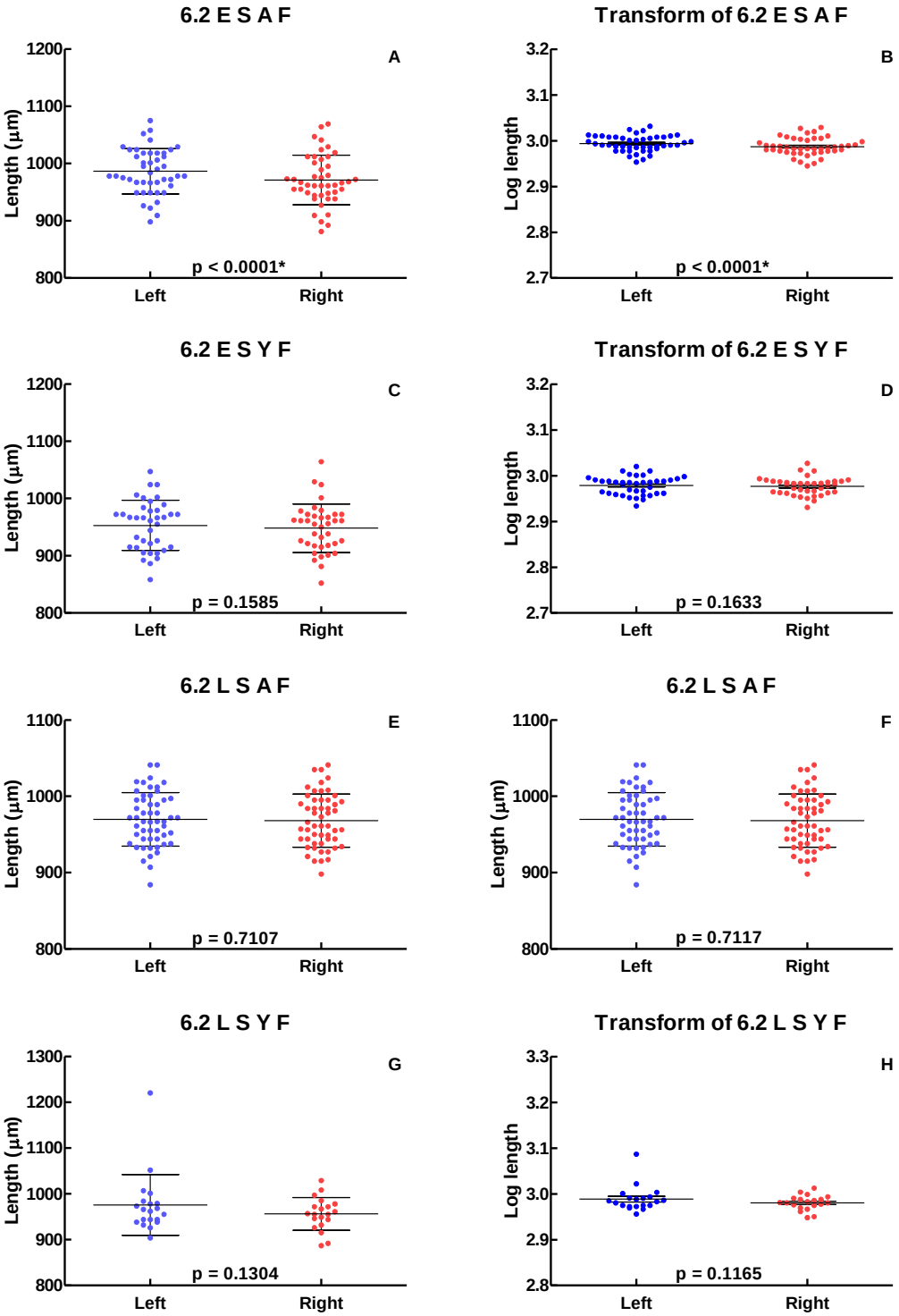

Fig 3.1.9: *Scatterplots of feather Variable 6.2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.9 Variable 6.2

Fig. 3.1.9 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. Variable 6.2 (non-transformed) was thicker on the left primaries in comparison with the right primaries.

Fig. 3.1.9 C and D: Data were normally distributed for non-transformed and log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.9 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. There were no significant differences for non-transformed data.

Fig 3.1.9 G and H: Data were not normally distributed for non-transformed or log-transformed data of late season young females. There were no significant differences for non-transformed data.

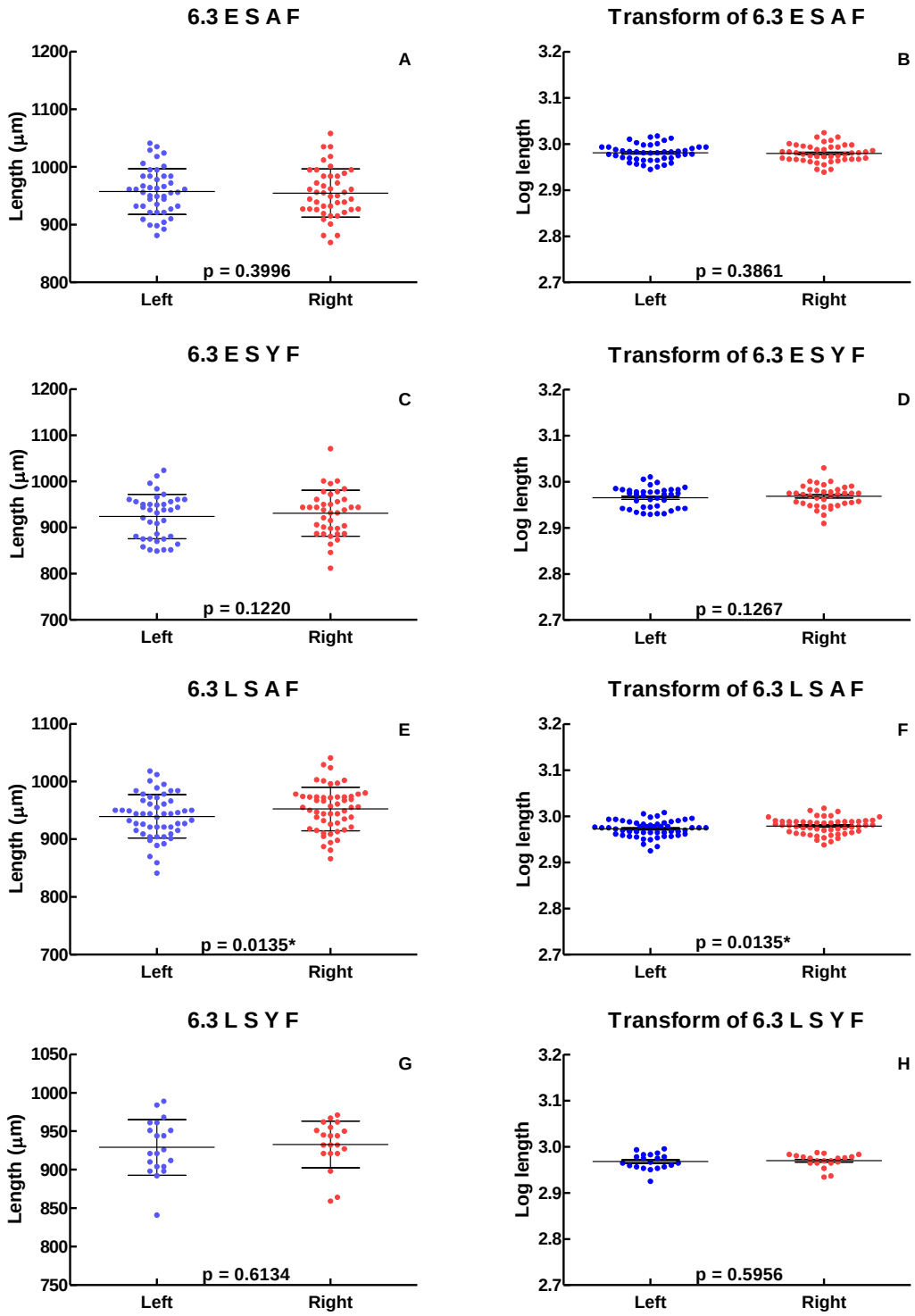

Fig 3.1.10: *Scatterplots of feather Variable 6.3 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.10 Variable 6.3

Fig. 3.1.10 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.10 C and D: Data were normally distributed for non-transformed and log-transformed data of early season young females. There were no significant differences for non-transformed data.

Fig 3.1.10 E and F: Data were normally distributed for non-transformed and log-transformed data of late season adult females. Variable 6.3 (non-transformed) was thicker on the left primaries in comparison with the right primaries.

Fig 3.1.10 G and H: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season young females. There were no significant differences for non-transformed data.

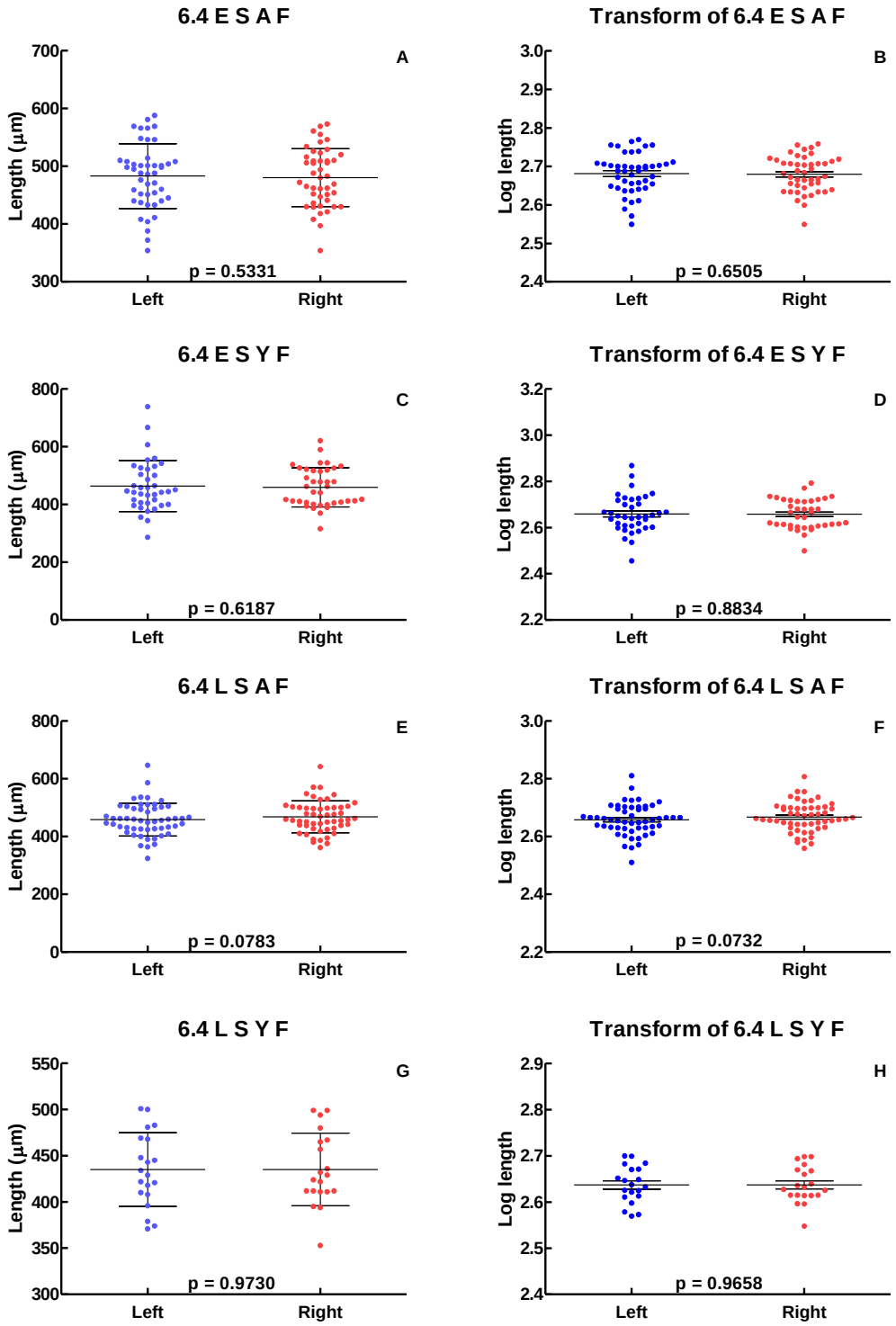

Fig 3.1.11: *Scatterplots of feather Variable 6.4 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.11 Variable 6.4

Fig. 3.1.11 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.11 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of early season young females. There were no significant differences for log-transformed data.

Fig 3.1.11 E and F: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult females. There were no significant differences for log-transformed data.

Fig 3.1.11 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

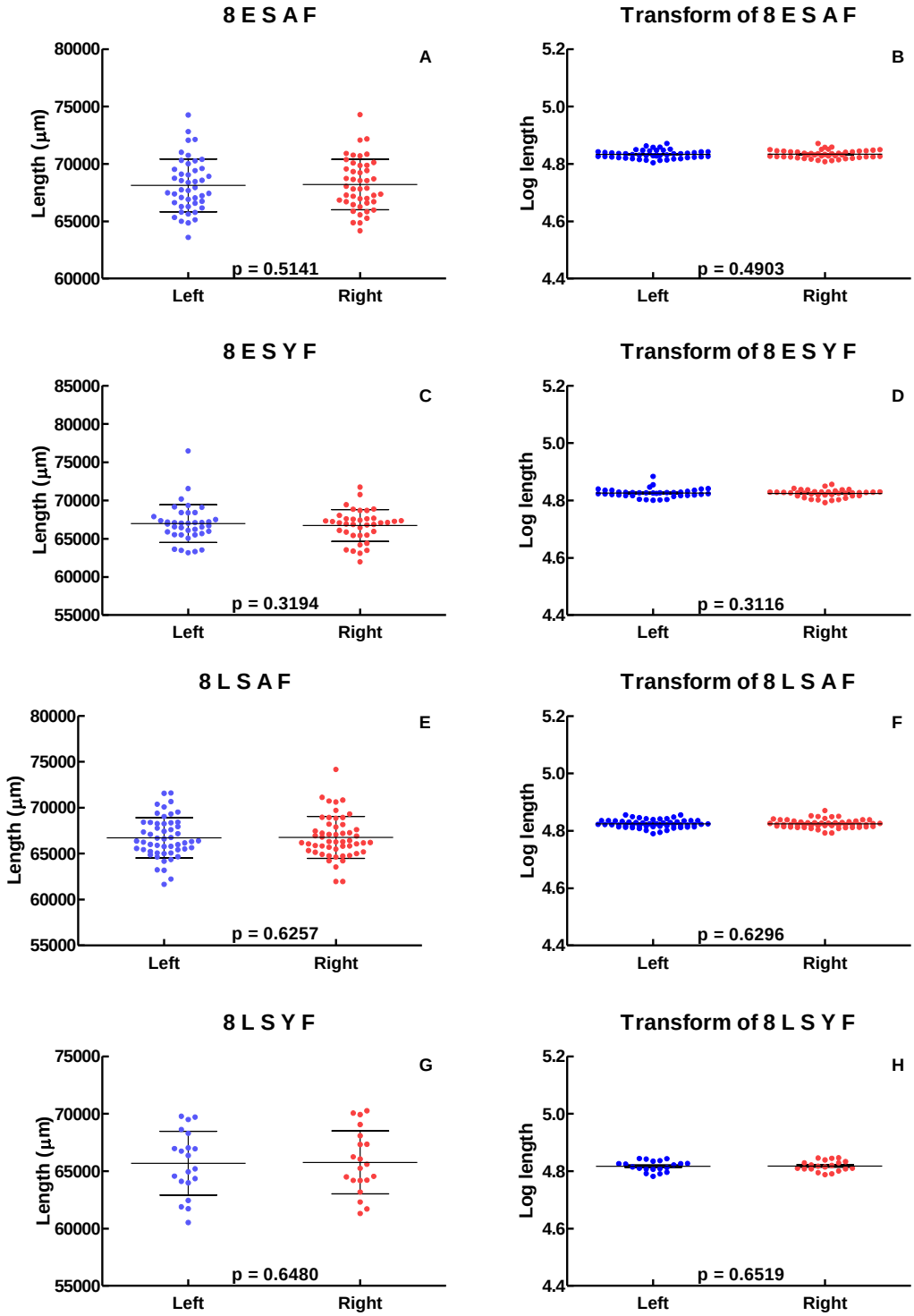

Fig 3.1.12: *Scatterplots of feather Variable 8 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young females between left and right P8 feathers (comparisons 1-4, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.1.1.* ***A****: Left and right primaries of early season adult females (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult females* ***C****: Left and right primaries of early season young females (non-transformed).* ***D****: Log-transform of left and right primaries of early season young females.* ***E****: Left and right primaries of late season adult females (non-transformed).* ***F****: Log-transform of left and right primaries of late season adult females.* ***G****: Left and right primaries of late season young females (non-transformed).* ***H****: Log-transform of left and right primaries of late season young females. Values that are both significant and normally distributed are marked with an asterisk.*

3.1.12 Variable 8

Fig. 3.1.12 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult females. There were no significant differences for non-transformed data.

Fig. 3.1.12 C and D: Data were not normally distributed for non-transformed or log-transformed data of early season young females. There were no significant differences for log-transformed data.

Fig 3.1.12 E and F Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult females. There were no significant differences for log-transformed data.

Fig 3.1.12 G and H: Data were normally distributed for non-transformed and log-transformed data of late season young females. There were no significant differences for non-transformed data.

Table 3.1.1: *Means (µm) and results (p-values) of transformed and non-transformed, paired, two-tailed, t-tests of females. The results are highlighted in red or blue for valid comparisons. Red indicates a significant result where the left primary dimensions were larger than right primaries for that Variable. Blue indicates a result where right primaries were larger than left primaries for that Variable. Variables that were not normally distributed or had p-values > 0.05 were not highlighted.*

Variable	Sex	Age	Season	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
				LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
1	Female	Adult	ES	7398	7453	3.869	3.872	NO	YES	YES	YES	0.0924	0.0767
	Female	Young	ES	7345	7409	3.865	3.869	YES	YES	YES	YES	0.0382	0.0415
	Female	Adult	LS	7029	7089	3.846	3.85	YES	YES	YES	YES	0.0703	0.0776
	Female	Young	LS	7255	7205	3.859	3.856	YES	YES	YES	YES	0.2077	0.2403
2	Female	Adult	ES	10150	10119	4.005	4.004	YES	YES	YES	YES	0.6507	0.6545
	Female	Young	ES	10444	10451	4.017	4.017	NO	YES	NO	YES	0.9294	0.9354
	Female	Adult	LS	10654	10552	4.025	4.021	NO	YES	YES	YES	0.3116	0.3851
	Female	Young	LS	10608	10700	4.025	4.028	YES	YES	YES	YES	0.4409	0.4689
3	Female	Adult	ES	8521	8483	3.93	3.928	YES	YES	YES	YES	0.2658	0.2937
	Female	Young	ES	8591	8582	3.933	3.933	YES	NO	YES	YES	0.8175	0.7442
	Female	Adult	LS	8483	8481	3.928	3.928	YES	YES	YES	YES	0.9431	0.9651
	Female	Young	LS	8539	8566	3.931	3.932	YES	YES	YES	YES	0.4491	0.4253
4.1	Female	Adult	ES	5905	5705	3.768	3.753	YES	YES	YES	YES	0.0145	0.0142
	Female	Young	ES	5850	5652	3.763	3.748	YES	YES	YES	YES	0.0721	0.0822
	Female	Adult	LS	5106	4871	3.7	3.68	YES	NO	NO	NO	0.0613	0.1015
	Female	Young	LS	5238	4834	3.713	3.674	YES	YES	YES	YES	0.0094	0.008
4.2	Female	Adult	ES	291	280.5	2.429	2.415	YES	NO	YES	YES	0.6128	0.6673
	Female	Young	ES	299.3	323.2	2.444	2.479	YES	NO	YES	YES	0.3825	0.351
	Female	Adult	LS	258.1	231.2	2.383	2.299	YES	NO	YES	YES	0.2192	0.025
	Female	Young	LS	246.1	235.4	2.362	2.343	YES	YES	YES	YES	0.5885	0.6297
5.1	Female	Adult	ES	1569	1531	3.193	3.183	NO	YES	YES	YES	0.0452	0.0527
	Female	Young	ES	1449	1477	3.154	3.162	NO	NO	NO	NO	0.329	0.3819
	Female	Adult	LS	1355	1389	3.12	3.135	NO	YES	NO	NO	0.3009	0.2059
	Female	Young	LS	1315	1408	3.106	3.138	NO	YES	NO	NO	0.1521	0.2464
5.2	Female	Adult	ES	6447	6424	3.808	3.807	YES	YES	YES	YES	0.7038	0.8015
	Female	Young	ES	6418	6494	3.807	3.812	YES	YES	YES	YES	0.1006	0.0904
	Female	Adult	LS	6466	6468	3.81	3.81	YES	YES	YES	YES	0.9645	0.8749
	Female	Young	LS	6326	6268	3.8	3.796	YES	YES	YES	YES	0.6357	0.6613
6.1	Female	Adult	ES	939.9	938.1	2.973	2.972	YES	YES	YES	YES	0.5822	0.5369
	Female	Young	ES	909.3	911.7	2.958	2.959	YES	NO	YES	NO	0.6168	0.57
	Female	Adult	LS	912.2	920.3	2.96	2.964	YES	YES	YES	YES	0.0957	0.0939
	Female	Young	LS	918.9	906	2.963	2.957	YES	YES	YES	YES	0.0875	0.0906
6.2	Female	Adult	ES	986.4	971.3	2.994	2.987	YES	YES	YES	YES	<0.0001	<0.0001
	Female	Young	ES	952.9	948.1	2.979	2.976	YES	YES	YES	YES	0.1585	0.1633
	Female	Adult	LS	969.8	968.2	2.986	2.986	YES	YES	YES	YES	0.7107	0.7117
	Female	Young	LS	975.8	956.4	2.988	2.98	NO	YES	NO	YES	0.1304	0.1165
6.3	Female	Adult	ES	957.6	954.6	2.981	2.979	YES	YES	YES	YES	0.3996	0.3861
	Female	Young	ES	923.9	931.1	2.965	2.968	YES	YES	YES	YES	0.122	0.1267
	Female	Adult	LS	939.5	952.3	2.973	2.978	YES	YES	YES	YES	0.0135	0.0135
	Female	Young	LS	929	932.9	2.968	2.97	YES	NO	YES	YES	0.6134	0.5956
6.4	Female	Adult	ES	483.1	480.3	2.681	2.679	YES	YES	YES	YES	0.5331	0.6505
	Female	Young	ES	463.8	459.7	2.659	2.658	NO	YES	YES	YES	0.6187	0.8834
	Female	Adult	LS	458.7	468.2	2.658	2.668	NO	YES	YES	YES	0.0783	0.0732
	Female	Young	LS	435	435.2	2.637	2.637	YES	YES	YES	YES	0.973	0.9658
8	Female	Adult	ES	68130	68204	4.833	4.834	YES	YES	YES	YES	0.5141	0.4903
	Female	Young	ES	66972	66718	4.826	4.824	NO	YES	NO	YES	0.3194	0.3116
	Female	Adult	LS	66727	66781	4.824	4.824	YES	NO	YES	YES	0.6257	0.6296
	Female	Young	LS	65681	65763	4.817	4.818	YES	YES	YES	YES	0.648	0.6519

3.2 Summary of significant differences between left and right Variables of females

Some Variables indicated significant differences for some groups, while other groups had differences between left and right primaries, but either lacked normal distribution or a significant p-value to statistically confirm this observation. A narrative summary of significantly different Variables is provided below. Tables 3.1.2 and 3.1.3 provide numeric summaries.

3.2.1 Variable 1

Variable 1 (length of calamus) of early season young females (non-transformed) were significantly longer on right primaries compared with left primaries (Fig 3.1.1 C). The remaining groups also had differences, but the t-tests were not significant. However, the mean lengths of the insignificant groups fluctuated between the left and right primaries.

3.2.2 Variable 2

Variable 2 (length of rachis to the start of anterior vane) indicated no significant differences, however the groups did vary between left and right being the largest. These differences were not significant.

3.2.3 Variable 3

Variable 3 (length of rachis to the start of posterior vane) indicated no significant differences, however the groups did vary between left and right being the largest. But these differences were not significant.

3.2.4 Variable 4.1

Variable 4.1 (length of plumaceous barbs, with respect to the rachis, on the anterior vane) of early season adult females (non-transformed) and late season young females (non-transformed) were significantly longer on the left primaries compared with the right primaries (Fig 3.1.4 A and Fig 3.1.4 G). The remaining groups also suggested (lacking either normal distribution or a significant p-value for the t-test) that Variable 4.1 was longer on the left primary in comparison with the right.

3.2.5 Variable 4.2

Log-transformed late season adult females (length of plumaceous barbs, with respect to the rachis, on the posterior vane) indicated that Variable 4.2 was significantly longer on the left primaries in comparison with the right (Fig 3.1.5 F). Early season adult females and late season young females suggested the same, but lacked normal distribution and a significant p-value for the t-tests. Conversely, Variable 4.2 was slightly longer on the right primaries for

early season young females, but lacked a significant p-value for the t-test. Although care was taken to measure Variable 4.1 and 4.2 consistently, it has to be noted that Variable 4.1 and Variable 4.2 were difficult to measure due to intertwined nature of the plumaceous barbs.

3.2.6 Variable 5.1

Early season adult females suggested that Variable 5.1 (width of the anterior vane from a proportionately consistent point on the rachis) was slightly wider on the left primaries in comparison with the right, while the remaining groups suggested the opposite. None of these groups had normal distributions or significant p-values for the t-tests.

3.2.7 Variable 5.2

Early season young females suggested that Variable 5.2 (width of the posterior vane from a proportionally consistent point on the rachis) was longer on the right primaries compared with the left primaries, but did not have significant p values. Late season young females suggested the opposite but lacked significant p values. Early season adult females and late season adult females had no significant differences for this Variable.

3.2.7 Variable 6.1

Late season adult females suggested that Variable 6.1 (width of rachis at the distal edge of the calamus) was wider on the right primaries compared with the left, but did not have significant p values. Late season young females suggested the opposite, but lacked significant p-values. The remaining groups indicated no significant difference between left and right primaries.

3.2.6 Variable 6.2

Non-transformed early season adult females (Fig 3.1.9 A), indicated that Variable 6.2 (width of the rachis at the start of the posterior vane) was wider on the left primaries in comparison with the right primaries. Late season young females also had a wider mean Variable 6.2, but lacked both normal distribution and a significant p-value. The remaining groups suggested no difference.

3.2.7 Variable 6.3

Variable 6.3 of late season adult females (width of the rachis at the start of the anterior vane) were wider on the right primaries in comparison with the left (Fig 3.1.10 E). Early season young females suggested the same, but did not have significant p-values. Late season young females, and early season adult females, indicated no significant differences between

left and right for Variable 6.3, did not have normal distributions, or significant p-values for t-tests. However, the mean widths of the insignificant groups fluctuated between the left and right primaries.

3.2.8 Variable 6.4

All four groups indicated no significant differences between left and right primaries for Variable 6.4 (width of the rachis at a proportionately consistent point) and did not have significant p-values for t-tests for the means. However, the mean widths of the insignificant groups fluctuated between the left and right primaries being the largest.

3.2.9 Variable 8

All four groups indicated no significant differences between left and right for Variable 8 (length of the rachis) and did not have significant p-values. However, the mean lengths of the insignificant groups fluctuated between the left and right primaries being the largest.

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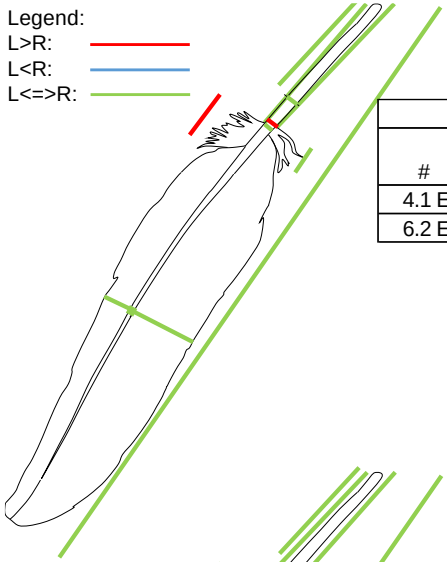
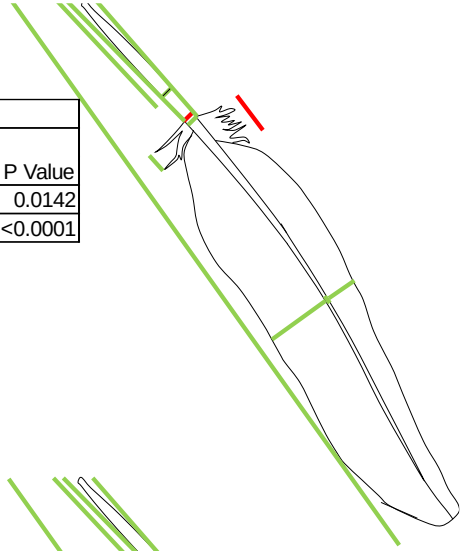
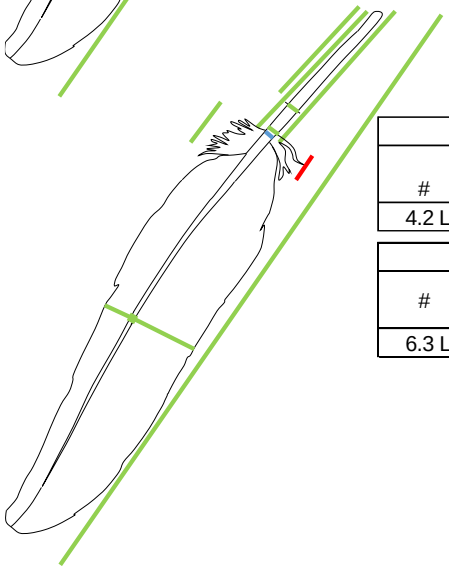
Table 3.1.2: *A schematic representation of the means and results of non-transformed and log-transformed, paired, two-tailed, t-test comparisons between left and right primaries of early season and late season adult females. Because this is a paired comparison, the left and right representations of the feathers are identical. Red indicates a significant result where the dimensions of the left primaries were larger than the right primaries. Blue indicates a significant result where the dimensions on the right primaries were larger than the left primaries. Green indicates no significant differences. The discussion of the detailed findings is presented in Section 3.2 and Section 4.1.*



Early season adult females

LEFT > RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
4.1 ES	5905	5705	3.768	3.753	YES	YES	YES	YES	0.0145	0.0142
6.2 ES	986.4	971.3	2.994	2.987	YES	YES	YES	YES	<0.0001	<0.0001





Late season adult females

LEFT > RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
4.2 LS	258.1	231.2	2.383	2.299	YES	NO	YES	YES	0.2192	0.025

LEFT < RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
6.3 LS	939.5	952.3	2.973	2.978	YES	YES	YES	YES	0.0135	0.0135

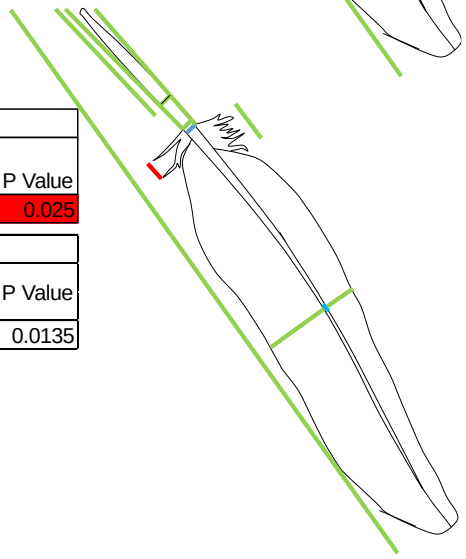
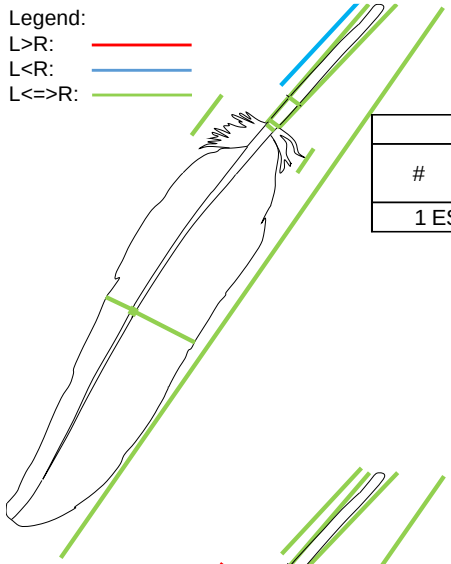


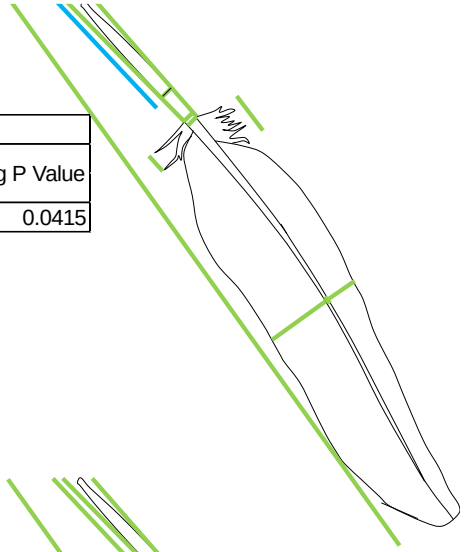
Table 3.1.3: *A schematic representation of the means and results of non-transformed and log-transformed, paired, two-tailed, t-test comparisons between left and right primaries of early season and late season young females. Because this is a paired comparison, the left and right representations of the feathers are identical. Red indicates a significant result where the dimensions of the left primaries were larger than the right primaries. Blue indicates a significant result where the dimensions on the right primaries were larger than the left primaries. Green indicates no significant differences. The discussion of the detailed findings is presented in Section 3.2 and Section 4.1.*

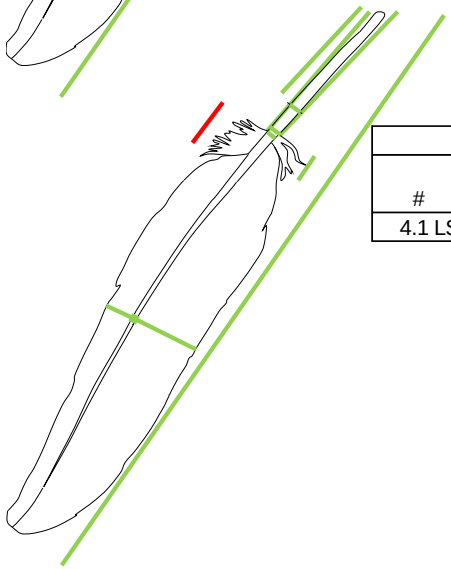
Legend:
L>R:
L<R:
L<=>R:



Early season young females

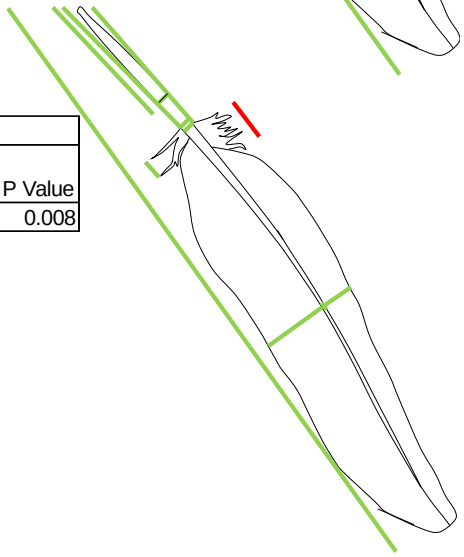
LEFT < RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
1 ES	7345	7409	3.865	3.869	YES	YES	YES	YES	0.0382	0.0415





Late season young females

LEFT > RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
4.1 LS	5238	4834	3.713	3.674	YES	YES	YES	YES	0.0094	0.008



3.5 Male left vs right (comparisons 5-7)

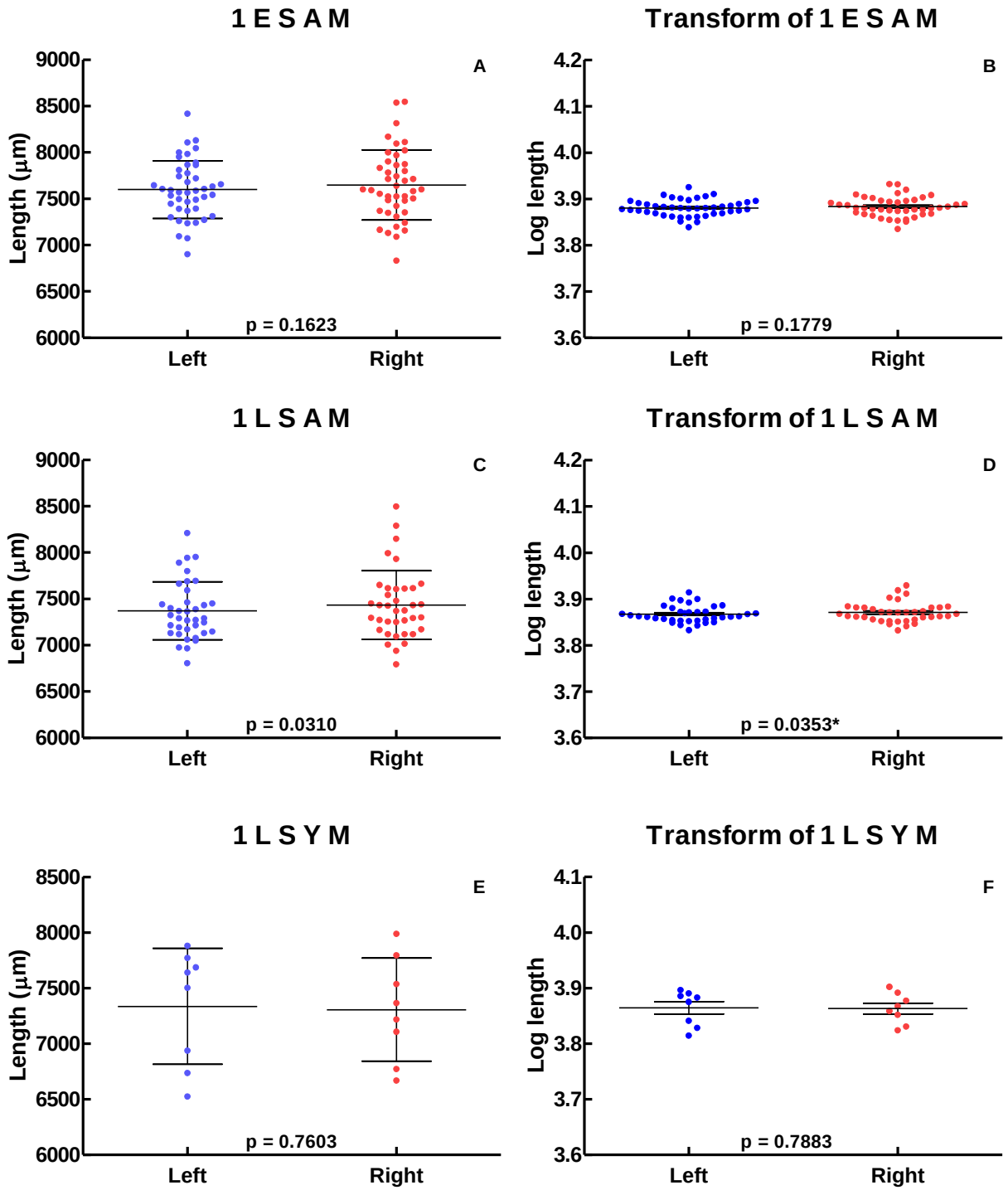


Fig 3.5.1: *Scatterplots of feather Variable 1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.1. Variable 1

Fig. 3.5.1 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.1 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult males. Variable 1 (log-transformed) was longer on right primaries in comparison with left primaries

Fig. 3.5.1 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

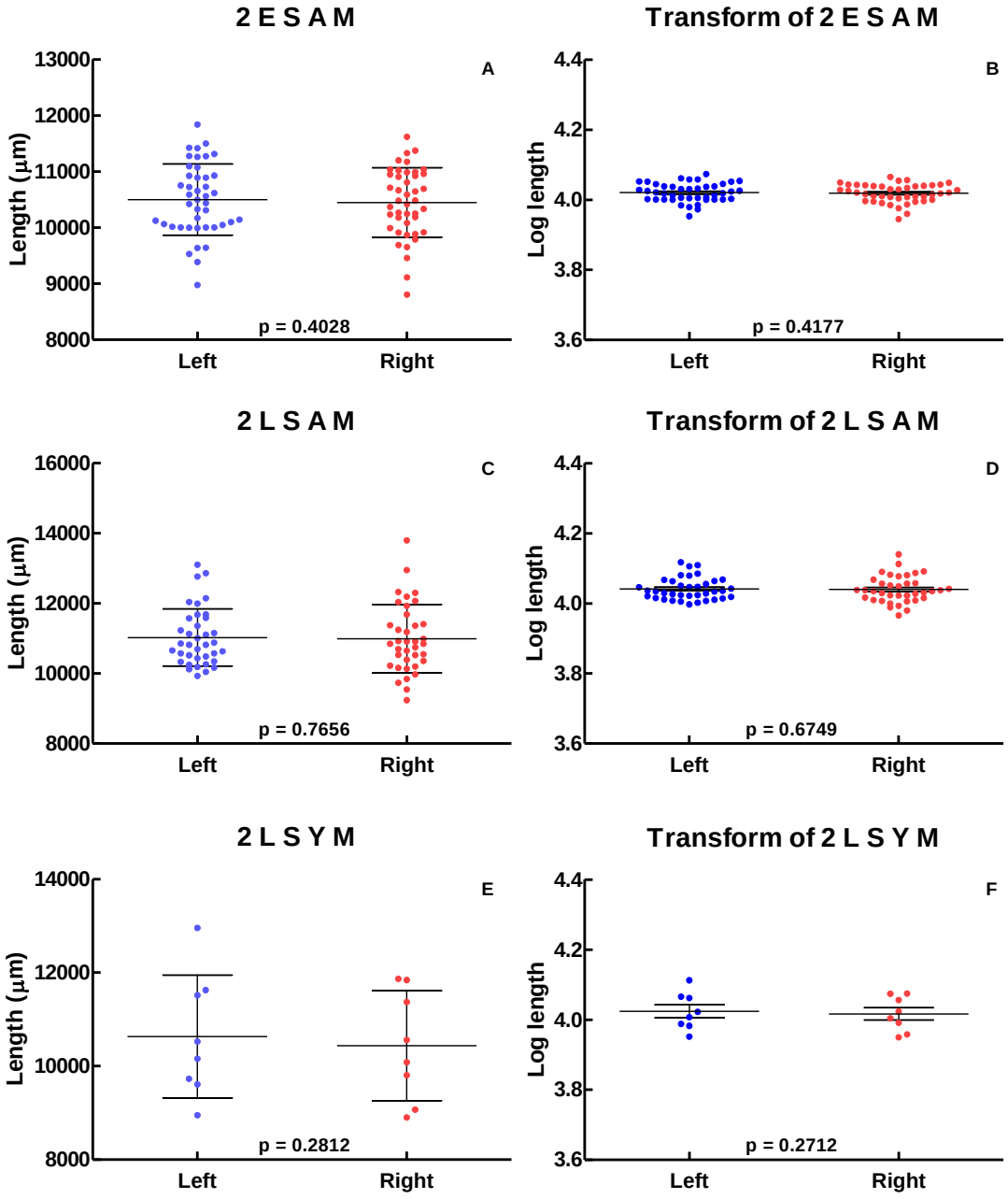


Fig 3.5.2: *Scatterplots of feather Variable 2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.2 Variable 2

Fig. 3.5.2 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.2 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult males. There were no significant differences for log-transformed data.

Fig. 3.5.2 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences non-transformed data.

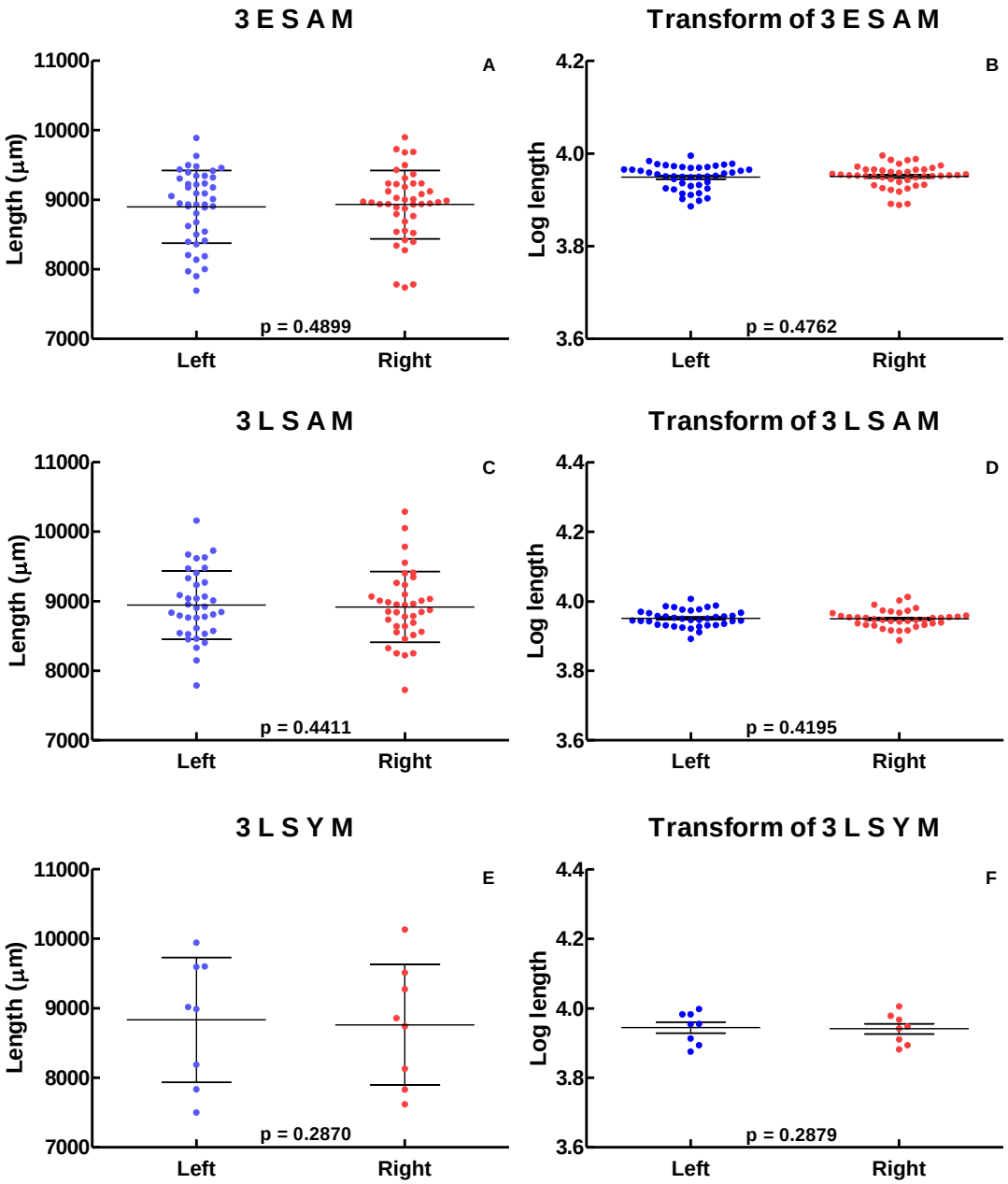


Fig 3.5.3: *Scatterplots of feather Variable 3 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

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3.5.3 Variable 3

Fig. 3.5.3 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.3 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.3 C and D: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

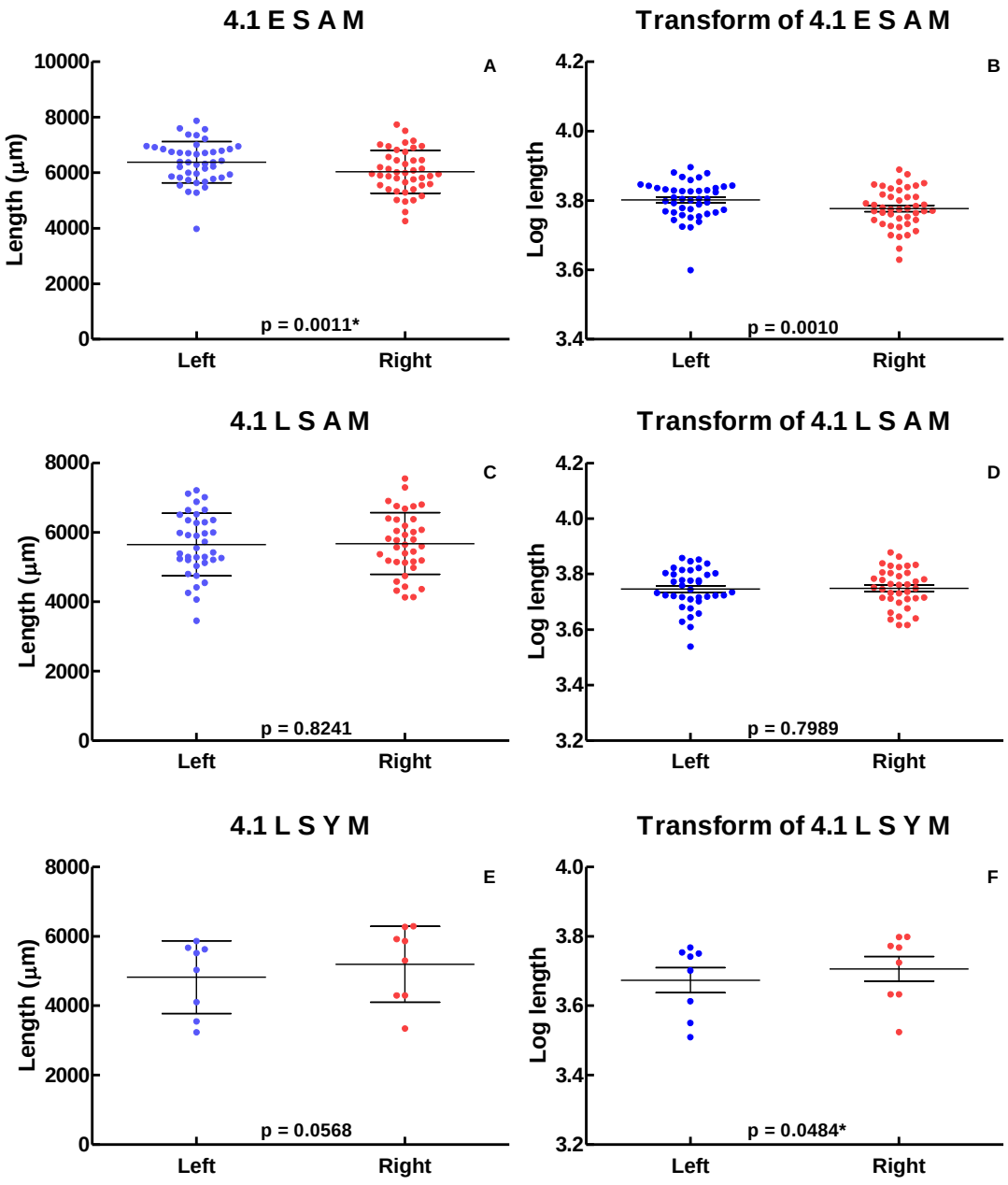

Fig 3.5.4: *Scatterplots of feather Variable 4.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.4 Variable 4.1

Fig. 3.5.4 A and B: Data were normally distributed for non-transformed data, but not for log-transformed data of early season adult males. Variable 4.1 (non-transformed) was longer on left primaries in comparison with right primaries.

Fig. 3.5.4 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. There were no significant differences for non-transformed data

Fig. 3.5.4 E and F: Data were normally distributed for non-transformed data and log-transformed data of late season young males. Variable 4.1 (log-transformed) was longer on right primaries in comparison with left primaries.

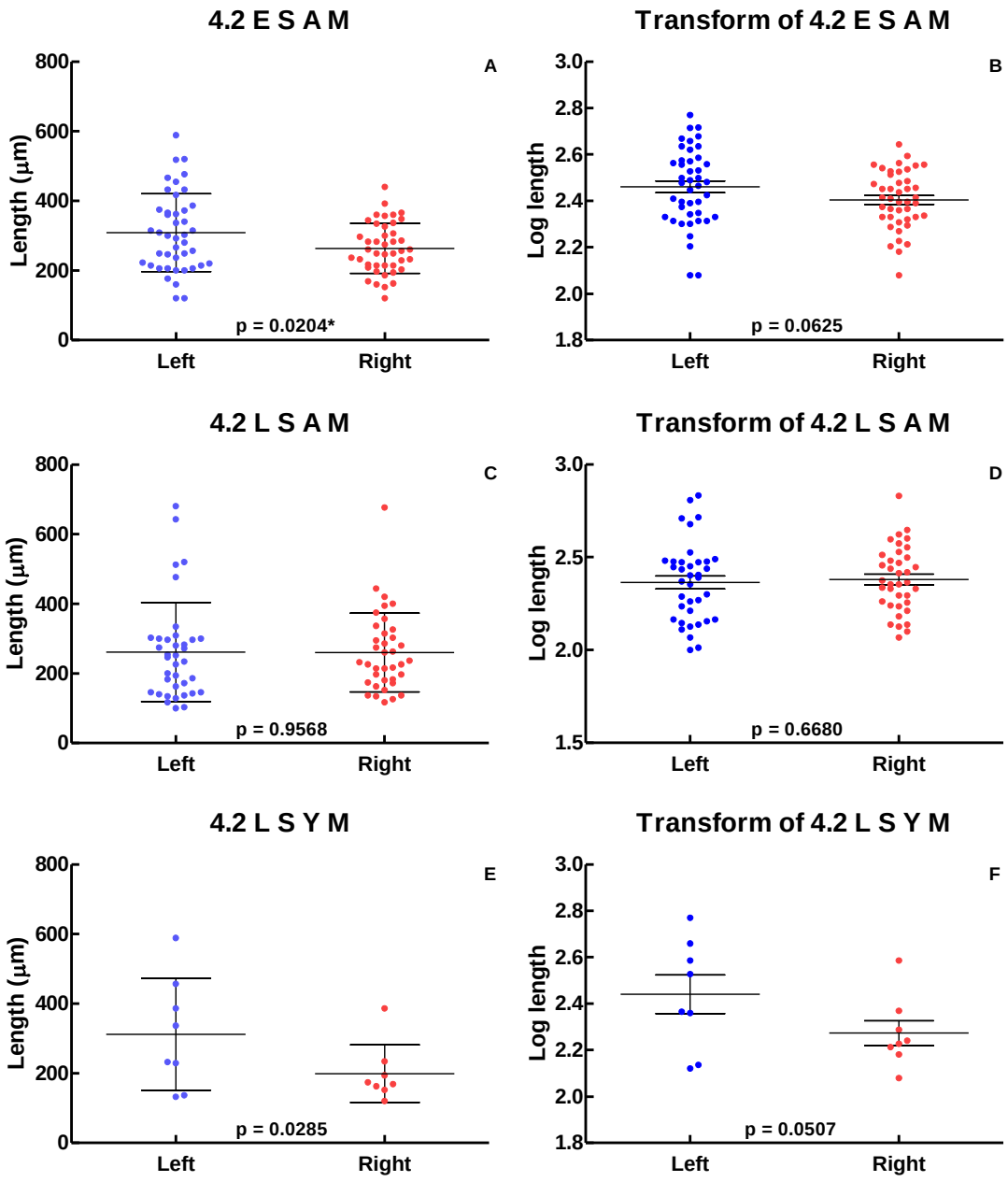

Fig 3.5.5: *Scatterplots of feather Variable 4.2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.5 Variable 4.2

Fig. 3.5.5 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. Variable 4.2 (non-transformed) was longer on left primaries in comparison with right primaries.

Fig. 3.5.5 C and D: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season adult males. There were no significant differences for log-transformed data.

Fig. 3.5.5 E and F: Data were not normally distributed for non-transformed data, but had normal distribution for log-transformed data of late season young males. Variable 4.1 (non-transformed) was significantly longer on left primaries in comparison with right primaries, but did not have normal distribution.

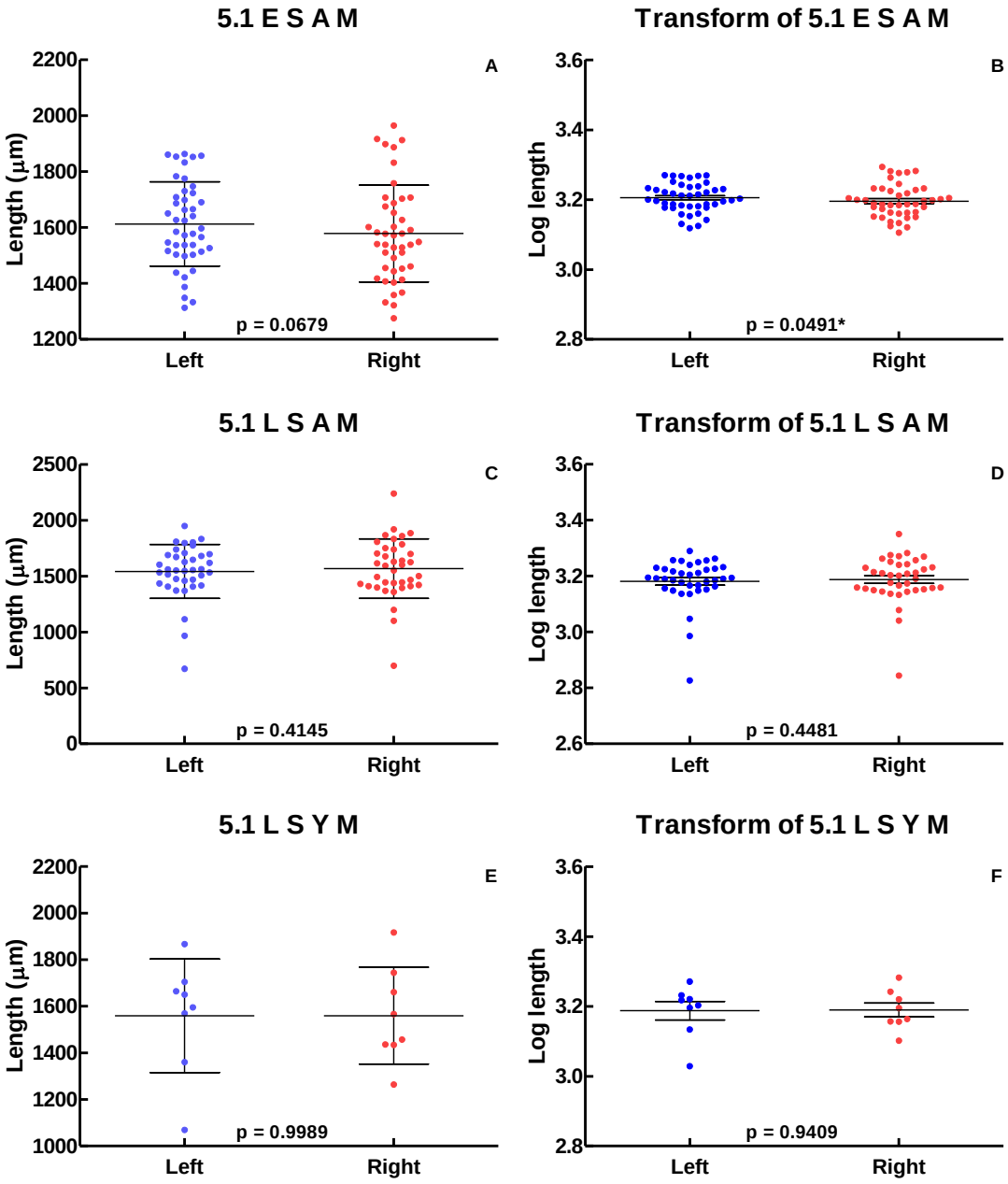

Fig 3.5.6: *Scatterplots of feather Variable 5.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.6 Variable 5.1

Fig. 3.5.6 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. Variable 5.1 (log-transformed) was significantly wider on left primaries in comparison with right primaries.

Fig. 3.5.6 C and D: Data were not normally distributed for non-transformed or log-transformed data of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.6 E and F: Data were normally distributed for non-transformed, but not for log-transformed data of late season young males. There were no significant differences for non-transformed data.

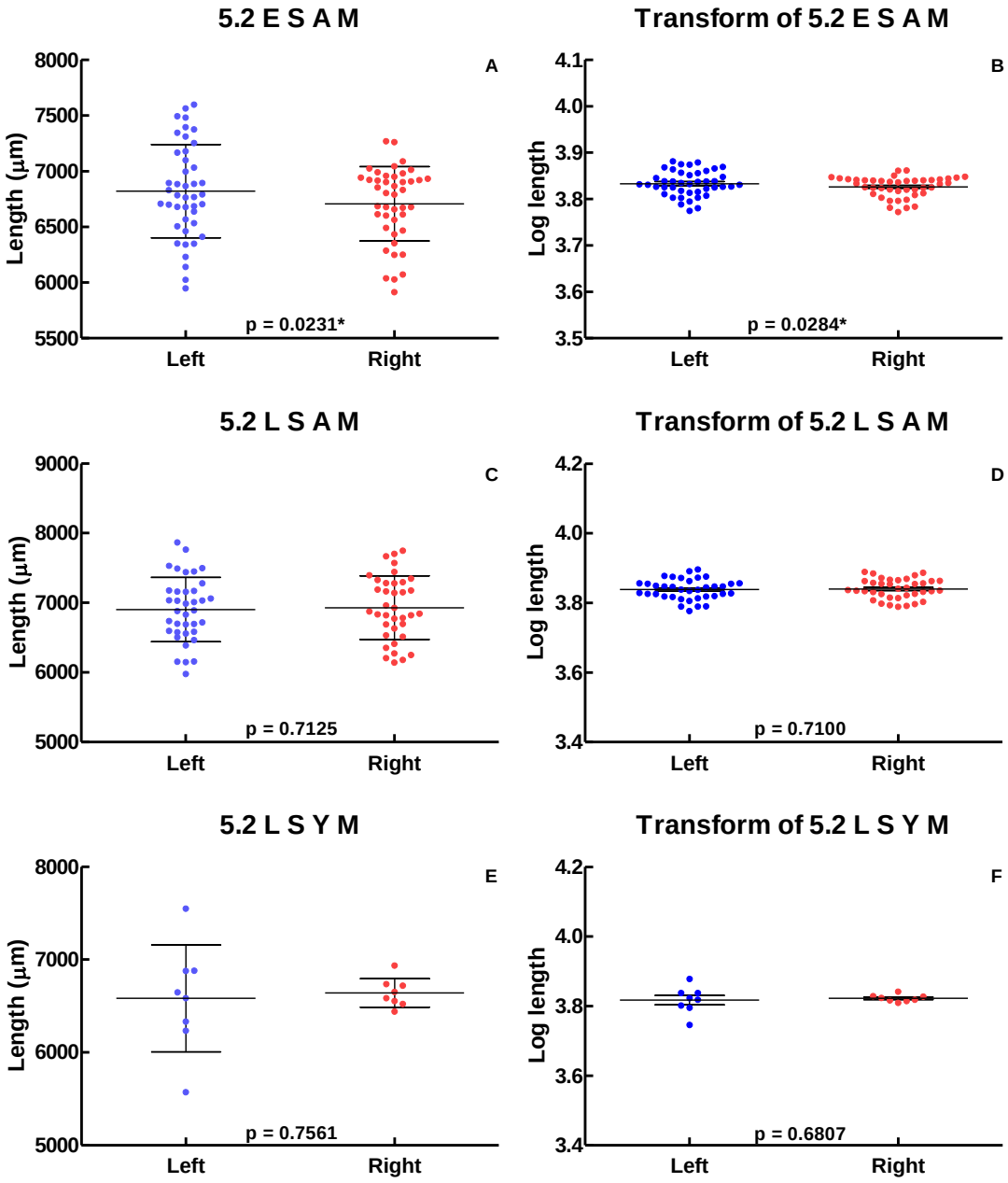

Fig 3.5.7: *Scatterplots of feather Variable 5.2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.7 Variable 5.2

Fig. 3.5.7 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. Variable 5.2 (non-transformed) was significantly wider on left primaries in comparison with right primaries.

Fig. 3.5.7 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.7 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

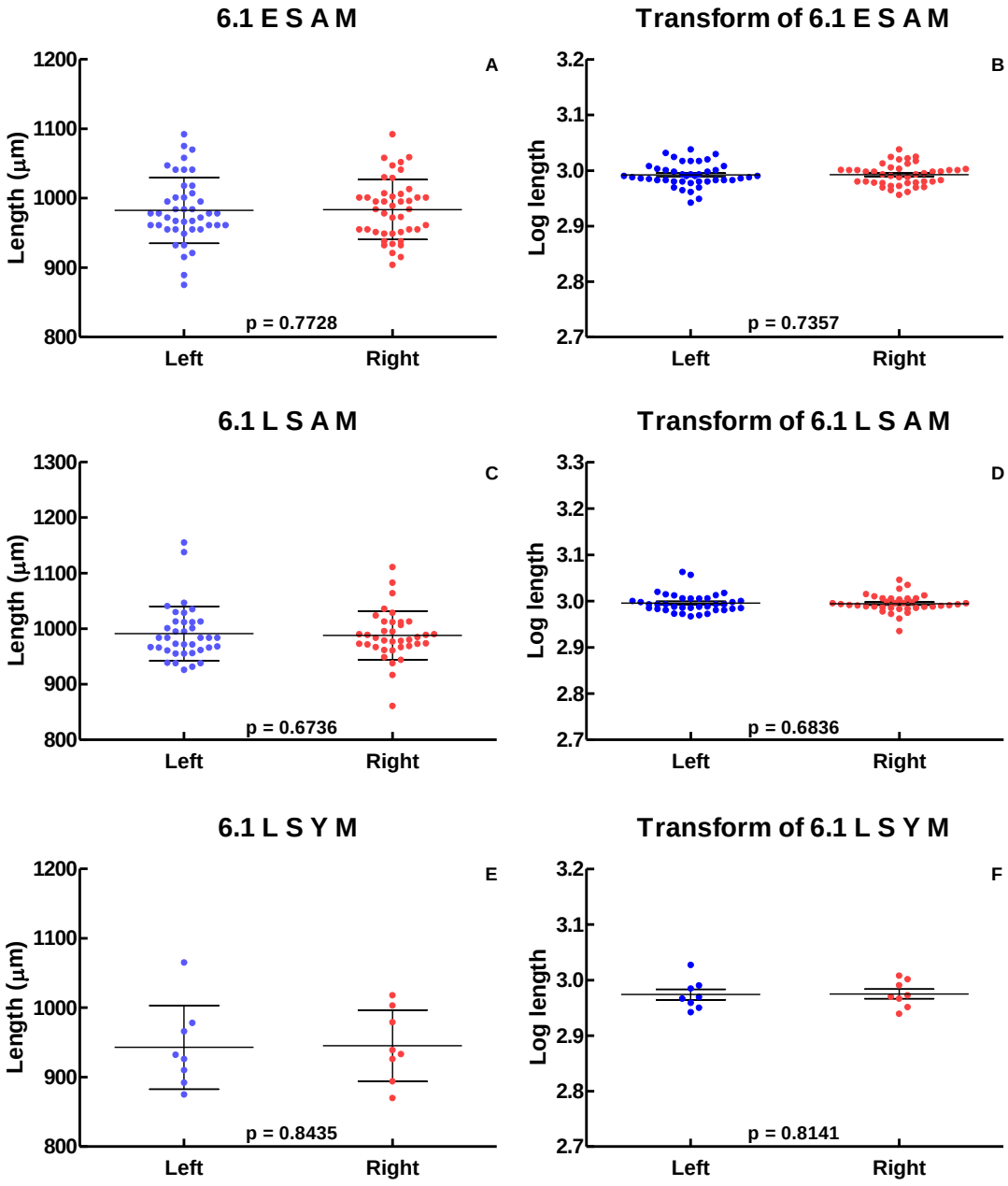

Fig 3.5.8: *Scatterplots of feather Variable 6.1 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

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3.5.8 Variable 6.1

Fig. 3.5.8 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.8 C and D: Data were not normally distributed for non-transformed or log-transformed of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.8 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

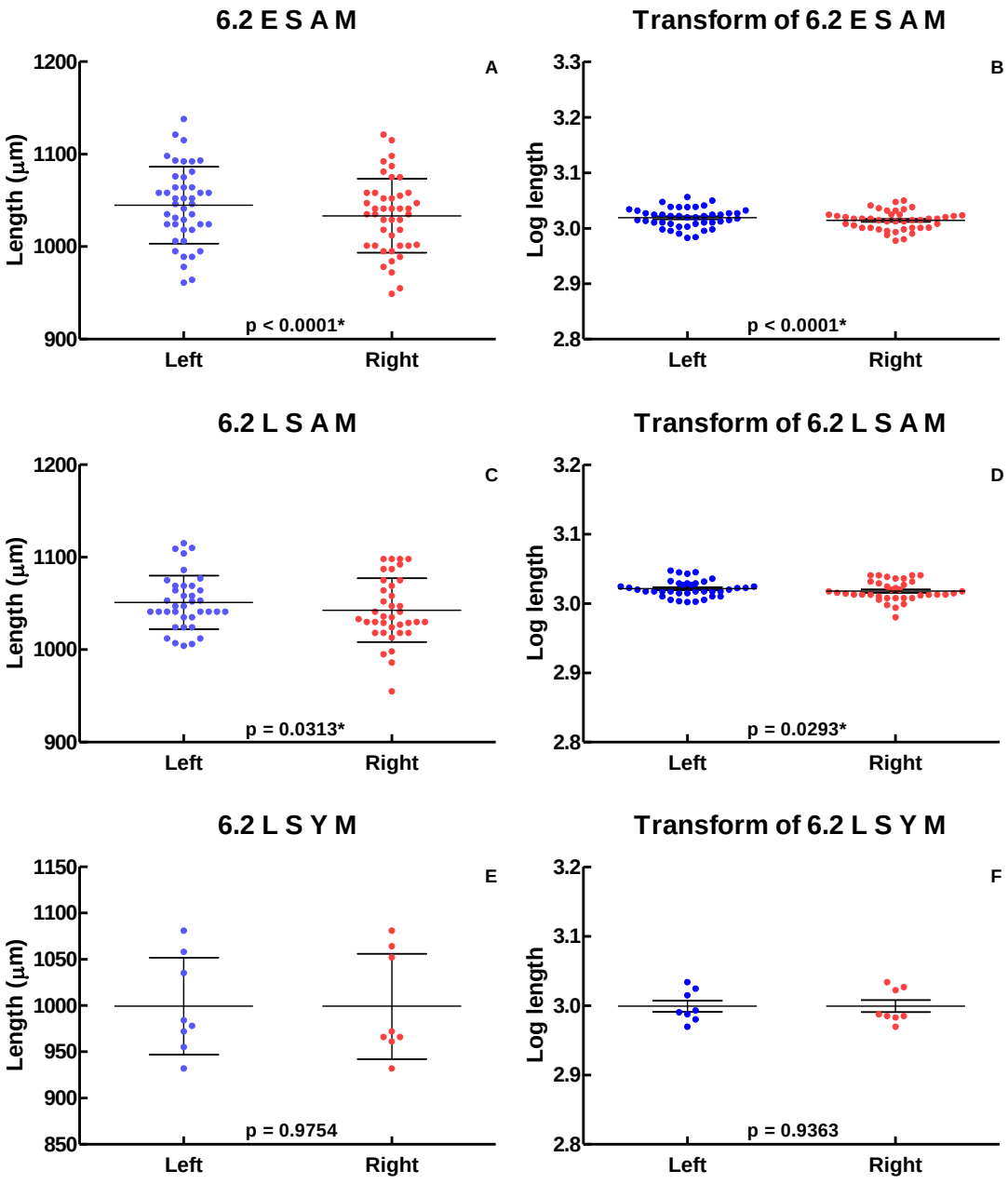


Fig 3.5.9: *Scatterplots of feather Variable 6.2 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.9 Variable 6.2

Fig. 3.5.9 A and B: Data were normally distributed for non-transformed data and log-transformed data of early season adult males. Variable 6.2 (non-transformed) was thicker on the left primaries in comparison with the right primaries.

Fig. 3.5.9 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. Variable 6.2 (non-transformed) was thicker on the left primaries in comparison with the right primaries.

Fig. 3.5.9 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

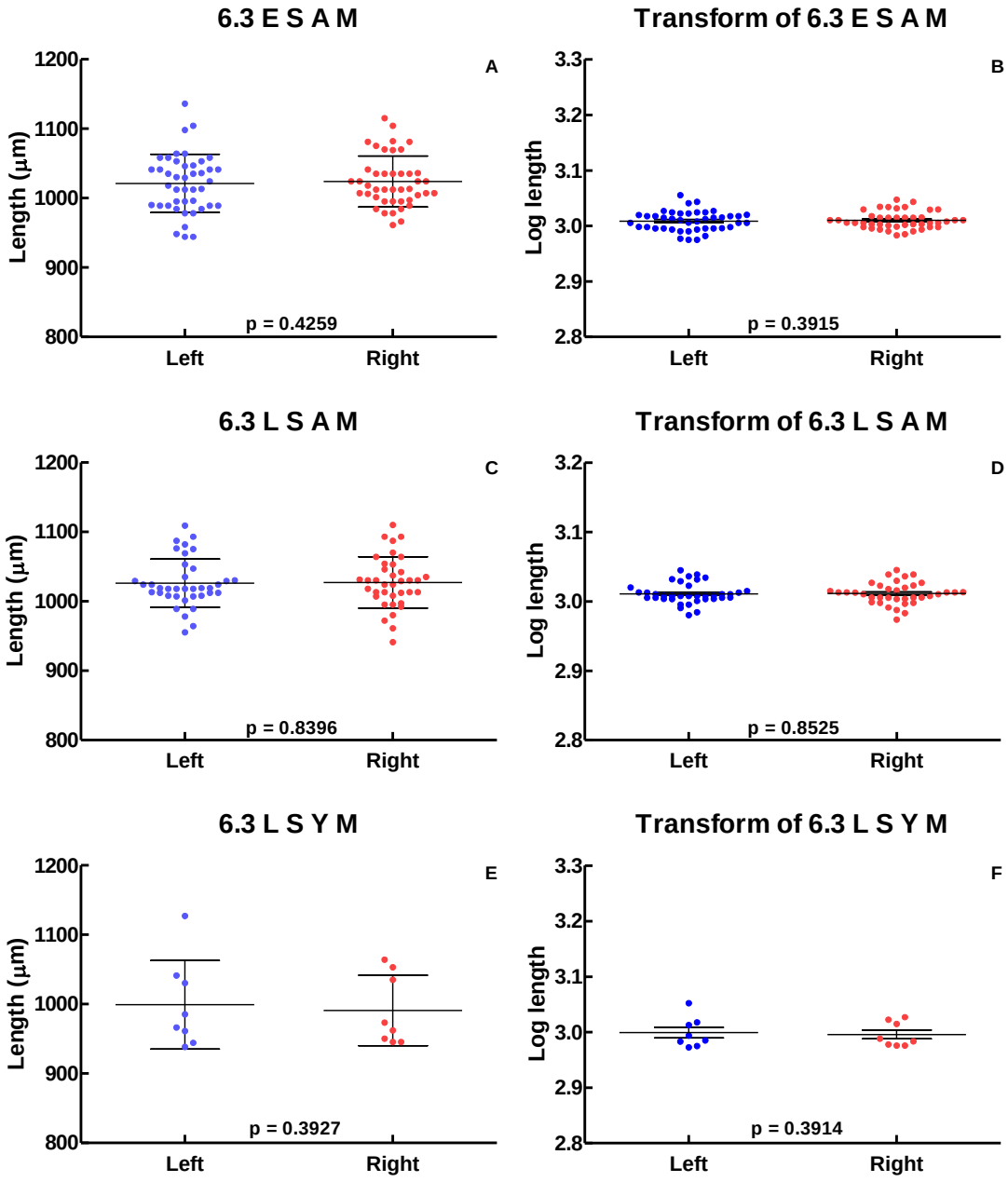


Fig 3.5.10: *Scatterplots of feather Variable 6.3 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.10 Variable 6.3

Fig. 3.5.10 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.10 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.10 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

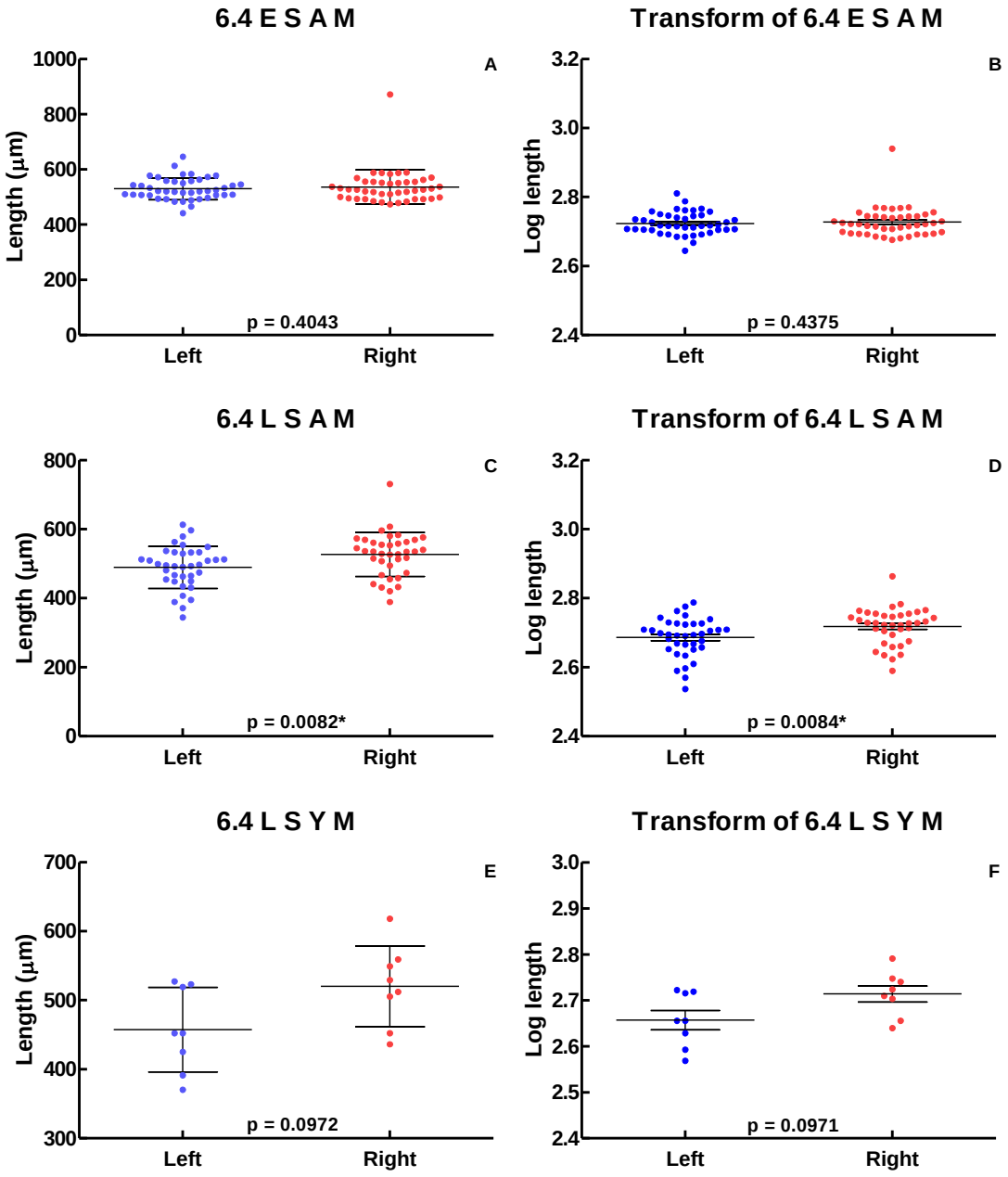


Fig 3.5.11: *Scatterplots of feather Variable 6.4 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.11 Variable 6.4

Fig. 3.5.11 A and B: Data were not normally distributed for non-transformed or log-transformed data of early season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.11 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. Variable 6.4 (non-transformed) was thicker on right primaries in comparison with left primaries.

Fig. 3.5.11 E and F: Data were normally distributed for non-transformed data and log-transformed data of late season young males. There were no significant differences for non-transformed data.

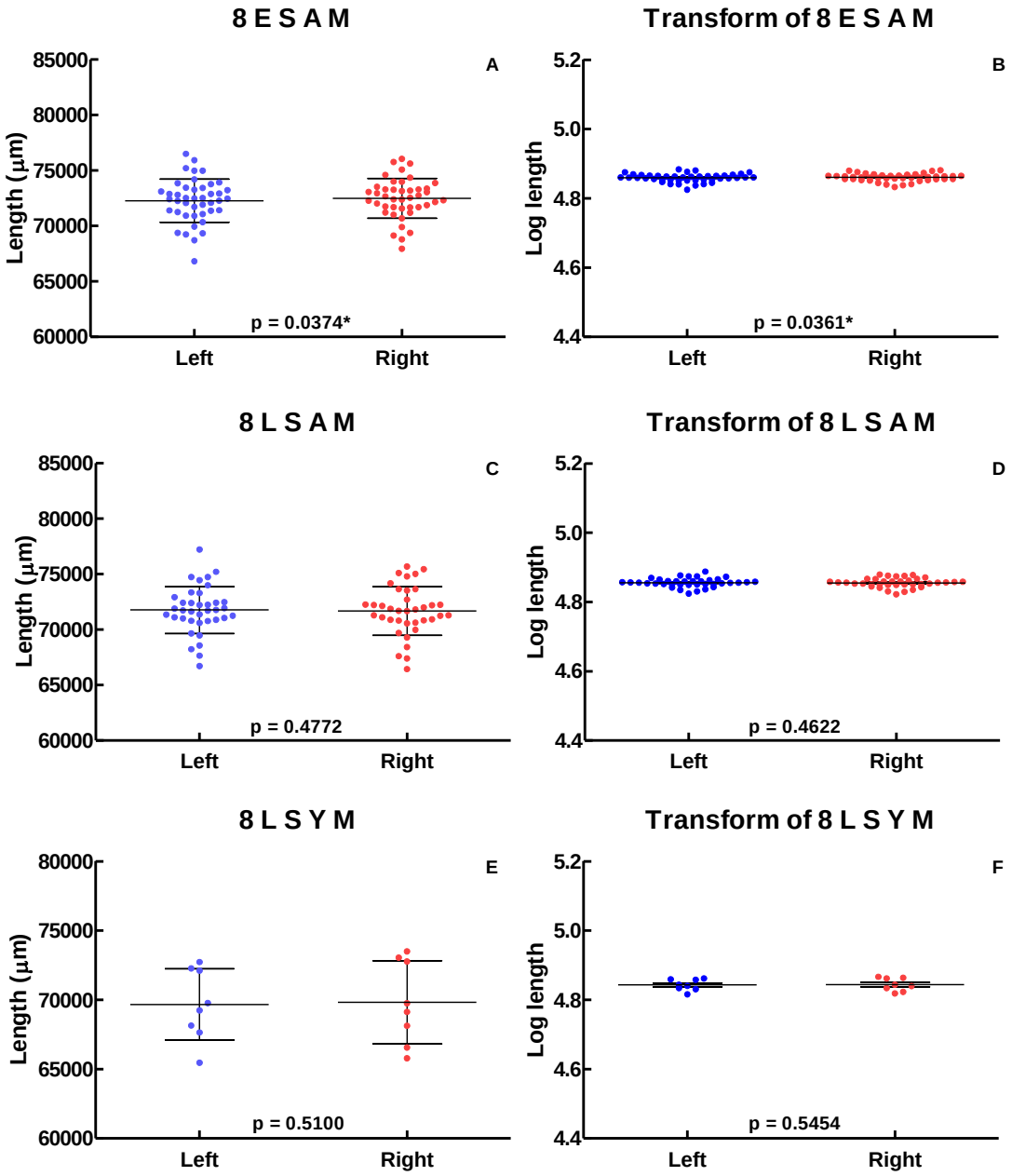


Fig 3.5.12: *Scatterplots of feather Variable 8 (see Fig 2.4.1). Means and standard deviations are indicated, as well as the results of the paired, two-tailed t-tests comparing adult and young males between left and right P8 feathers (comparisons 5-7, see Fig 1.8.1) across late season and early season. Corresponding data are presented in Table 3.5.1.* ***A****: Left and right primaries of early season adult males (non-transformed).* ***B****: Log-transform of left and right primaries of early season adult males* ***C****: Left and right primaries of late season adult males (non-transformed).* ***D****: Log-transform of left and right primaries of late season adult males.* ***E****: Left and right primaries of late season young males (non-transformed).* ***F****: Log-transform of left and right primaries of late season young males. Values that are both significant and normally distributed are marked with an asterisk.*

3.5.12 Variable 8

Fig. 3.5.12 A and B: Data were normally distributed for non-transformed and log-transformed data of early season adult males. Variable 8 (non-transformed) was longer on right primaries in comparison with left primaries.

Fig. 3.5.12 C and D: Data were normally distributed for non-transformed and log-transformed data of late season adult males. There were no significant differences for non-transformed data.

Fig. 3.5.12 E and F: Data were normally distributed for non-transformed and log-transformed data of late season young males. There were no significant differences for non-transformed data.

Table 3.5.1: *P-values and means of log-transformed and non-transformed, paired, two-tailed, t-tests of males. The results were highlighted in red or blue for valid comparisons. Red indicates a significant result where the left primaries were larger than right primaries for that Variable. Blue indicates a result where right primaries were larger than primaries for that Variable. Variables that were not normally distributed or had p-values > 0.05 were not highlighted.*

Variable	Sex	Age	Season	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
				LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
1	Male	Adult	ES	7601	7650	3.88	3.883	YES	YES	YES	YES	0.1623	0.1779
	Male	Adult	LS	7371	7434	3.867	3.871	YES	NO	YES	YES	0.031	0.0353
	Male	Young	LS	7335	7306	3.864	3.863	YES	YES	YES	YES	0.7603	0.7883
2	Male	Adult	ES	10499	10445	4.02	4.018	YES	YES	YES	YES	0.4028	0.4177
	Male	Adult	LS	11023	10992	4.041	4.039	NO	YES	YES	YES	0.7656	0.6749
	Male	Young	LS	10634	10437	4.024	4.016	YES	YES	YES	YES	0.2812	0.2712
3	Male	Adult	ES	8899	8931	3.949	3.95	YES	YES	YES	YES	0.4899	0.4762
	Male	Adult	LS	8946	8918	3.951	3.95	YES	YES	YES	YES	0.4411	0.4195
	Male	Young	LS	8834	8762	3.944	3.941	YES	YES	YES	YES	0.287	0.2879
4.1	Male	Adult	ES	6372	6027	3.801	3.777	YES	YES	NO	YES	0.0011	0.001
	Male	Adult	LS	5647	5679	3.746	3.749	YES	YES	YES	YES	0.8241	0.7989
	Male	Young	LS	4824	5198	3.673	3.706	YES	YES	YES	YES	0.0568	0.0484
4.2	Male	Adult	ES	309	263.7	2.461	2.405	YES	YES	YES	YES	0.0204	0.0625
	Male	Adult	LS	261.8	260.6	2.365	2.381	NO	NO	YES	YES	0.9568	0.668
	Male	Young	LS	312.4	199	2.441	2.273	YES	NO	YES	YES	0.0285	0.0507
5.1	Male	Adult	ES	1613	1579	3.206	3.196	YES	YES	YES	YES	0.0679	0.0491
	Male	Adult	LS	1543	1567	3.182	3.188	NO	NO	NO	NO	0.4145	0.4481
	Male	Young	LS	1560	1560	3.188	3.19	YES	YES	NO	YES	0.9989	0.9409
5.2	Male	Adult	ES	6820	6706	3.833	3.826	YES	YES	YES	YES	0.0231	0.0284
	Male	Adult	LS	6903	6930	3.838	3.84	YES	YES	YES	YES	0.7125	0.71
	Male	Young	LS	6584	6642	3.817	3.822	YES	YES	YES	YES	0.7561	0.6807
6.1	Male	Adult	ES	982.5	983.6	2.992	2.992	YES	YES	YES	YES	0.7728	0.7357
	Male	Adult	LS	991.4	988.2	2.996	2.994	NO	YES	NO	YES	0.6736	0.6836
	Male	Young	LS	943	945.3	2.974	2.975	YES	YES	YES	YES	0.8435	0.8141
6.2	Male	Adult	ES	1045	1033	3.019	3.014	YES	YES	YES	YES	<0.0001	<0.0001
	Male	Adult	LS	1051	1043	3.021	3.018	YES	YES	YES	YES	0.0313	0.0293
	Male	Young	LS	999.4	999.3	2.999	2.999	YES	YES	YES	YES	0.9754	0.9363
6.3	Male	Adult	ES	1021	1024	3.009	3.01	YES	YES	YES	YES	0.4259	0.3915
	Male	Adult	LS	1026	1027	3.011	3.011	YES	YES	YES	YES	0.8396	0.8525
	Male	Young	LS	999	990.9	2.999	2.996	YES	YES	YES	YES	0.3927	0.3914
6.4	Male	Adult	ES	530.2	536.6	2.723	2.727	YES	NO	YES	NO	0.4043	0.4375
	Male	Adult	LS	489.4	526.8	2.686	2.719	YES	YES	YES	YES	0.0082	0.0084
	Male	Young	LS	457.4	520	2.657	2.714	YES	YES	YES	YES	0.0972	0.0971
8	Male	Adult	ES	72288	72484	4.859	4.86	YES	YES	YES	YES	0.0374	0.0361
	Male	Adult	LS	71772	71690	4.856	4.855	YES	YES	YES	YES	0.4772	0.4622
	Male	Young	LS	69663	69826	4.843	4.844	YES	YES	YES	YES	0.51	0.5454

3.6 Summary of significant differences between left and right Variables of males

Some Variables had significant differences for some groups, while other groups had differences between left and right primaries, but lacked either normal distribution or a significant p-value to statistically confirm this observation. A narrative summary of significantly different Variables is provided below. Tables 3.5.2 and 3.5.3 provide numerical summaries.

3.2.1 Variable 1

Log-transformed Variable 1 (length of calamus) of late season adult males had significantly longer right primaries compared with left primaries (Fig 3.5.1 D). Early season adult males suggested the same result, but were not statistically significant. Late season young males suggested the opposite, but did not have significant p-values.

3.2.2 Variable 2

Variable 2 (length of rachis to the start of anterior vane) indicated no significant differences, however all groups suggested that the left primaries were slightly longer. These differences were not significant.

3.2.3 Variable 3

Variable 3 (length of rachis to the start of posterior vane) indicated no significant differences, however the groups did vary between left and right being the largest. These differences were not significant.

3.2.4 Variable 4.1

Non-transformed Variable 4.1 (length of plumaceous barbs, with respect to the rachis, on the anterior vane) of early season adult males were significantly longer on the left primaries compared with right primaries (Fig 3.5.4 A). Conversely, log-transformed late season young males had a result were right primaries were longer than left primaries (Fig 3.5.4 F). Late season adult males suggested the same result, but lacked significant p-values.

3.2.5 Variable 4.2

Non-transformed early season adult males indicated that Variable 4.2 (length of plumaceous barbs, with respect to the rachis, on the posterior vane) was significantly longer on the left primaries in comparison with the right primaries (Fig 3.5.5 A). Late season young males suggested the same, but lacked normal distribution and a significant p-value for the t-tests. Late season adult males suggested no difference. Although care was taken to measure

Variable 4.1 and 4.2 consistently, it has to be noted that Variable 4.1 and Variable 4.2 were difficult to measure due to intertwined nature of the plumaceous barbs.

3.2.6 Variable 5.1

Log-transformed early season adult males had a result were Variable 5.1 (width of the anterior vane from a proportionately consistent point on the rachis) was wider on the left primaries in comparison with the right (Fig: 3.5.6 B). Late season adult males suggested the opposite, but lacked normal distribution and significant p-values. Late season young males indicated no difference between left and right primaries; this observation was not significant.

3.2.7 Variable 5.2

Early season adult males had significantly wider left primaries for Variable 5.2 (width of the posterior vane from a proportionally consistent point on the rachis) compared with the right primaries, based on non-transformed data (Fig 3.5.7 G). Late season adult males and late season young males had no significant differences for this Variable.

3.2.8 Variable 6.1

Variable 6.1 (width of rachis at the distal edge of the calamus) indicated no significant differences.

3.2.9 Variable 6.2

Non-transform of early season adult males (Fig 3.1.9 A) and late season adult males (Fig 3.1.9 C) indicated that Variable 6.2 was thicker on the left primaries in comparison with the right primaries. Late season young males had no significant differences.

3.2.10 Variable 6.3

Variable 6.3 (width of the rachis at the start of the anterior vane) indicated no significant differences.

3.2.11 Variable 6.4

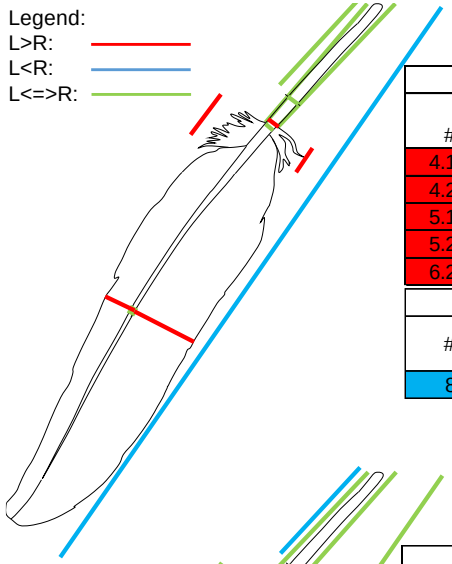
Non-transformed late season adult males indicated that Variable 6.4 (width of the rachis at a proportionately consistent point) was thicker on the right primaries in comparison with the left (Fig 3.1.11 C). Late season young males suggested the same result, but lacked significant p-values. Early season adult males suggested no difference.

3.2.9 Variable 8

The non-transformed length of the right rachis (Variable 8) was significantly longer on right primaries compared with left primaries for early season adult males (Fig 3.1.12 A). Late

season young males suggested the same, but these differences were not significant. Late season adult males suggested the opposite, but these differences were not significant.

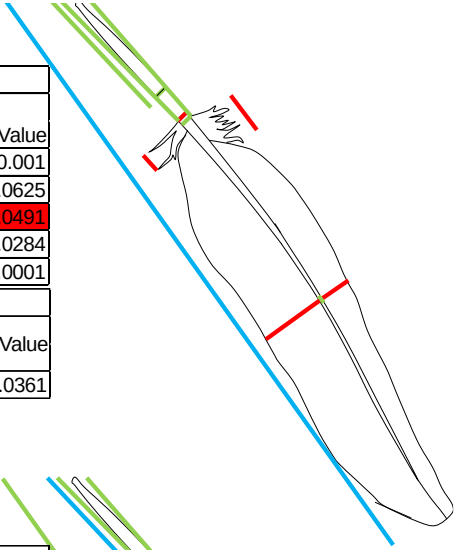
Table 3.5.2: *A schematic representation of the means and results of non-transformed and log-transformed, paired, two-tailed, t-test comparisons between left and right primaries of early season and late season adult males. Because this is a paired comparison, the left and right representations of the feathers are identical. Red indicates a significant result where the dimensions of the left primaries were larger than the right primaries. Blue indicates a significant result where the dimensions on the right primaries were larger than the left primaries. Green indicates no significant differences. The discussion of the detailed findings is presented in Section 3.6 and Section 4.2.*



Early season adult males

LEFT > RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
4.1 ES	6372	6027	3.801	3.777	YES	YES	NO	YES	0.0011	0.001
4.2 ES	309	263.7	2.461	2.405	YES	YES	YES	YES	0.0204	0.0625
5.1 ES	1613	1579	3.206	3.196	YES	YES	YES	YES	0.0679	0.0491
5.2 ES	6820	6706	3.833	3.826	YES	YES	YES	YES	0.0231	0.0284
6.2 ES	1045	1033	3.019	3.014	YES	YES	YES	YES	<0.0001	<0.0001

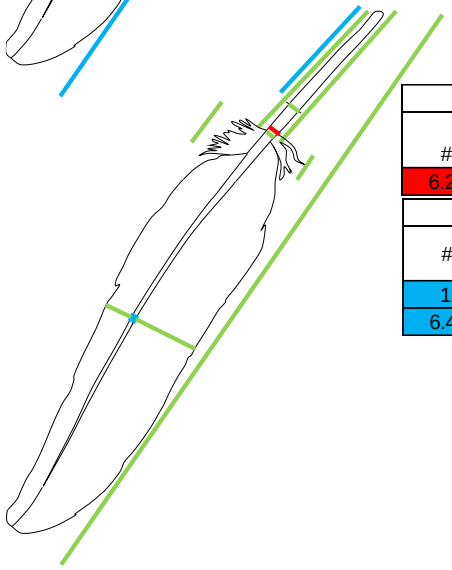
LEFT < RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
8 ES	72288	72484	4.859	4.86	YES	YES	YES	YES	0.0374	0.0361



Late season adult males

LEFT > RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
6.2 LS	1051	1043	3.021	3.018	YES	YES	YES	YES	0.0313	0.0293

LEFT < RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
1 LS	7371	7434	3.867	3.871	YES	NO	YES	YES	0.031	0.0353
6.4 LS	489.4	526.8	2.686	2.719	YES	YES	YES	YES	0.0082	0.0084



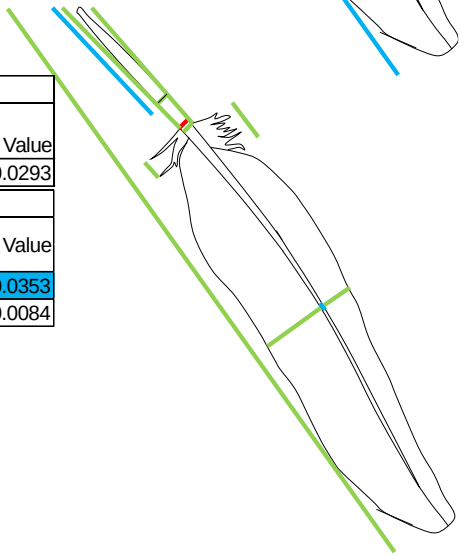
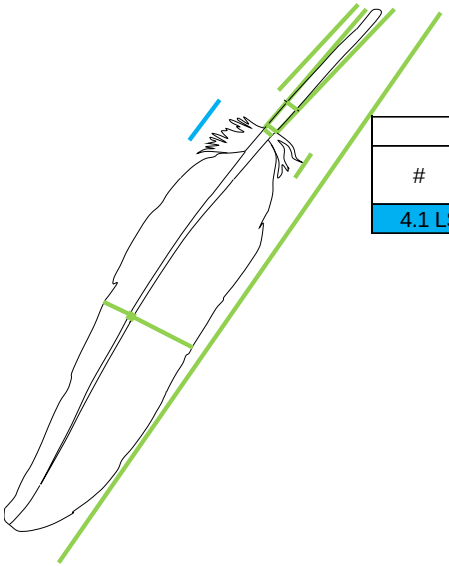


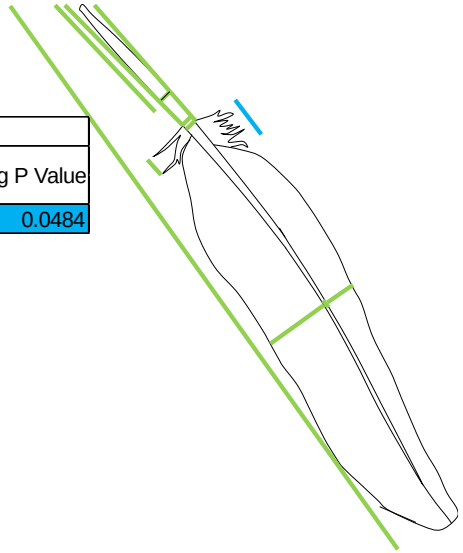
Table 3.5.3: *A schematic representation of the means and results of non-transformed and log-transformed, paired, two-tailed, t-test comparisons between left and right primaries of early season and late season young males. Because this is a paired comparison, the left and right representations of the feathers are identical. Red indicates a significant result where the dimensions of the left primaries were larger than the right primaries. Blue indicates a significant result where the dimensions on the right primaries were larger than the left primaries. Green indicates no significant differences. The discussion of the detailed findings is presented in Section 3.6 and Section 4.2.*

Legend:
L>R:
L<R:
L<=>R:

Late season young males



LEFT < RIGHT										
#	Mean (µm)		Mean Log		Pass normality test		Log Pass normality test		P Value	Log P Value
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT		
4.1 LS	4824	5198	3.673	3.706	YES	YES	YES	YES	0.0568	0.0484



Chapter 4: Discussion

I will first discuss the differences between means of the left and right Variables between early season and late season P8 feathers of adult and young females (comparisons 1-4). Secondly, I will discuss the differences between means of the left and right Variables between early season and late season P8 feathers of adult and young males (comparisons 5-7). Hereafter, the possible causation of LADPFS will be discussed.

Causal factors mentioned in the introduction (Section 1.6) that could be discounted was the handedness of the measurer and measurement error (Helm & Albrecht, 2000; Watson & Thornhill, 1994), since the measurements were not taken with the feather on the wing, but taken microscopically where handedness of the measurer did not play a role. The differences found could possibly be attributed to AS, DA or FA, due to a directional and non-directional nature; but this needs to be determined through subsequent tests for normality and variance.

SMWs have limited local movement (Section 2.1), therefore a homogenous exposure to stressors can safely be assumed since all individuals were captured at PBS. However, it is not impossible that some individuals might have moved into the sampling area.

4.1 Left versus right comparisons (comparisons 1-7, see Fig 1.8.1)

Perfect bilateral symmetry has been assumed as normal, but one cannot entirely dispute the notion that a small measure of deviation from symmetry is the norm (Kellner & Alford, 2003). However, in the scope of the present study, I assume bilateral symmetry as normal, and any deviation from this is considered LADPFS, most probably as a function of AS, DA, FA or mixtures thereof.

4.1.1 Adult females (comparisons 1 & 3, see Fig 1.8.1)

Comparing the left and right primaries of early season adult females (Table 3.1.2), Variables 4.1 and 6.2 were respectively wider and thicker on the left primaries. In late season adult females (Table 3.1.2), Variable 4.2 was wider on the left primaries, while Variable 6.3 was significantly thicker on the left primaries

4.1.2 Young females (comparisons 2 & 4, see Fig 1.8.1)

Comparing left and right primaries of early season young females (Table 3.1.3), Variable 1 was longer on the right primaries. In late season young females (Table 3.1.3), Variable 4.1 was wider on the left primaries.

4.1.3 Adult males (comparisons 5 & 6, see Fig 1.8.1)

Comparing the left and right primaries of early season adult males (Table 3.5.2), left primaries were larger than right primaries for Variables 4.1, 4.2, 5.1, 5.2, and 6.2; but Variable 8 was longer on the right primaries. In late season adult males (Table 3.5.2), Variables 1 and 6.4 were respectively longer and thicker on the right primaries while Variable 6.2 was thicker on the left.

4.1.4 Young males (comparison 7, see Fig 1.8.1)

Comparing the left and right primaries of early season young males (Table 3.5.2), Variable 4.1 was wider on the right primaries.

4.1.5 Summary of LADPFS

Having asymmetry favouring the left side of the bilateral trait included Variable 4.1 and 6.2 of early season adult females, Variable 4.2 of late season adult females, Variables 4.1, 4.2, 5.1, 5.2, and 6.2 of early season adult males, and Variable 6.2 of late season adult males.

Having asymmetry favouring the right side of the bilateral trait included Variable 1 of early season young females, Variable 6.3 of late season adult females, Variable 8 of early season adult males, Variables 1 and 6.4 of late season adult males, and Variable 4.1 of late season young males. The opposing directions in adult and young males of the early season could be attributed to AS, FA or a structural adaptation where males have a different function or preference for each wing. In early season adult males, the directional results (Table 3.5.3) suggest a more prominent role for these Variables (4.1, 4.2, 5.1, 5.2, and 6.2) on the left wing in comparison with the right wing. However, the opposite is also true for Variable 8 of early season males, where the length of the rachis could have a more prominent role on the right primaries in comparison to the left primaries. However, this has yet to be confirmed.

The present study investigated the presence of LADPFS. No prior knowledge existed on this aspect (Section 1.8). Therefore, it was not possible to *a priory* identify and measure possible causes at this stage, since I had to assume symmetry. The results of this study did present LADPFS that is expressed differently between age, sex, and season. The difference in direction of LADPFS within the same trait was intriguing. Determining the underlying influences (AS, FA, DA, or mixtures thereof) could become insightful in future studies on asymmetry.

I therefore conclude that some dimensions of left and right P8 feathers of SMWs are significantly asymmetric and that age, sex, and season have been accounted for. LADPFS were present in this study and could be a function of AS, FA, DA, or mixtures thereof.

Bilateral traits can be influenced by a variety of factors. Below, I will summarize these factors.

4.2 Possible causes of LADPFS

4.2.1 Genetic stress

Genetic stress includes factors such as maternal age and a genetic predisposition for asymmetry, even in unstressed environments (Møller, 1996; Parsons, 1990; Watson & Thornhill, 1994). Furthermore, individual genetic composition can influence the ability of an organism to buffer stressors that are commonly associated with FA (Jenni-Eiermann *et al*., 2015; Rohwer & Rowher, 1994; Swaddle & Witter, 1994; Watson & Thornhill, 1994). In the present study, genetic variation was not measured. Since all the birds were from the same area and presumably freely interbreeding, a uniformly buffered population may be assumed. However, this needs to be determined.

4.2.2 Developmental and environmental stress

Current literature provides a variety of potentially causal developmental and environmental stressors associated with FA.

Developmental and environmental stressors include:

- Exposure to heavy metal pollution (Bustnes *et al*., 2002; Eeva *et al*., 2000; Herring *et al*., 2017),
- Exposure to certain POPs (Bustnes *et al*., 2002; Bustnes *et al*., 2007; Jenssen *et al*., 2010),
- Reduced time available to complete moult (Rohwer & Rohwer, 2013; Susanna & Hall, 2000),
- Nutritional and energetic stress (e.g. lack of food, drought, and mating), as indicated by Swaddle & Witter (1994),
- Elevated CORT levels as a response to developmental and environmental stress (Jenni-Eiermann *et al*., 2015; Romero *et al*., 2000),
- Predation risk, intraspecific competition, or other environmental perturbations.

The impact(s) of these factors are not known for the present study, as it assumes differential individual exposure to stressors. This is unlikely, given the small area from where the birds were sampled. Homogenous exposure to the same stressors may therefore be assumed. This assumption could be tested by analysing the feathers for, say, heavy metals, POPs, and/or CORT and regress the differences between feather Variables with stressor levels.

4.2.3 Behavioural factors

Behavioural factors could affect the susceptibility to factors that promote asymmetry. An example of this is that some male SMWs are lateralized (direction in which they insert grass) in nest building. Lateralized males tended to build nests faster than non-lateralized males (Walsh *et al*., 2011). They conclude that this could be attributed to increasing dexterity during nest building and that lateralization is experience-dependant. As in the study conducted by Walsh *et al* (2011) there may be more unknown factors in behaviour that could promote DA or FA, but what these factors might be for the present study has yet to be ascertained.

4.2.4 Changes in keratin over time

Regardless of the expected wear, it is not impossible that the keratin structures of feathers changes with time. This might explain some of the phenomena observed where asymmetries were expressed differently between early season and late season feathers. The mechanical properties of β-keratin (of which feathers are based) are susceptible to the effects of aging and relative humidity (Wang *et al*, 2016). Most studies focussed on the mechanical properties and inner structure of feathers, but what the effects are on the outer structure needs more attention. It is possible that factors such as humidity and aging could induce the differences found between early and late season feathers, but more study is needed to confirm this observation.

4.2.5 Summary of possible causal factors

In summery the observation of LADPFS in SMWs could possibly be attributed to one or a combination of the following:

- Antisymmetry,
- Behavioural factors (Walsh *et al*., 2011),
- Climate perturbations (Watson & Thornhill, 1994),
- Directional asymmetry,
- Elevated CORT levels (Jenni-Eiermann *et al*., 2015; Romero *et al*., 2000),
- Heavy metal pollution (Bustnes *et al*., 2002; Eeva *et al*., 2000; Herring *et al*., 2017),
- POPs pollution (Bustnes *et al*., 2002; Bustnes *et al*., 2007; Jenssen *et al*., 2010),
- Genetic predisposition to FA (Møller, 1996; Watson & Thornhill, 1994),
- Induced need for rapid moulting (Jenni-Eiermann *et al*., 2015; Rohwer & Rohwer, 2013; Susanna & Hall, 2000),
- Lack of fitness (Parsons, 1990; Rohwer & Rowher, 1994; Swaddle & Witter, 1994),
- Maternal age (Parsons, 1990; Watson & Thornhill, 1994),

- High BMR that increases cost of moulting (Lasiewski & Dawson 1967; Lindström *et al*., 1993), and
- Nutritional and energetic costs (Swaddle & Witter, 1994).

None of the above-mentioned factors could be discounted, as the assumed homogenous exposures to these factors were not measured. Furthermore, irregular intra-species responses have been observed before (Swaddle & Witter, 1994; Swaddle *et al*., 1994). Therefore, different groups of the same species could respond different from other groups (e.g. irregular expression between sexes and age as seen in the current study).

However, the most likely causal factors of the differences in expression and direction of LADPFS includes behaviour that promotes energetic stress at the time of moult, irregular response to the same stressors between groups, antisymmetry, and directional asymmetry.

4.3 Current hypothesis on the origin of FA

In Section 1.7.5, I listed seven hypotheses in current literature. I cannot discount any of these hypotheses. However, since I measured asymmetry in fully-grown feathers of adult SMWs and did not focus on growth or timeframes of growth I cannot comment on the following four:

- *The accumulation of accidents hypothesis*,
- *The persistent asymmetry hypothesis*,
- *The coin toss hypothesis*, and
- *The magnification of asymmetry hypothesis*.

The remaining three hypotheses named in Section 1.7.5, however, deserve some comment for consideration in future study of LADPFS.

- *The directional external cues hypothesis* suggests that asymmetry can be induced by side-biased environmental influences (Kellner & Alford, 2003). In the present study, I did not account for directional cues, but an adaption to handedness in flight or nest building (Walsh *et al*., 2011), or the influence of an external directional cue such as synoptic conditions during moult or embryonic development might be involved (Kellner & Alford, 2003; van Dongen, 2006).
- *The compensatory growth hypothesis* suggests that large deviations between left and right symmetry is not the norm - there would be feedback mechanisms (Kellner & Alford, 2003). This can occur through increased growth on the lagging side, or halted growth on the larger side (Kellner & Alford, 2003). Since fully-grown feathers of adult

SMWs were measured in this study, it could not account for compensatory feedback mechanisms. However, future studies on FA should consider LADPFS and should include feathers at different stages of development.

- *The* r*esidual growth hypothesis* suggests that asymmetry is influenced by compensatory mechanisms that counter developmental stressors (Kellner & Alford, 2003). Therefore, asymmetry reflects only recent exposure to developmental stressors as individuals have the ability to correct the deviation (Kellner & Alford, 2003). Since fully-grown feathers of adult SMWs were measured in this study, it could not account for compensatory feedback mechanisms. However, future studies on FA should consider LADPFS and should include feathers at different stages of development.

Chapter 5: Conclusions and considerations

5.1 Conclusions

The methods employed and the numbers sampled were robust enough to identify P8 feather structure Variables that did or did not differ between left and right primaries.

5.1.1 Left vs right

LADPFS were present and could possibly be a function of AS, DA, FA, or mixtures thereof; but further investigation is still needed to determine causation.

My first hypothesis was that there are laterally-associated differences in primary feather structure (LADPFS) between the left and right P8 feathers of SMWs for both males and females, and that the LADPFS patterns will differ between sex and age.

My second hypothesis was that LADPFS is expressed differently between early season (new) primaries and late season (old) primaries for both sexes and age.

Perfect bilateral symmetry can be influenced by a variety of causal factors. Some of causal factors could include:

- Antisymmetry,
- Behavioural patterns,
- Developmental stress,
- Genetic stress,
- Handedness (directional asymmetry), and
- Pollution.

However, LADPFS may most likely be attributed to handedness and behavioural factors as homogenous exposure to the other stressors could be assumed. Directional asymmetry (handedness) is likely an adaptation with some form of unknown function. Furthermore, behavioural factors can make a group (e.g. males) more susceptible to FA through increased energetic stress for the particular group. This is also dependent on the group's ability to cope with these stressors. Finally, the possibility that the differences found are attributable to AS could not be discounted.

In absolute terms, the asymmetries found in the structure of P8 feathers were relatively small. Albeit small, these differences may be biologically significant, especially if the other nine primary, six secondary, and three tertial feathers per wing also display LADPFS. Small differences in one feather likely become biologically significant if the trend persists within the 38 flight feathers of the SMW. Wing aspect ratios, wing loadings, flight muscle ratios, and

power/mass ratios might be affected by asymmetric tendencies (Maclean, 1990), presumably requiring more energy to maintain stable flight.

The results found in this study do not disprove the hypothesis that there are laterally-associated differences in primary feather structure (LADPFS) between the left and right P8 feathers of SMWs for both males and females, and that the LADPFS patterns will differ between sex and age.

The results found in this study also does not disprove the hypothesis that LADPFS will be expressed differently between early season (new) primaries and late season (old) primaries throughout both sexes and age.

It is clear that future studies on LADPFS should control for feather age, sex, and individual age. Finally, it is not sufficient to only include length measurements when identifying patterns of asymmetry in bird feathers as a structural approach such as LADPFS would give more reliable results

5.2 Considerations

5.2.1 Left vs right

LADPFS are undoubtedly an area that requires further investigation to explain the anomalies found in this study. Further study should be focussed on determining associations between LADPFS and asymmetry (AS, DA, and FA), keeping in mind the limitations listed in Section 1.7.4.

Some considerations include:

- Multiple geographic reference groups,
- Include more species from the area,
- Compare LADPFS between seasons,
- Inclusion of environmental factors that could contribute (e.g. heavy metal pollution),
- Inclusion of secondary feathers, tertiary feathers, and tail feathers,
- Age of the matured feather,
- Include associations between gross morphological measurements (e.g. wing length, body length, and weight) and LADPFS,
- Measurement of barbs and barbules (e.g. density, width, and length),
- Measurement of feathers at different stages of development, and
- Study LADPFS as a structural response to AS, DS, FA, or mixtures thereof.

Now that it has been shown that there are differences in the expression and direction of LADPFS between early season and late season primaries, between males and females, and age of individuals; future studies can account for these differences.

5.3 Testable hypotheses

5.3.1 LADPFS

Although some previous workers have looked at asymmetry in feathers, none in the detail as this study. Further study is needed to ascertain the extent, biological relevance, and reasons for these differences. I present the following predictions and testable hypotheses (some are contradictory since we just do not know which are correct), and there may be many more:

- In environments where birds are homogenously exposed to factors that may cause FA, the most likely cause for irregular intra-species expression are behavioural factors that promote energetic stress.
- LADPFS are a structural-level form of FA and is induced by the same causal factors.
- LADPFS are the result of DS and AS.
- The measure of deviation (for instance using percentage coefficients of variation) from perfect symmetry may be normal.
- Factors that contribute to FA influence a bird's ability to adapt structurally to seasons.
- Asymmetry is associated with behavioural traits such a preferred direction of flight after take-off (left or right), orientation towards the sun, or direction taken to fly around obstacles.
- Birds display handedness in flight feathers (primaries, secondaries, and tertiaries), favouring the side the embryo faces during embryonic development.
- The embryo orientation in the egg is not symmetrical. Directional cues play a role in the expression of LADPFS.
- Handedness (directional asymmetry) is embedded in the structural composition of the feather primaries, to such an extent that it would also be found in the barbs and barbules.
- The handed side of the bird will have a stronger capacity to buffer FA.
- Birds depending on soaring as a major mode of locomotion will have less asymmetry than birds using powered flight.
- Larger birds will have less asymmetry than smaller birds (or the other way round).
- Most bird species that moult twice a year modify the structural composition of feathers to adapt to seasons and climate.
- Sedentary species in regions experiencing large differences between summer and winter temperatures will have more pronounced structural adaptation.

- Structural adaptations will be more pronounced in passerines than in non-passerines as they are generally smaller.
- Structural adaptation will express differently in migratory and non-migratory birds that are closely related.
- Structural adaptation will be present in diving birds such as penguins and cormorants, but I have no idea how this will be expressed.
- Gross morphological traits (e.g. body weight, feather length, sex, and maternal age) are significantly associated with the ability of the bird to adapt structurally to seasonal changes.
- Structural adaptation will also be present in barb and barbule structure.

References

Abbasi, N.A., Eulaers, I., Jaspers, V.L.B., Chuadry, M.J.I., Frantz, A., Ambus, P.L., Covaci, A. & Malik, R.N. 2016. Use of feathers to assess polychlorinated biphenyl and organochlorine pesticide: exposure in top predatory bird species of Pakistan. *Science of the Total Environment*, 569-570:1408-1417.

Abbasi, N.A., Arukwe, A., Jaspers, V.L.B., Eulaers, I., Mennilo, E., Ibor, O.R., Frantz, A., Covaci, A. & Malik, R.N. 2017. Oxidative stress responses in relationship to persistent organic pollutant levels in feathers and blood of two predatory bird species from Pakistan. *Science of the Total Environment*, 580:26-33.

Able, K.P. 1973. The role of weather variables and flight direction in determining the magnitude of nocturnal bird migration. *Ecology*, 54:1031-1041.

Aparicio, J.M. 1998. Patterns of fluctuating asymmetry in developing primary feathers: a test of the compensational growth hypothesis. *Proceedings of the Royal society of London B: Biological Sciences*, 265:2353-2357.

Aparicio, J.M. & Bonal, R. 2002. Why do some traits show higher fluctuating asymmetry than others? A test of hypothesis with tail feathers of birds. *Heredity*, 89:139-144.

Benderlioglu, Z. 2010. Fluctuating asymmetry and steroid hormones: a review. *Symmetry,* 2:541-553.

Birkhead, T. & Brooke, M. 1991. *The Cambridge encyclopaedia of ornithology*. 1st ed. Cambridge: Cambridge University Press.

Bize, P., Roulin, A. & Richner, H. 2004. Parasitism, developmental plasticity and bilateral asymmetry of wing feathers in alpine swift, *Apus melba*, nestlings. *OIKOS*, 106:317-323.

Brown, C.R. & Brown, M.B. 2002. Ectoparasites can cause increased bilateral asymmetry of naturally selected traits in a colonial bird. *Journal of Evolutionary Biology*, 15:1067-1075.

Bustnes, J.O., Folkstad, I., Erikstad, J.E., Fjeld, M., Miland, Ø.O. & Skaare, J.U. 2002. Blood concentration of organochlorine pollution and wing feather asymmetry in Glaucous gulls. *Functional Ecology*, 16:617-612.

Bustnes, J.O., Kristiansen, K.O. & Helberg, M. 2007. Immune status, carotenoid coloration, and wing feather growth in relation to organochlorine pollutants in Great Black-Backed gulls. *Archives in Environmental Contamination and Toxicology*, 53:96-102.

Chittenden, H., Allan, D. & Weiersbye, I. 2012. *Roberts Geographic Variation of South African Birds*. 1st ed. Cape Town: The John Voelcker Bird Book Fund.

Clarke, G.M. 1995. Relationships between developmental stability and fitness: application for conservation biology. *Conservation Biology*, 9:18-24.

Croxall, J.P. 1982. Energy costs of incubation and moult in petrels and penguins. *Journal of Animal Ecology*, 51:177-194.

De Beer, S.J., Lockwood, G.M., Raijmakers, J.H.F.A., Raijmakers, J.M.H., Scott, W.A., Oschadleus, H.D. & Underhill, L.G. 2001. *SAFRING bird ringing manual. ADU Guide 5*. (eds) Cape Town: Avian Demography Unit, University of Cape Town.

Dietz, M.W., Daan, S. & Masman, D. 1992. Energy requirements for molt in the kestrel, *Falco tinnunculus*. *Physiological Zoology*, 65:1217-1235.

Dolnik, V.R. & Gavrilov, V.M. 1979. Bioenergetics of molt in the Chaffinch (*Fringilla coelebs*). *The Auk*, 96:253-264.

Eeva, T., Tanhuapaa, S., Råbergh, C., Airaksinen, S., Nikinmaa, M. & Lehikoinen, E. 2000. Biomarkers and fluctuating asymmetry as indicators of pollution-induced stress in two hole-nesting passerines. *Functional Ecology*, 14:235-243.

Eulaers, I., Jaspers, V.L.B., Halley, D.J., Lepoint, G., Nygàrd, T., Pinxton, R., Covaci, A. & Eens, M. 2014. Brominated and phosphorus flame retardants in white-tailed eagle *Haliaeetus albicilla* nestlings: bioaccumulation and associations with dietary proxies (δ^{13}C, δ^{15}N, δ^{34}S). *Science of the Total Environment*, 478:48-57.

García-Fernández, A.J., Espín, S. & Martínez-López, E. 2013. Feathers as a biomonitoring tool of polyhalogenated compounds: a review. *Environmental Science and Technology*, 47:3028-3043.

Graham, J.H., Raz, S., Hel-Or, H. & Nevo, E., 2010. Fluctuating asymmetry: methods, theory, and applications. *Symmetry*, 2:466-540.

Grieco, F. 2003. Greater food availability reduces tarsus asymmetry in nestling blue tits. *The Condor*, 105:599-603.

Griggio, M., Serra, L., Licheri, D., Campomori, C. & Pilastro, A. 2009. Moult speed affects structural feather ornaments in the blue tit. *Journal of Evolutionary Biology*, 22:782-792.

Helm, B. & Albrecht, H. 2000. Human handedness causes directional asymmetry in avian wing length measurements. *Animal Behaviour*, 60:899-902.

Herremans, M. 1994. Partial migration in the Masked Weaver <Ploceus velatus> in south-eastern Botswana. *Ostrich*, 65: 79-85.

Herring, G., Eagles-Smith, C.A. & Ackerman, J.T. 2017. Mercury exposure may influence fluctuation asymmetry in water-birds. *Environmental Toxicology and Chemistry*, 36:1599-1605.

Hickman, C.P., Jr, Roberts, L.S., Keen, S.L., Eisenhour, D.J., Larson, A. & l'Anson, H. 2011. *Integrated principles of zoology*. 15th ed. New York: McGraw-Hill.

Hockey, P.A.R., Dean, W.R.J., & Ryan, P.G. 2005. *Roberts – Birds of Southern Africa*. 7th ed. Cape Town: The Trustees of the John Voelcker Bird Book Fund.

IUCN. 2015. Global IUCN Red List for birds. http://www.birdlife.org/datazone/species/search (Last visited 15/06/2016).

Jenni-Eiermann, S., Helfenstein, F., Vallat, A., Glauser, G. & Jenni, L. 2015. Corticosterone: effects on feather quality and deposition into feathers. *Methods in Ecology and Evolution*, 6:237-246.

Jensson, B.M., Aarnes, J.B., Murvoll, K., Herzke, D., & Nygàrd, T. 2010. Fluctuating wing asymmetry and hepatic concentrations of persistent organic pollutants are associated in European shag (*Phalacrocorax aristotelis*). *Science of the Total Environment*, 408:478-485.

Kellner, J.R. & Alford, R.A. 2003. The ontogeny of fluctuating asymmetry. *The American Naturalist*, 161:931-947.

Kendeigh, S.C. 1970. Energy requirements for existence in relation to size of bird. *The Condor*, 72:60-65.

Kidson, S. & van Niekerk, H. 2014. *Birds of South Africa*. 1st ed. Pretoria: Briza Publications.

Klingenberg, C.P. & Nijhout, H.F. 1999. Genetics of fluctuating asymmetry: a developmental model of developmental instability. *Evolution*, 52:358-375.

Knierim, U., van Dongen, S., Forkman, B., Tuyttens, F.A.M., Špinka, M., Campo, J.L. & Weissengruber, G.E. 2007. Fluctuating asymmetry as an animal welfare indicator: A review of methodology and validity. *Physiology & Behaviour*, 92:398-421.

Lasiewski, R.C. & Dawson, W.R. 1967. A re-examination of the relation between standard metabolic rate and body weight in birds. *The Condor*, 69:13-23.

Leamy, L.J. & Klingenberg, C.P. 2005. The genetics and evolution of fluctuating asymmetry. *Annual Review of Ecology, Evolution, and Systematics*, 36:1-21.

Lens, L., van Dongen, S., Wilder, C.M., Brooks, T.M. & Matthysen, E. 1999. Fluctuating asymmetry increases with habitat disturbance in seven bird species of a fragmented afrotropical forest. *Proceedings of the Royal Society of London B: Biological Sciences*, 266:1241-1246.

Lens, L., van Dongen, S., Kark, S. & Matthysen, E. 2001. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biological Reviews of the Cambridge Philosophical Society*, 77:27-38.

Leung, B. 1998. Correcting for allometry in studies of fluctuating asymmetry and quality within samples. *Proceedings of the Royal Society of London B: Biological Sciences*, 265: 1623-1629.

Leung, B. & Forbes, M.R. 1996. Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. *Écoscience*, 3:400-413.

Leung, B., Forbes, M.R. & Houle, D. 2000. Fluctuating asymmetry as a bio-indicator for stress: comparing efficiency of analysis involving multiple traits. *The American Naturalist*, 155:101-115.

Lindström, Å., Visser, G.H. & Daan, S. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. *Physiological Zoology*, 66:490-510.

Lingham-Solair, T. 2015. *The vertebrate integument*. vol 2. Verlug Berlin Heidelberg. DOI: 10.1007/978-3-662-46005-4_1.

Lingham-Solair, T. 2017. Microstructural tissue-engineering in the rachis and barbs of bird feathers. *Scientific Reports*, 7:451-462.

McKittrick, J., Chen, P.Y., Bodde, S.G., Yang, W., Novitskaya, E.E. & Meyers, M.A. 2012. The structure, functions, and mechanical properties of keratin. *Journal of the Minerals, metals, and Materials Society*, 4:449-467.

Maclean, G.L. 1990. *Ornithology for Africa*. Pietermaritzburg: University of Natal Press.

Møller, A.P. & Hoglund, J. 1991. Patterns of fluctuating asymmetry in avian feather ornaments: implications for models of sexual selection. *Proceedings of the Royal Society B: Biological Sciences*, 245:1-5.

Møller, A.P. 1992. Female swallow preference for symmetrical male sexual ornaments. *Nature,* 357:238-240.

Møller A.P. & Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments? *Behavioural Ecology and Sociobiology*, 32:167-176.

Møller, A.P. 1996. Development of fluctuating asymmetry in tail feathers of the barn swallow *Hirundo rustica*. *Journal of Evolutionary Biology*, 9:677-694.

Murphy, M.E. & King, J.R. 1984. Sulfur amino acid nutrition during molt in the white-crowned sparrow: does dietary sulfur amino acid concentration affect the energetics of molt as assayed by metabolized energy? *Condor*, 86:314-323.

Müller, W. & Patone, G. 1998. Air transmissivity of feathers. *The Journal of Experimental Biology*, 201:2591-2599.

Newton, I. 1968. The temperatures, weights, and body composition of moulting bullfinches. *The Condor*, 70:323-232.

Palestis, B.G. 2009. Fluctuating asymmetry in common tern (*Sterna hirundo*) chicks varies with hatching order and clutch size. *The Auk*, 126:815-822.

Palmer, A.R. 1994. Fluctuating asymmetry: a primer. pp. 335-364. In Markow, T.A. *Developmental Instability: Its Origins and Evolutionary Implications.* (eds). Dordrecht: Kluwer.

Palmer, A.R. 1996. Waltzing with asymmetry. *BioScience*, 46:518-532.

Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology, Evolution, and Systematics*, 17:391-421.

Palmer, A.R. & Strobeck, C. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. *Acta Zoologica Fennica*, 191:57-72.

Palmer, A.R. & Strobeck, C. 2001. Fluctuating asymmetry analyses revisited. *Developmental Instability (DI): Causes and Consequences*. Oxford: Oxford University Press. pp. 279-319.

Parsons, P.A. 1990. Fluctuating asymmetry. an epigenetic measure of stress. *Biological Reviews*, 65:131-145.

Parsons, P.A. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, 68:361-364.

Price, T., Pavelka, E.Chi.M. & Hack, M. 1991. Population and developmental variation in the feather tip. *Evolution*, 45:518-533.

Rohwer, V.G. & Rowher, S. 2013. How do birds adjust the time required to replace their flight feathers? *The Auk*, 130:699-707.

Romero, L.M., Reed, J.M. & Wingfield, J.C. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. *General and Comparative Endocrinology*, 118:113-122.

Schmidt-Nielsen, K. 2007. *Animal physiology: adaption and environment*. 5th ed. Cambridge: Cambridge University Press.

Shamoun-Baranes, J., van Loon, E., van Gasteren, H., van Belle, J., Bouten, W. & Buurma, L. 2006. A comparative analysis of the influence of weather patterns on the flight altitudes of birds. *American Meteorological Society*, 87:47-61.

Shykoff, J.A. & Møller, A.P. 1999. Fitness and asymmetry under different environmental conditions in the barn swallow. *OIKOS*, 86:152-158.

Sinclair, I., Hockey, P., Tarboton, W. & Ryan, P. 2011. *Sasol birds of Southern Africa*. 4th ed. Cape Town: Struik Nature.

Strochlic, D.E. & Romero, L.M. 2008. The effects of chronic psychological and physical stress on feather replacement in European starlings (*Sturnus vulgarus*). *Comparative Biochemistry and Physiology*, Part A, 149:68-79.

Susanna, K. & Hall, S. 2000. Lesser whitethroats under time-constraint moult more rapidly and grew shorter wing feathers. *Journal of Avian Biology*, 31:583-587.

Swaddle, J.P., Witter, M.S. & Cuthill, I.C. 1994. The analysis of fluctuation asymmetry. *Animal Behaviour*, 48:986-989.

Swaddle, J.P. & Witter, M.S. 1994. Food, feathers and fluctuating asymmetries. *Proceedings: Proceedings of the Royal Society of London B: Biological Sciences*, 255:147-152.

Swaddle, J.P. & Witter, M.S. 1998. Cluttered habitats reduce wing asymmetry and increase flight performance in European starlings. *Behavioral Ecology and Sociobiology*, 42:281-287.

Thomas, A.L.R. 1993. The aerodynamic costs of asymmetry in the wings and tails of birds – asymmetric birds can't fly round tight corners. *Proceedings of the Royal Society of London B: Biological Sciences*, 254:181-189.

Thornhill, R. 1992a. Female preference for pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). *Behavioural Ecology*, 3:277-283.

Thornhill, R. 1992b. Fluctuating asymmetries, intraspecific aggression and male mating tactics in two species of Japanese scorpionflies. *Behavioural Ecology and Sociobiology*, 30:357-363.

Underhill, L.G. 1999. Avian demography: statistics and ornithology. *Ostrich*, 70:61-70.

Van Dongen, S. 2006. Fluctuating asymmetry in and developmental instability in evolutionary biology: past, present and future. *The Author*, 19:1727-1743.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution*, 16:125-142.

Walsh, P.T., Hansell, M., Borello, W.D. & Healy, S.D. 2011. Individuality in nest building: do Southern Masked-Weaver (*Ploceus velatus*) males vary in nest-building behaviour. *Behavioural Processes*, 88:1-6.

Wang, B., Yang, Y., McKittric, J. & Meyers, M.A. 2016. Keratin: structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science*, 76:229-319.

Watson, P.J. & Thornhill, R. 1994 Fluctuating asymmetry and sexual selection. *TREE*, 9:21-25.

Zakharov, V.M. 1992. Population phenogenetics: analyses of developmental stability in natural populations. *Acta Zoologica Fennica*, 191:7-30.

Appendix A: *Presents the p-values, means, standard deviation, and CV of transformed and non-transformed, two-tailed, paired t-tests of females. The significant results were highlighted in red or blue for valid comparisons. Red indicates a significant result where the left primaries were longer than right primaries for that Variable. Blue indicates a result where right primaries were longer than left primaries for that Variable. Variables that were not normally distributed or had p-values > 0.05 were not highlighted.*

Variable	Age	Sex	Season	Mean Left (µm)	SD	Mean Right (µm)	SD	Median Left (µm)	Median Right (µm)	CV Left (%)	CV Right (%)	Log Mean Left	SD	Log Mean Right	SD	Log Median Left	Log Median Right	CV Left (%)	CV Right (%)
1	Adult	F	ES	7398	381.3	7453	342.2	7331	7428	5.15	4.59	3.869	0.02203	3.872	0.02001	3.865	3.871	0.57	0.52
	Young	F	ES	7345	398.8	7409	431.4	7355	7451	5.43	5.82	3.865	0.02363	3.869	0.02564	3.867	3.872	0.61	0.66
	Adult	F	LS	7029	384.6	7089	421.4	6959	7016	5.47	5.94	3.846	0.02378	3.85	0.02583	3.843	3.846	0.62	0.67
	Young	F	LS	7255	662.2	7205	617.4	7002	7046	9.13	9.47	3.859	0.03895	3.856	0.03677	3.845	3.848	1.01	0.95
2	Adult	F	ES	10150	914.4	10119	885.4	9796	10196	9.01	8.75	4.005	0.03854	4.004	0.03798	3.991	4.008	0.96	0.95
	Young	F	ES	10444	1050	10451	1036	10076	10449	10.06	9.91	4.017	0.04179	4.017	0.04255	4.003	4.019	1.04	1.06
	Adult	F	LS	10654	1217	10552	1047	10488	10430	11.43	9.92	4.025	0.04802	4.021	0.04529	4.021	4.018	1.19	1.06
	Young	F	LS	10608	751.4	10700	812.2	10542	10568	7.08	7.59	4.025	0.03061	4.028	0.0326	4.023	4.024	0.76	0.81
3	Adult	F	ES	8521	577.6	8483	514.7	8503	8509	6.78	6.07	3.93	0.0294	3.928	0.02662	3.93	3.93	0.75	0.68
	Young	F	ES	8591	499.8	8582	564.5	8569	8452	5.82	6.58	3.933	0.02511	3.933	0.02784	3.933	3.927	0.64	0.71
	Adult	F	LS	8483	529.5	8481	512.7	8458	8538	6.24	6.05	3.928	0.02712	3.928	0.02613	3.927	3.931	0.69	0.67
	Young	F	LS	8539	847.7	8566	502.6	8378	8472	6.41	5.87	3.931	0.02734	3.932	0.02516	3.923	3.928	0.7	0.64
4.1	Adult	F	ES	5905	758.9	5705	713.9	5750	5781	12.85	12.51	3.768	0.05583	3.753	0.05474	3.76	3.762	1.48	1.46
	Young	F	ES	5850	833	5652	783.4	6077	5593	14.24	13.86	3.763	0.06432	3.748	0.06136	3.784	3.748	1.71	1.64
	Adult	F	LS	5106	922.6	4871	837.6	5124	4904	18.07	17.2	3.7	0.08793	3.68	0.08954	3.71	3.691	2.38	2.43
	Young	F	LS	5238	915.7	4834	1010	5324	5112	17.48	20.9	3.713	0.0765	3.674	0.09816	3.726	3.709	2.06	2.67
4.2	Adult	F	ES	291	112.4	280.5	113.7	286	260	38.63	40.53	2.429	0.1806	2.415	0.1708	2.456	2.415	7.43	7.07
	Young	F	ES	299.3	113.9	323.2	123.6	300	316.5	38.05	38.24	2.444	0.1725	2.479	0.1675	2.477	2.5	7.06	6.76
	Adult	F	LS	258.1	91.18	231.2	151.3	257	194	35.33	65.44	2.383	0.1662	2.299	0.2305	2.41	2.288	6.97	10.02
	Young	F	LS	246.1	86.19	235.4	88.65	247.5	220	35.03	37.66	2.362	0.171	2.343	0.16	2.394	2.341	7.24	6.83
5.1	Adult	F	ES	1569	170.7	1531	161.8	1553	1527	10.88	10.57	3.193	0.04611	3.183	0.04545	3.191	3.184	1.44	1.43
	Young	F	ES	1449	231.1	1477	242.1	1479	1535	15.95	16.39	3.154	0.08446	3.162	0.08759	3.17	3.186	2.68	2.77
	Adult	F	LS	1355	282.8	1389	245.9	1404	1388	20.86	17.7	3.12	0.1144	3.135	0.08277	3.147	3.142	3.67	2.64
	Young	F	LS	1315	273.3	1408	283.4	1382	1451	20.78	20.12	3.106	0.1175	3.138	0.1028	3.14	3.161	3.78	3.28
5.2	Adult	F	ES	6447	519.7	6424	406.8	6479	6402	8.06	6.33	3.808	0.03482	3.807	0.02729	3.812	3.806	0.91	0.72
	Young	F	ES	6418	397.1	6494	365.8	6413	6485	6.19	5.63	3.807	0.02663	3.812	0.02431	3.807	3.812	0.7	0.64
	Adult	F	LS	6466	453.6	6468	469.2	6555	6466	7.02	7.25	3.81	0.03083	3.81	0.03133	3.817	3.811	0.81	0.82
	Young	F	LS	6326	463.1	6268	400.9	6430	6168	7.32	6.4	3.8	0.03283	3.796	0.02783	3.808	3.79	0.86	0.73
6.1	Adult	F	ES	939.9	44.08	938.1	48.4	938	926	4.69	5.16	2.973	0.02016	2.972	0.02221	2.972	2.967	0.68	0.75
	Young	F	ES	909.3	45.81	911.7	38.64	901	907	5.04	4.24	2.958	0.02167	2.959	0.01789	2.955	2.958	0.73	0.6
	Adult	F	LS	912.2	41.11	920.3	38.35	915	917	4.51	4.17	2.96	0.01977	2.964	0.01807	2.961	2.962	0.67	0.61
	Young	F	LS	918.9	45.47	906	42.96	898	898.5	4.95	4.74	2.963	0.0212	2.957	0.02055	2.953	2.954	0.72	0.7
6.2	Adult	F	ES	986.4	39.66	971.3	43.25	978	966	4.02	4.45	2.994	0.01751	2.987	0.01928	2.99	2.985	0.58	0.65
	Young	F	ES	952.9	43.87	948.1	42.51	966	955.5	4.6	4.48	2.979	0.02005	2.976	0.01938	2.985	2.98	0.67	0.65
	Adult	F	LS	969.8	34.94	968.2	34.92	967	961	3.6	3.61	2.986	0.01567	2.986	0.01563	2.985	2.983	0.52	0.52
	Young	F	LS	975.8	66.24	956.4	35.54	964	956	6.79	3.72	2.988	0.02731	2.98	0.0162	2.984	2.98	0.91	0.54
6.3	Adult	F	ES	957.6	39.74	954.6	41.91	956	950	4.15	4.39	2.981	0.01798	2.979	0.01898	2.98	2.978	0.6	0.64
	Young	F	ES	923.9	48.13	931.1	49.58	938	938	5.21	5.32	2.965	0.02267	2.968	0.02311	2.972	2.972	0.76	0.78
	Adult	F	LS	939.5	37.49	952.3	37.94	944	955	3.99	3.98	2.973	0.01743	2.978	0.01733	2.975	2.98	0.59	0.58
	Young	F	LS	929	36.04	932.9	30.52	923.5	938	3.88	3.27	2.968	0.01699	2.97	0.01449	2.965	2.972	0.57	0.49
6.4	Adult	F	ES	483.1	56.15	480.3	50.19	488	480	11.62	10.45	2.681	0.0516	2.679	0.0462	2.688	2.681	1.92	1.72
	Young	F	ES	463.8	88.21	459.7	67.75	446	453	19.02	14.74	2.659	0.07961	2.658	0.06388	2.649	2.656	2.99	2.4
	Adult	F	LS	458.7	56.99	468.2	55.27	456	460	12.42	11.8	2.658	0.05354	2.668	0.05066	2.659	2.663	2.01	1.9
	Young	F	LS	435	39.8	435.2	39.19	431.5	426.5	9.15	9.01	2.637	0.03988	2.637	0.03924	2.635	2.63	1.51	1.49
8	Adult	F	ES	68130	2297	68204	2195	67744	67870	3.37	3.22	4.833	0.01454	4.834	0.0139	4.831	4.832	0.3	0.29
	Young	F	ES	66972	2458	66718	2074	66968	67070	3.67	3.11	4.826	0.01558	4.824	0.01335	4.826	4.827	0.32	0.28
	Adult	F	LS	66727	2203	66781	2283	66392	66295	3.3	3.42	4.824	0.01432	4.824	0.01471	4.822	4.821	0.3	0.3
	Young	F	LS	65681	2771	65763	2742	65772	65415	4.22	4.17	4.817	0.01841	4.818	0.01807	4.818	4.816	0.38	0.38

Appendix B: *Presents the p-values, means, standard deviation, and CV of transformed and non-transformed, two-tailed, paired t-tests of males. The significant results were highlighted in red or blue for valid comparisons. Red indicates a significant result where the left primaries were longer than right primaries for that Variable. Blue indicates a result where right primaries were longer than left primaries for that Variable. Variables that were not normally distributed or had p-values > 0.05 were not highlighted.*

Variable	Age	Sex	Season	Mean Left (µm)	SD	Mean Right (µm)	SD	Median Left (µm)	Median Right (µm)	CV Left (%)	CV Right (%)	Log Mean Left	SD	Log Mean Right	SD	Log Median Left	Log Median Right	CV Left (%)	CV Right (%)
1	Adult	M	ES	7601	311.7	7650	378.3	7588	7600	4.1	4.95	3.88	0.01777	3.883	0.02134	3.88	3.881	0.46	0.55
	Adult	M	LS	7371	312.7	7434	369.9	7291	7377	4.24	4.98	3.867	0.01817	3.871	0.02115	3.863	3.868	0.47	0.55
	Young	M	LS	7335	521.8	7306	463.2	7572	7291	7.11	6.34	3.864	0.03152	3.863	0.02757	3.879	3.863	0.82	0.71
2	Adult	M	ES	10499	636	10445	617.9	10493	10476	6.06	5,92	4.02	0.02646	4.018	0.02615	4.02	4.021	0.66	0.65
	Adult	M	LS	11023	816.7	10992	976.9	10819	10842	7.41	8.89	4.041	0.03127	4.039	0.03777	4.034	4.035	0.77	0.93
	Young	M	LS	10634	1316	10437	1175	10342	10319	12.37	11.25	4.024	0.05275	4.016	0.04919	4.015	4.014	1.31	1.22
3	Adult	M	ES	8899	524.2	8931	490.8	8949	8966	5.89	5.5	3.949	0.02606	3.95	0.02435	3.952	3.953	0.66	0.62
	Adult	M	LS	8946	492.8	8918	509.9	8904	8875	5.51	5.72	3.951	0.02389	3.95	0.02463	3.95	3.948	0.6	0.62
	Young	M	LS	8834	898	8762	869	9004	8801	10.17	9.92	3.944	0.04501	3.941	0.04307	3.954	3.944	1.14	1.09
4.1	Adult	M	ES	6372	746.4	6027	772.6	6373	5950	11.71	12.82	3.801	0.05385	3.777	0.05655	3.804	3.775	1.42	1.5
	Adult	M	LS	5647	899.9	5679	889.4	5550	5644	15.94	15.66	3.746	0.07267	3.749	0.0692	3.744	3.752	1.94	1.85
	Young	M	LS	4824	1044	5198	1095	5274	5583	21.65	21.06	3.673	0.1017	3.706	0.0995	3.722	3.746	2.77	2.68
4.2	Adult	M	ES	309	112.3	263.7	72.12	300	257	36.34	27.35	2.461	0.1639	2.405	0.1237	2.477	2.41	6.66	5.14
	Adult	M	LS	261.8	142.5	260.6	113.3	246	232	54.41	43.48	2.365	0.2139	2.381	0.1751	2.391	2.365	9.05	7.35
	Young	M	LS	312.4	160.4	199	82.37	284.5	171.5	51.36	41.36	2.441	0.2368	2.273	0.1514	2.447	2.234	9.7	6.66
5.1	Adult	M	ES	1613	151.1	1579	173.5	1597	1549	9.37	10.99	3.206	0.04105	3.196	0.04688	3.203	3.19	1.28	1.47
	Adult	M	LS	1543	241.2	1567	266.6	1556	1593	15.64	17.01	3.182	0.08303	3.188	0.08395	3.192	3.202	2.61	2.63
	Young	M	LS	1560	243.9	1560	207.3	1623	1512	15.64	13.29	3.188	0.07495	3.19	0.05714	3.21	3.179	2.35	1.79
5.2	Adult	M	ES	6820	419	6706	334.1	6782	6803	6.14	4.98	3.833	0.02676	3.826	0.02066	3.831	3.833	0.7	0.58
	Adult	M	LS	6903	460.9	6930	457.3	6892	6875	.6.68	6.6	3.838	0.02908	3.84	0.02881	3.838	3.837	0.76	0.75
	Young	M	LS	6584	576.1	6642	155.1	6614	6617	8.75	2.33	3.817	0.03851	3.822	0.01007	3.82	3.821	1.01	0.26
6.1	Adult	M	ES	982.5	47.23	983.6	43.29	978	984	4.81	4.4	2.992	0.02081	2.992	0.019	2.99	2.993	0.7	0.63
	Adult	M	LS	991.4	48.98	988.2	43.83	984	984	4.94	4.44	2.996	0.02071	2.994	0.01295	2.993	2.993	0.69	0.64
	Young	M	LS	943	60.19	945.3	51.57	929	936	6.38	5.46	2.974	0.02703	2.975	0.02371	2.968	2.971	0.91	0.8
6.2	Adult	M	ES	1045	41.56	1033	40.19	1046	1035	3.98	3.89	3.019	0.01729	3.014	0.01689	3.02	3.025	0.57	0.56
	Adult	M	LS	1051	29.05	1043	34.36	1047	1035	2.76	3.3	3.021	0.01193	3.018	0.01434	3.02	3.015	0.39	0.48
	Young	M	LS	999.4	52.54	999.3	56.82	981	969	5.26	5.69	2.999	0.02265	2.999	0.02445	2.992	2.986	0.76	0.82
6.3	Adult	M	ES	1021	42.12	1024	36.69	1024	1013	4.12	3.58	3.009	0.01784	3.01	0.0154	3.01	3.006	0.59	0.51
	Adult	M	LS	1026	34.71	1027	36.9	1019	1029	3.38	3.59	3.011	0.01459	3.011	0.0156	3.008	3.012	0.48	0.52
	Young	M	LS	999	63.9	990.9	50.97	975.5	967.5	6.4	5.14	2.999	0.02705	2.996	0.0221	2.989	2.986	0.9	0.74
6.4	Adult	M	ES	530.2	39.76	536.6	62.32	522	527	7.5	11.61	2.723	0.03219	2.727	0.04327	2.718	2.722	1.18	1.59
	Adult	M	LS	489.4	61.01	526.8	63.88	495	535	12.47	12.13	2.686	0.05629	2.719	0.05297	2.695	2.728	2.1	1.95
	Young	M	LS	457.4	61.06	520	58.57	452	520.5	13.35	11.26	2.657	0.05889	2.714	0.04919	2.655	2.716	2.22	1.81
8	Adult	M	ES	72288	1950	72484	1793	72473	72543	2.7	2.47	4.859	0.01178	4.86	0.01079	4.86	4.861	0.24	0.22
	Adult	M	LS	71772	2115	71690	2201	71742	71702	2.95	3.07	4.856	0.01282	4.855	0.0134	4.586	4.586	0.26	0.28
	Young	M	LS	69663	2573	69826	2995	69489	69434	3.69	4.29	4.843	0.01612	4.844	0.01865	4.842	4.842	0.33	0.38