Life history optimisation in response to environmental change

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I declare that this thesis is entirely my own work, and except where otherwise stated, describes my own research. This work has not been submitted for any degree or professional qualification.

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The truth is, birds and feathers haven't always been my obsession. I distinctly remember my grandmother constantly sending me videos and articles about cool birds doing cool stuff, and me, being a moody teenager that has 'better' things to do, leaving all those emails sitting in my inbox (she sent so many). It turns out, I could not escape my fate, and ended up being the bird-obsessed lady in her absence. How I wish I could tell you about all the fun bird facts I've learnt over the years!

So here they are, all those interesting birds facts. Most of which I learnt from my wonderful supervisors - Ben, Ella and Charlotte - it has truly been a privilege and a pleasure working with you over these past years. Thank you Ben, for always keeping an open mind to explore new questions and for guiding me through them all. We did not initially intend for this project to be so feather-focused, but once I showed interest in them, you gave me room to explore at my heart's desire, and provided me the tools and skill set to develop a true researcher's mindset. I don't think I will ever have such a profound understanding of biology and birds as you do, but thank you for sharing some of that knowledge with me.

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ABSTRACT

Organisms living in seasonal habitats face trade-offs between optimising different life-history traits due to energy and time restrictions imposed by the environment. We have ample evidence for the existence of trade-offs, however, we do not fully understand the mechanisms underlying them. In birds, variation in plumage quality has been suggested as a potential mediator linking life-history traits over various timescales, but studies investigating the role of feather structure are limited. In this thesis, I applied a combination of theoretical, experimental, and observational approaches to explore how life-history traits are coupled in response to environmental variation, and tested plumage quality as a candidate mechanism linking life-history trade-offs across time. In Chapter 2, I developed an agent-based model simulating the daily behaviours of a seasonally breeding iteroparous bird, the great tit (*Parus major*), and showed that different environmental conditions (temperature and resource availability) favour different optimal combinations of seasonal reproductive timing (lay date) and reproductive effort (clutch size) in terms of maximising the number and quality of offspring raised. In Chapter 3, I introduced a novel data collection and automated analysis framework for extracting feather structural information from images taken on live birds, providing the tools for exploring how variation in plumage quality might mediate life-history trade-offs over different timescales. In Chapter 4, I tested the role of feather quality in the short-term trade-off between the number and quality of offspring by manipulating the brood sizes of blue tits (*Cyanistes caeruleus*) and found significant impacts on both the feather and body condition of chicks. And in Chapter 5, I investigated how feather structure relates to insulation efficiency in zebra finches (*Taeniopygia guttata*) to begin to understand how plumage quality might link life-history traits over longer time frames through survival. To conclude, in Chapter 6, I summarised the key findings from each chapter and their potential to address the questions set out in this thesis, besides highlighting new questions that emerged from the results. I discussed how life-history traits are intricately linked under environmental variation and how we can improve models to address remaining gaps in our understanding. I also assessed the strength of evidence presented about the role of plumage quality in mediating life-history trade-offs over both short and long timescales. Finally, I highlighted the benefits of developing tools that will allow more efficient data collection and analysis of feather structural variation for future research

Contents

1 General introduction **2**
- 1.1 An introduction to life history theory . . . 2
- 1.2 Life-history trade-offs in changing environments . . . 2
- 1.3 Linking life-history trade-offs across time . . . 3
- 1.4 Plumage quality as a mediator of life-history trade-offs . . . 3
  - 1.4.1 The growth and structure of feathers . . . 4
  - 1.4.2 Why moult? . . . 5
  - 1.4.3 The demands and costs of feather growth . . . 5
  - 1.4.4 Optimal timing of moult within the annual cycle . . . 6
  - 1.4.5 Plumage quality trade-offs with other life-history traits . . . 7
- 1.5 Study species and field sites . . . 8
  - 1.5.1 Ethical note . . . 10
- 1.6 Aims of this thesis . . . 11

2 Life history optimisation in response to temperature variation in seasonal environments **19**
- Appendix A: Supplementary material for chapter 2 . . . 34

3 featheRscan: a field method and R package for collecting feather quality metrics **41**
- Appendix B: Supplementary material for chapter 3 . . . 51

4 Plumage structure as a mediator of trade-offs between offspring quantity and quality: an experimental study of blue tits, *Cyanistes caeruleus* **52**
- Appendix C: Supplementary material for chapter 4 . . . 65

5 Manipulated foraging costs alter individual morphology and thermoregulation in a small passerine: linking feather structure and insulation effi-ciency **67**
- Appendix D: Supplementary material for chapter 5 . . . 80

6 General discussion **82**
- 6.1 Questions addressed in this thesis . . . 82
  - 6.1.1 How are life-history traits linked in seasonal environments? . . . 82
  - 6.1.2 How can we collect and analyse large amounts of feather quality data fast? . . . 83
  - 6.1.3 Can plumage quality mediate life-history trade-offs over different timescales? . . . 84
- 6.2 Additional questions raised . . . 85
  - 6.2.1 Why is feather structure highly variable within individuals? . . . 85
  - 6.2.2 Why do decreased broods behave opposite to our expectations? 86
  - 6.2.3 How might plumage characteristics vary under continued environmental change? . . . 86
- 6.3 Future directions . . . 88

1 | GENERAL INTRODUCTION

1.1 | An introduction to life history theory

There is astounding variation in environments all across the globe continuously shaping how organisms survive and reproduce. In theory, every organism could maximise their fitness if they lived forever and reproduced prolifically (Smith (1978)). Instead, we live in a world with limited resources, requiring a wide array of unique solutions to the many challenges of life. Understanding why and how this diversity in adaptations exists in the context of ecology is at the heart of life history theory.

Life history theory, formally described in Stearns (1992) and Roff (1992), proposes that traits important for the survival and reproduction of organisms are shaped by both the extrinsic elements of their ecological background, as well as the intrinsic trade-offs between traits due to underlying constraints (Stearns (2000)). This implies that organisms are unable to simply maximise all aspects of their life cycle. Instead, the optimal solution(s) to the ecological challenges faced relies on a careful balance between the pros and cons of each morphological, physiological and behavioural adaptation in a complex, intricately-linked feedback-system (Smith (1978)). Therefore, answers to the 'why' and 'how' in the field of life history theory are often sought after using a series of optimisation problems (Lotka (1907), McNamara and Houston (1996), Barta et al. (2006)).

1.2 | Life-history trade-offs in changing environments

A classic example of a life-history trade-off is the balance between the quantity and quantity of offspring, where organisms need to consider how to distribute finite resources to maximise both the number of descendants but also their ability to survive and reproduce thereby contributing to the overall fitness score (Stearns (1989)). Moreover, iteroparous species that reproduce several times in their life need to allocate resources not only to their offspring, but also to themselves, as maintaining somatic health can improve the chances of survival and future reproduction - another key trade-off linked to significant differences in fitness (Lessells (1991)).

Resource limitations are especially prevalent in seasonal ecosystems where the periodic fluctuations in environmental conditions require organisms to continuously fine-tune different aspects of their life history, potentially disrupting previously held balances between traits (Ejsmond et al. (2015)). For instance, they often face strong selection pressures to time energetically

demanding activities to coincide with periods of favourable conditions (Varpe (2017)). This time and energy limitation can create trade-offs between alternative life-history decisions about the timing and effort dedicated to fitness-defining activities, such as balancing the number and quality of offspring or current reproduction and future survival.

1.3 | Linking life-history trade-offs across time

Predicting how environmental variation may shift the balance of life-history trade-offs requires a profound understanding of the mechanisms that link them. Often, adjustment to one life-history trait can have knock-on consequences on fitness acting over both short- and long timescales within an individual's lifetime (Harrison et al. (2011)). Continuing with the key trade-off examples described earlier, adjustment to the number of offspring produced carries immediate consequences for the success of the current reproductive event (i.e. number of independent offspring produced), as well as impacting the long-term survival and future reproduction of both offspring and parent (Lessells (1991)). Therefore, we need to identify mechanisms that can link trade-offs over various timescales (McNamara and Houston (2008)).

A large body of research has investigated immune function-related costs of reproductive behaviour as a mechanism mediating life-history trade-offs over both short and long timescales (e.g. resistance to oxidative stress: (Alonso-Alvarez et al. (2006), Casagrande and Hau (2018), Hasselquist and Nilsson (2012), Yang et al. (2013)), control of infections: (Contreras-Garduño et al. (2014), Knowles et al. (2010)). However, these physiological traits are often underpinned by complex pathways and require invasive techniques to measure. Instead, the periodic replacement of key morphological traits linked to survival and/or reproduction - such as the leaves of deciduous trees or the fur and plumage of animals - has been suggested as an alternative mechanism linking trade-offs over different timescales.

1.4 | Plumage quality as a mediator of life-history trade-offs

In birds, moult is the periodic replacement of feathers that ensures plumage integrity for the fulfillment of various fitness-defining functions, such as those essential for survival (e.g. flight, insulation, water repellency, and camouflage), and reproduction (e.g. sexually selected ornamental traits) (Jenni and Winkler (2020a)). There is vast diversity in moult strategies relating to the timing and intensity of feather replacement, but most species will undergo a complete moult about once a year (Newton (2009)). This periodic nature of feather moult and regrowth creates a dichotomy: on the one hand enabling individuals to respond to changing ecological pressures within their lifetime, but on the other hand, the feathers produced during moult

remain fixed until the next cycle, thereby profoundly impacting several fitness-related processes during that time. This dual aspect of feathers makes them a good candidate mechanism linking life-history trade-offs over different timescales.

Defining feather quality can be approached from different perspectives. A large body of research on individual variation in plumage quality has so far focused on visual characteristics, such as colouration (Garcia-Navas et al. (2012), Charmantier et al. (2017), Berzins and Dawson (2019), reviewed in Hill and McGraw (2006) and Roulin (2016)), leaving the causes and consequences of variation in its structural properties rather unexplored (but see: Vagasi et al. (2012), Gamero et al. (2015)). Therefore, the following introduction to the dynamics of plumage growth, and the questions addressed in this thesis, focus on feather structure.

1.4.1 | The growth and structure of feathers

Feathers consist of beta-keratin chains that are assembled within a protective sheath, innervated with a pulp of nutrients (called the blood quill) protruding from an epidermal follicle. During feather growth, new cells are continuously added at the base of the follicle, differentiating into elements such as the rachis, barbs and barbules (figure 1.1), while pushing already deposited structures out of this sheath. Once exposed, the cells are no longer connected to the blood supply; hence, a feather is made of a dead keratin matrix prone to wear and tear, with its structure remaining fixed until the old feather is dropped and a new one begins to grow in its place in the process of moult (Jenni and Winkler (2020a)).

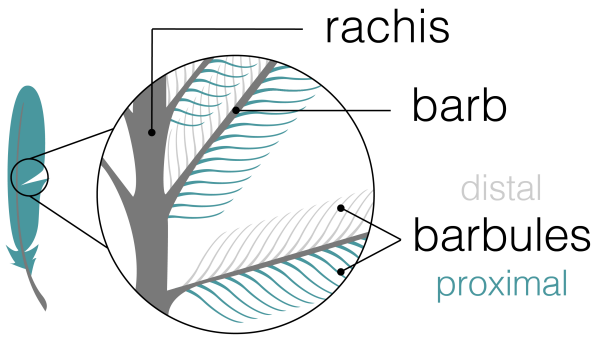


FIGURE 1.1 Schematic of feather structural elements visible at x100 magnification, often used to characterise feather structural quality.

1.4.2 | Why moult?

The purpose of moult is two-fold: it maintains plumage functionality, but also allows adjustments to novel ecological pressures and life stages. Firstly, feather wear, for instance through physical damage (e.g. intensive foraging during breeding Merilä and Hemborg (2000)) or due to parasites (e.g. feather lice Barbosa et al. (2002)) results in a loss of feather material (e.g. lighter and shorter feathers Newton (1968), Flinks and Salewski (2012)) and impairs essential plumage functions, such as insulation (e.g. lighter plumage structure of tawny owl brown morphs selected against in cold environments Koskenpato et al. (2016); northern cold-adapted populations of great tits have denser plumage Broggi et al. (2011)) and flight (e.g. short flight feathers were linked to diminished take-off speed Swaddle et al. (1996), and a larger wing beat amplitude after moult in common starlings Williams and Swaddle (2003)). Secondly, plumage requirements may shift with age, such as switching from juvenile to adult plumage to adopt sexually-selected colouration (Hill and McGraw (2006)) and improve structural integrity (Nielsen (1993), Butler et al. (2008)), as well as in response to varying environmental pressures, including seasonal changes to insulatory (Middleton (1986)) and camouflage needs (Montgomerie et al. (2001)).

1.4.3 | The demands and costs of feather growth

Feather production entails a time-consuming and energetically demanding process that necessitates significant physiological and behavioural adjustments. During moult, individuals replace 20-25% of their lean dry body mass in feathers that consist of 90-95% protein and make up about 25% of the total protein content of a small bird, requiring elevated protein turnover rates and an increase in vascularisation to transport nutrients to the feather follicles (Chilgren (1977), Jenni and Winkler (2020a)). However, these physiological changes during feather morphogenesis carry high energetic costs (e.g. as seen by an increase in resting metabolic rates of 12-36% in common kestrels during moult, Dietz et al. (1992)), and insufficient energy allocation towards feather growth can impair the quality of the plumage produced (e.g. lower food intake causes slower growth rate Murphy et al. (1988), low protein diet results in shorter, lighter feathers Pap et al. (2008)).

Birds have evolved several mechanisms to mitigate these increased costs; many species decrease their mass during moult (e.g. the body mass of barnacle geese drops by 25%, Portugal et al. (2007)), as an adaptation to lower maintenance costs, and decrease wing loading to compensate for an impaired flight capacity. In addition, individuals often show behavioural adjustments, primarily a decrease in activity levels (e.g. many seabirds spend more time floating rather than flying during moult Gutowsky et al. (2014), barnacle geese increase resting time Portugal (2008)), which helps to protect growing feathers, reduce the risk of predation (Ben-Hamo et al. (2016)) and further decreases energy expenditure. Some species also need to adjust

their foraging preferences to fulfill the special dietary requirements of feather morphogenesis (e.g. granivorous house sparrows switch to eating insects during moult Pap et al. (2008)).

Besides the nutrient and metabolic demands, birds also face time constraints with respect to the time it takes to grow feathers of a certain size (Rohwer et al. (2009a), e.g. large vulture primaries often take over 100 days to grow Houston (1975), while small passerines may take only 20 Jenni and Winkler (2020a)), and environmental factors, such as temporally limited availability of food. A shorter moult is often linked to an increase in moult speed, which, however, carries negative consequences for plumage quality (e.g. shorter and lighter primary feathers in starlings Dawson et al. (2000), Dawson (2004), shorter feathers with fewer plumulaceous barbs in house sparrows Vagasi et al. (2012)) and subsequent plumage function.

1.4.4 | Optimal timing of moult within the annual cycle

The ability of birds to accurately time feather growth is controlled primarily by an internal circannual rhythm (birds moult periodically even in captivity (Gwinner (2003), Piersma et al. (2008)) synchronised to seasonal changes in photoperiod (Dawson (2007)), which is in part genetically determined (e.g. 'clock' genes in barn swallows Saino et al. (2013); further demonstrated by hybrids of two populations of Eurasian black caps showing intermediate moult duration Gwinner (1996)), as well as cued by environmental factors (e.g. food availability: supplemental feeding advances moult initiation in tropical species Danner et al. (2015)) and modulated by hormones (e.g. moult prevented until gonadal regression and decrease in sex hormones following breeding, demonstrated by testosterone-injected starlings delaying moult Dawson (1994)).

Because of the high costs associated with feather production and the impaired functions provided by the plumage during moult, most species generally adopt strategies that avoid any significant overlap between moult and other demanding activities, such as breeding and migration. Therefore, the optimal timing and duration of moult forms a crucial element in the organisation of the annual life cycle. Due to the increased energetic burden and compromised plumage function, moult is often coincided with periods of favourable environmental conditions, such as mild temperatures (with moult best carried out close to thermoneutrality Jenni and Winkler (2020a)) allowing for a reduction in insulatory capacity (e.g. plumage conductance and therefore heat loss increases during moult in laughing kookaburra Buttemer et al. (2003)), and abundant resources to support feather synthesis (e.g. common kestrels time moult during periods of rodent abundance peaks Masman and Klaassen (1987)), and compensate for poorer foraging efficiency (e.g. common kestrel and buzzards show lower rates of hunting success during moult, Masman (1986)). Feather replacement is most beneficial after periods of intense wear, such as during reproduction and offspring care, therefore most species moult after the breeding season (in late summer in seasonal environments), in agreement with theoretical predictions of the most stable strategy (Barta et al. (2006)).

1.4.5 | Plumage quality trade-offs with other life-history traits

Moult strategies vary between species, individuals, and even within an individual between moult cycles, depending on the energetic and time constraints faced, and considering the environmental conditions (for a comprehensive overview of all factors influencing moult strategies, see Jenni and Winkler (2020a) p177). As feather production is costly, both in terms of time and energy, it directly competes with other demanding activities in the annual cycle, leading to trade-offs between key life-history traits, such as reproduction and migration (though this thesis only deals with non-migratory populations of birds).

For species that moult after the breeding season, a delay in the end of the reproductive event (either through late initiation or a longer duration, for instance, via raising multiple broods within the same season) can subsequently delay the onset of moult, leading to time constraints to complete moult before daylengths shorten and environmental conditions deteriorate in the autumn. In response, individuals often increase the speed of moult, but at the expense of feather quality (e.g. fast moulting house sparrows grow shorter feathers with fewer plumulaceous barbs, Vagasi et al. (2012)), which may in turn influence their future survival and performance in other life-history events (e.g. reproductive success). For instance, blue tits induced to lay a second brood delayed their moult and had lower over-winter survival (potentially through impaired insulation as shown by an increase in energy expenditure), as well as lower reproductive success (delayed egg laying, smaller clutch sizes) in the following breeding season (Nilsson and Svensson (1996)).

Complete separation of moult and other activities in the annual cycle may not always be possible or carry the highest fitness benefits, therefore, individuals may overlap breeding and feather replacement (moult-migration overlaps are much less frequent) under certain circumstances (e.g. when food abundance is only high during the breeding season in tropical species Rohwer et al. (2009b); when both breeding and moult takes long in many species of corvids Zuberogoitia et al. (2018); when flight is not necessary during certain stages of reproduction, such as the case for many species of hornbills during incubation Kemp (1969)). However, moult-breeding overlaps create a trade-off between investing in the current reproductive event and self-maintenance for future survival and reproduction. For instance, northern temperate small passerines constrained by the length of summer may begin moult before the offspring have fledged, therefore balancing the risks of lower feather quality (e.g. shorter, lighter feathers Echeverry-Galvis and Hau (2013)) or reduced success of the current breeding attempt (e.g. by reducing chick provisioning rates Svensson and Nilsen (1997)). As such, decisions about moult, combined with the many factors (genetic, physiological, environmental) that influence feather growth, and the significant consequences of variation in plumage quality for fitness (survival, reproduction), may provide a link between life-history trade-offs over both short and long timescales.

1.5 | Study species and field sites

For the majority of work presented in this thesis, I studied the great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) at the mixed deciduous forest of Wytham Woods, near Oxford, UK (51°46'N, 1°20'W), which covers 385 ha and is surrounded by farmland (for a detailed description of the land, see Perrins (1965)). This population of tits has been studied here since 1947 (Lack (1964), Perrins (1965)), making it the longest continuous-running individual-based study of any animal population in the world, providing 77 years of data on various aspects of their life history (1.2). As such, they make an excellent model species and have been (and continue to be) the focus of numerous, diverse research questions over the decades (Perrins (1965), Krebs (1971), Charmantier et al. (2008), Aplin et al. (2015)).

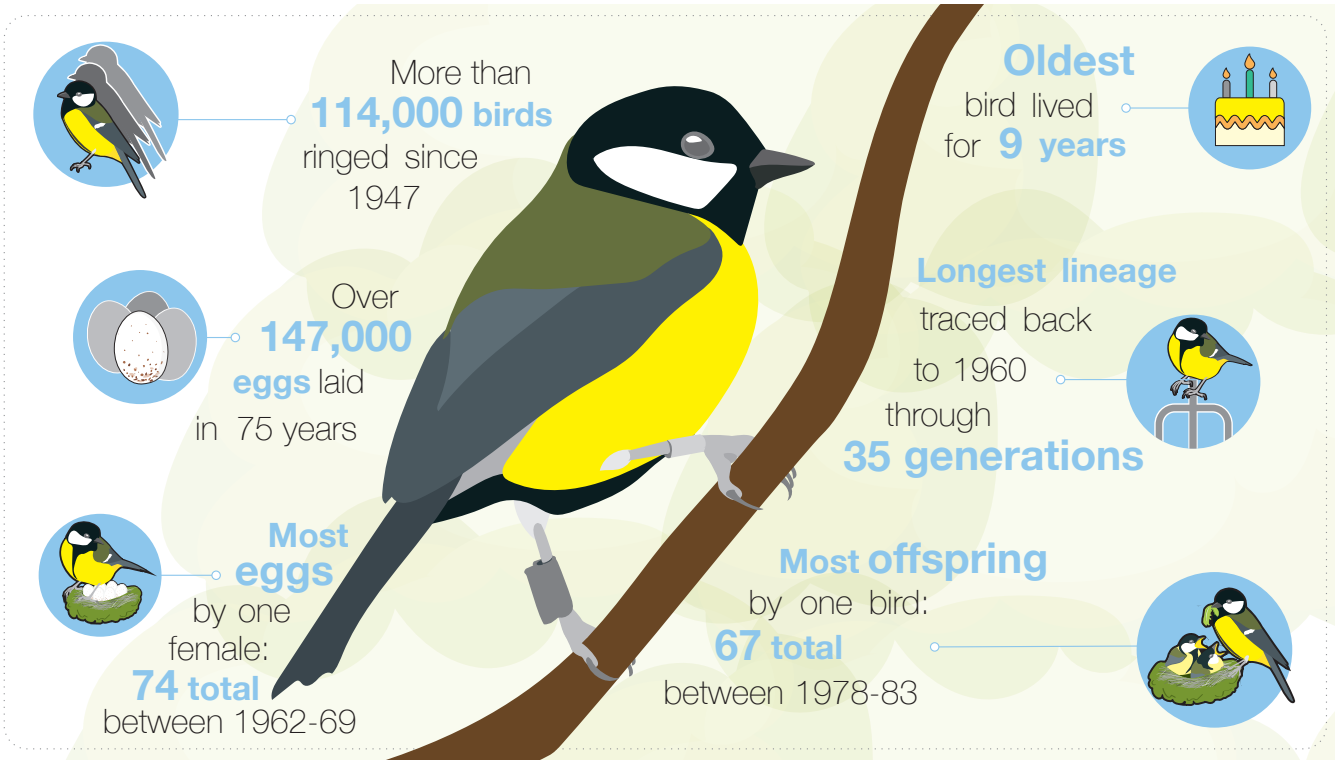


FIGURE 1.2 Interesting facts about the Wytham Tit Study, showcasing the wealth of data in this 77-year-long study system.

Blue tits and great tits are short-lived, seasonally breeding, socially monogamous small passerines found across much of the Western Palearctic. At Wytham Woods, they live on average 1.9 years (Bouwhuis et al. (2009)), they are resident all-year-round and produce one (occasionally two, Perrins (1965)) clutches of 8-10 eggs on average during the breeding season that lasts from April to June, followed by a moult cycle between June to October. Newly fledged young undergo a partial post-juvenile moult, replacing most of their body plumage but retaining their flight feathers, whereas adults perform a complete post-breeding moult, replacing all their feathers annually (Jenni and Winkler (2020b)).

As hole-nesting birds, tits readily use the 1209 fixed location nest boxes placed across Wytham Woods, making it easy to collect repeated information on the progress of individual breeding attempts across time, providing standardised records of lay dates (first egg in the clutch, where tits lay one egg on each consecutive day until clutch completion, Perrins (1996)), incubation durations (the interval between clutch completion and hatch date), clutch sizes (total number of eggs incubated), hatch dates (the hatch date of the oldest nestling), as well as the number of chicks successfully fledged and their condition (body mass, wing length). The majority of the breeding population, as well as nestlings at the age of two weeks, are fitted with uniquely numbered British Trust for Ornithology issued metal leg rings, enabling detailed observation of life-history and other traits at the individual level. In combination with the vast research on great tits across their species range, this system provided me the opportunity to study the intricate links between life-history traits and how they interact with the environment in a natural setting.

Besides using data from the long-term monitoring of great tits (in **Chapter 2**), I also collected feather quality information, using images taken with a digital microscope, on both great tits and blue tits throughout the non-breeding seasons (July - March) between 2021-2023 (in **Chapter 3**), and on two-week-old blue tit nestlings across two breeding seasons (April - June) of 2022 and 2023, following brood size manipulation experiments, where I partially cross-fostered chicks between pairs of nests to either increase or decrease their total brood size by two offspring (in **Chapter 4**). As highlighted in **section 1.4**, feather structure and the ecological pressures shaping it can vary greatly between species depending on their habitat and general life history (Pap et al. (2017), Barve et al. (2021)). Therefore, I additionally collected feather quality information on zebra finches (*Taeniopygia guttata*) across two years (March of 2023 and 2024), and on mute swans (*Cygnus olor*) in September of 2023 (in **Chapters 3 and 5**). This allowed me to compare whether any potential trends in the causes and consequences of variation in feather structure may reflect broader patterns generalisable across multiple species with different life histories and moult strategies.

Mute swans are long-lived (7.6 years on average; Woodman et al. (2023)), colony breeding, socially monogamous waterfowl, native to Eurasia. The individuals studied in this thesis originate from Abbotsbury Swannery, coast of Dorset, UK (50°35'N, 2°30'W), formed of a 14 km long tidal lagoon, the Fleet, partially connected to the sea, but mostly separated from it by a high pebble beach, the Chesil Bank (Perrins and Ogilvie (1981)). The breeding population here has been consistently managed and monitored since 1977 (Perrins et al. (1994)), collecting individual-level information on several life-history traits, including lay dates, clutch sizes and hatch dates. The breeding season lasts from April to June, during which females lay an average of 5-6 eggs (Perrins et al. (1994)), followed by a 6-8 week complete post-breeding moult cycle that lasts until September. As opposed to tits that moult their flight feathers sequentially, adult mute swans replace all their primary feathers at once, rendering them flightless. Because of this, breeding males delay their moult to be able to defend the family while the female and cygnets are flightless (McCleery et al. (2007)). First-year birds also undergo a partial post-juvenile moult in the summer and grow their first

flight feathers over the following 4-5 months. Plumage structure information collected in September for **Chapter 3** therefore characterises the quailty of newly grown feathers.

Zebra finches are a relatively short-lived (on average 5.5 years, Berntsen and Bech (2021)), colony living, socially monogamous, granivorous passerines, originally native to the arid grasslands of Australia, but have been widely domesticated and used as a model species across numerous European and North American research laboratories (Forstmeier et al. (2007), Griffith et al. (2017)). In the wild where resources are unpredictable, they breed opportunistically at any time of year cued by the availability of grass seeds following periods of rain (Zann et al. (1995)), laying clutches of 4-6 eggs on average (Haywood (1993)). Similarly, they display a unique moult strategy that may be initiated at any time of year, lasting over 200 days, and characterised by a slow sequential replacement of primary feathers, even while caring for young (Zann (1985)). In captivity, they breed and moult whenever adequate food and nesting materials are provided. The individuals studied in this thesis are from a captive-bred, individually marked population, housed in outdoor aviaries (3.1 x 2.1 x 1.5 m) at the University of Groningen, The Netherlands (53°13'N, 6°33'E), and have been the subject of long-term foraging cost (Koetsier and Verhulst (2011)) and brood size manipulations (Briga et al. (2017)). Here, individuals reproduce once a year, between spring and summer (whenever nest boxes are provided), roughly coinciding with a slow primary moult initiated in April (van der Heide, unpublished). Plumage structure information collected in March for **Chapter 5** therefore characterises the quality of old feathers before moult. In addition, I investigated the thermoregulatory capacity of their plumage, using thermal imaging and respiromerty.

1.5.1 | Ethical Note

All activities involving bird handling and experimentation were conducted by trained fieldworkers holding appropriate licences (personal BTO-issued permit number: C/6982) and carried out under appropriate ethical review, while adhering to agreed guidelines. The long-term monitoring of the Wytham tit population was approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/TitBreedingEcology). Brood size manipulation experiments involving blue tits were also approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/BlueTitBreedingManipulation), which included considerations such as; keeping clutch sizes within the normal range observed in the wild, and transporting nestlings in heated artificial nests, as well as limiting maximum transport time to minimise the risk of hypothermia and starvation. Additionally, Special Methods endorsement for the use of claw-clipping for the individual identification of nestlings was provided by the BTO (under permit: C/6982, project code: 12131). All methods and experiments using zebra finches were performed under approval of the Central Committee for

Animal Experiments (Centrale Commissie Dierproeven) of the Netherlands, under license AVD1050020174344. Individuals were transported between the outdoor aviaries and indoor observation rooms in under five minutes, held for a maximum of three hours and provided ad libitum food and water during the first hour. No injuries or mortality occurred as a result of the observations.

1.6 | Aims of this thesis

Throughout this thesis, I aim to investigate both the extrinsic and intrinsic factors that underlie life history variation. Through a combination of theoretical, experimental and observational approaches, I seek to understand the broader patterns of how life-history traits are coupled in response to environmental variation, as well as investigating plumage quality as a candidate mechanism linking life-history trade-offs across time.

In **Chapter 2**, I start by exploring how the optimal combination(s) of life-history traits vary under different environmental conditions. To do this, I built an agent-based model (ABM) that simulates the daily behavioural decisions of a population of birds living in a seasonal ecosystem with temperature cuing their breeding behaviours and an ephemeral food source required to feed their offspring. I used the vast empirical research on great tits and data from the long-term monitoring of the population at Wytham Woods to parameterise the model. The modular nature of the ABM allowed me to test how variations in temperature annual mean and seasonality (the range between annual minimum and maximum) might favour different optimal strategies of clutch sizes and lay dates that maximise fitness in terms of both offspring number and quality. Although the model is tuned to the breeding biology of the great tit, its findings can be generalised to other similar systems where reproductive success is linked to a seasonally available ephemeral food source. As a whole, this chapter showcases the complexity of how life-history traits are connected and helps further our understanding of the potential challenges individuals might face under climate change.

In **Chapter 3**, I introduce a novel research framework that combines a field data collection protocol with an automated analysis tool for studying feather macrostructure in vivo. By using an inexpensive portable digital microscope alongside an automated image analysis pipeline, I collected and analysed over 1500 images from 500 individual birds of 4 species (blue tit, great tit, zebra finch and mute swan) to estimate key feather structural quality metrics. Once published as an open-source R package with its full functionality, 'featheRscan', and the associated data collection protocol, could offer a non-invasive and time-efficient approach for collecting large amounts of detailed feather quality information on live birds of various species. I hope that it might enable a significantly faster research on a wide range of questions around the ecology and evolution of feathers, especially encouraging studies to further investigate the causes and consequences of individual variation in feather structure.

In **Chapter 4**, I begin to test how variation in feather structure might mediate life-history trade-offs in birds, by investigating whether an experimental change in offspring number impacts offspring morphology, with an emphasis on the structural quality of their feathers. I conducted a cross-fostering and brood size manipulation experiment, and followed the individual growth trajectories of c. 800 wild blue tit chicks across two years at Wytham Woods. I used the data collection protocol and semi-automated functionality of 'featheRscan' from **Chapter 3** to characterise the structural makeup of feathers grown in the nest. The results from this work provide an insight into how nestlings balance resource allocation towards feather growth during early development and its consequences for fledging success. This chapter therefore addresses how feather structure might mediate life-history trade-offs over short timescales.

In **Chapter 5**, I continue to investigate the role of feather structure in linking life-history trade-offs over longer timescales, by testing how variation in feather quality might impact survival through its functions in thermoregulation. I followed methodology described in **Chapter 3** to collect feather structure information across two years on c. 100 zebra finches that have been subjected to experimental foraging cost treatments. In addition to information about their feather quality, I also measured individual variation in thermoinsulative traits, using infrared thermography to derive a non-invasive proxy for insulation via the plumage, as well as recording resting metabolic rates. Together, these results help bridge the gap in our understanding of how variation in feather structure might influence the insulating capacity of birds' plumage, providing a test of the proposed mechanism by which feather quality could link life-history trade-offs over long timescales.

In **Chapter 6**, I reflect on the general conclusions of the thesis, while also discussing the main assumptions and limitations of its methodology and findings. I highlight new questions that emerged from the results. Finally, I suggest areas for future research to refine and build upon the work presented here, aiming to deepen our understanding and improve the models and techniques used in the study of life-history trade-offs in birds.

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Chapter 2

Life history optimisation in response to temperature variation in seasonal environments



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ABSTRACT

Individuals living in seasonal habitats must navigate complex trade-offs between optimising multiple life-history traits in response to changing environmental conditions. In this study, we developed an agent-based model (ABM) simulating the daily behavioural decisions of a seasonally breeding iteroparous bird, to investigate how variation in temperature annual mean and seasonality might drive optimal life history strategies, teasing apart the relationship between the timing (lay date) and effort (clutch size) of reproduction. We found that optimal lay dates were more strongly influenced by temperature variation, showing advances in response to an increase in annual mean temperatures, modulated by the degree of seasonality. On the other hand, optimal clutch sizes were primarily influenced by seasonality, with higher seasonality selecting for smaller clutches, likely as a result of the shorter window of food availability. Comparison against breeding data on the long-term population of great tits (*Parus major*) at Wytham Woods, UK, revealed that observed lay dates in this population aligned closely with predicted optima, although clutch sizes laid by wild tits were larger than those predicted in our model, perhaps indicating that natural populations can utilise other reproductive adjustments to respond to environmental uncertainty. We discuss the implications of these findings in the context of shifting temperature regimes under ongoing climate change, and highlight how the current framework of our model could be expanded to study life history responses to environmental variation across other systems and species that rely on an ephemeral food source.

1 | INTRODUCTION

Organisms living in seasonal ecosystems face numerous challenges requiring the periodic fine-tuning of different aspects of their life history – physiological and behavioural traits associated with survival and reproduction. Environmental fluctuations often impose strong selection pressures on individuals to perform energetically demanding activities within a restricted window of favourable conditions. However, the inherent uncertainty surrounding environmental variation, coupled with a finite availability of time and resources, means life-history responses are rarely perfect. Instead, fitness maximisation hinges on achieving an optimal balance between the costs and benefits underlying a suite of intricately linked traits (Stearns (1992)). The exact mechanisms through which individuals navigate these trade-offs remains a central focus of studies within the framework of life history theory.

Responses to environmental variation often require simultaneous considerations of both biotic and abiotic factors that can, together and/or independently, influence the optimal expression of life-history traits (Stearns (2000)). This creates particular challenges in multi-trophic food webs, where the phenology (timing of key life events) of one species directly influences the optimal timing for species at higher trophic levels (McMeans et al. (2015)). In such systems, consumers must time energetically demanding activities, such as reproduction, to align not only with favorable abiotic conditions (e.g., temperature) but also with biotic factors, particularly the timing and abundance of prey (Durant et al. (2007), Visser and Both (2005)). The correct timing of a life-history event relies on the optimal sequence of behavioural decisions preceding it, while considering the associated costs and benefits at each step. The trade-offs between different decisions are influenced by the individual's state, their previous actions as well as the environmental conditions they are faced with (McNamara and Houston (2008)). For temperate species relying on an ephemeral food source to raise their young, parents face a trade-off between the number and quality of offspring they can rear under the given environmental conditions, whereby breeding success relies on optimising both the seasonal timing and effort of reproduction. Moreover, iteroparous breeders need to also balance the trade-offs between current and future breeding attempts to maximise lifetime reproductive success (Stearns (1989)).

In birds, balancing these trade-offs is achieved through decisions about the lay date (start of egg laying) and clutch size (number of eggs laid) of a breeding attempt. For instance, woodland passerines, such as tits in the *Paridae* family (e.g. great and blue tits), maximise their reproductive success when the provisioning of young coincides with the peak in (primarily winter moth; *Operophtera brumata*) caterpillar abundance (Burgess et al. (2018), Van Noordwijk et al. (1995)). The optimal timing of the nestling stage is preceded by decisions about reproductive timing and effort including (1) the laying date (i.e. when the first egg is laid), (2) the clutch size (i.e. the total number of eggs laid) and (3) the incubation period (i.e. between clutch completion and hatching). These traits are inherently linked, as tits lay consecutive eggs at approximately one day intervals, and a completed clutch needs to be incubated for 13-14 days to hatch (Perrins (1996)). Therefore, every additional egg in a clutch delays the date of hatching by a day (although this can be somewhat buffered if incubation is commenced before the last egg is laid, Simmonds et al. (2017a)).

Empirical evidence shows that there are often strong selection pressures on the timing of reproduction, with even small deviations from the optimal lay date carrying significant fitness costs (Verhulst and Nilsson (2008)). Similarly, experiments that manipulated the number of offspring have also demonstrated detrimental fitness consequences (Parejo and Danchin (2006)). This highlights the importance of precise life history adjustments in seasonal environments. In response to an increase in temperatures in spring, females may (1) advance their laying date and/or (2) reduce their clutch size and/or (3) shorten their incubation period (e.g. start incubation before the last egg is laid, incubate more intensely) in anticipation of an earlier caterpillar peak (Cresswell and Mccleery (2003), Phillimore et al. (2016a), Simmonds et al. (2017b)). In this example, the quantity of offspring (smaller clutch size) may be traded for their quality (increased synchrony with resource).

In this example trophic system, temperature is an important cue for the reproduction of both tits as well as caterpillars. As such, year-to-year variations in annual mean and seasonality in temperatures can influence the phenological match between them (Burgess et al. (2018)). However, recent changes in temperature regimes have prompted many species, including tits and caterpillars, to shift their phenology (Parmesan and Yohe (2003)) in response. In some populations (though not all: Charmantier et al. (2008)), this has led to disruptions in established phenological matches between the two species, due to their differences in temperature sensitivity, with potentially profound impacts on individual fitness (e.g. offspring number) and demography (e.g. population size) (Visser et al. (2004)). As both mean and seasonality in temperatures are projected to change, many species will likely face novel pressures to adjust to potential shifts in multiple life history optima.

The extent to which an individual is able to adjust different aspects of their life cycle depends on the costs and benefits associated with each life-history stage, though the exact processes underlying these 'decisions' are not well understood (Stearns (1989)). While several mechanisms have been proposed to explain how individuals mitigate the trade-offs between competing behaviours and their consequences for fitness, many of these conclusions were drawn from studies that focus on a few traits in isolation (Flatt and Heyland (2011)). This approach overlooks the complexity of how interconnected traits might be co-optimised in response to environmental variability. Instead, we need a more holistic framework that simultaneously considers multiple life-history traits and the external factors that influence them.

An agent-based model (ABM) provides an ideal framework to test the effects of different ecological variables on behavioural decisions and study the resulting fitness consequences. ABMs offer a unique advantage of allowing multiple aspects of an individual's life history (such as the timing of reproduction and clutch size) to be independently varied, along with the ability to incorporate and control responses to both

abiotic (eg. temperature) and biotic factors (such as prey availability). This allows us to simulate dynamic interactions between traits and environmental conditions, offering a level of experimental control that would be difficult to achieve in the wild. Extensive research on great tits (*Parus major*) over many decades and across a variety of habitats has provided a wealth of data on various aspects of their behaviour, life history, and responses to environmental change (Perrins (1970), Nager and van Noordwijk (1995), Garant et al. (2004), Visser and Lessells (2001)). In particular, the population of great tits at Wytham Woods, Oxford, UK, has been the subject of the oldest continuous-running study of an individually-marked population of animals in the world (Lack (1964)), providing long-term, fine-scale data to parameterise an ABM with biologically relevant values.

We used 61 years of this long-term population data on the phenology and reproductive metrics of great tits to build an ABM that simulates daily behavioural decisions made by individual tits living in a seasonal woodland ecosystem. These models allow us to study life-history trade-offs in a more holistic context, rather than relying on one-to-one comparisons between selected traits. We aimed to explore how differences in annual mean temperature coupled with variation in the rate of spring warming might shape the theoretical optimum sequence of behaviours that maximises fitness (defined as the number of successfully fledged young scaled by their quality) in this population. Finally, we also compare how closely the annual strategies observed in the wild actually align to the expected optima derived from our models, and therefore discuss any potential shortfalls in adjusting to future predicted temperature change. In particular, we explore how different environments might influence; (1) the number of emergent optimal strategies predicted from theory, (2) the strength of selective pressures on lay date and clutch size, and (3) the ability of individuals to attain these predicted optima in the wild. While our model is tailored to great tits, this framework can be easily modified and its findings generalised to other species in seasonal environments where reproductive success is tightly coupled to an ephemeral resource.

2 | METHODS

2.1 | Model framework

We modelled the daily actions of a population of birds using an agent-based modelling (ABM) framework in R (v4.4.0, R Core Team (2024)). For simplicity, we only considered the actions of females, as they are primarily in control of behaviours related to laying, incubation and clutch size. At the start of each day, an individual may perform two, non-mutually exclusive, classes of behaviours; (1) self-maintenance and (2) reproduction (see detailed description at section 2.1.3 below). The available actions to perform depend on the time of year, the individual's energy reserves and their previous behaviours. The action taken by an individual affects their future energy reserves and thus behaviours on different days are linked to one another.

2.1.1 | Environmental variables

Temperature

The daily temperature cycle is simulated using a sinusoidal function:

$$T_t = A \times \sin\left(t \times \frac{2\pi}{365} - \frac{\pi}{2}\right) + H$$

where A represents the amplitude of temperature variation (i.e. annual seasonality in temperature), t is the time in days, and H is the annual mean temperature. The temperature reaches its maximum at $H + A$ in midsummer and is at its lowest at $H - A$ in midwinter (figure 2.1).

Caterpillar availability

Parents provision their young exclusively with caterpillars. The start and duration of caterpillar availability (figure 2.1) is simulated using a cumulative growing degree days (GDD) function;

$$\text{GDD} = \sum_{t=1}^{n} (T_t - T_{\text{base}})$$

where temperature values (T_t) above a base temperature (T_{base}) of 10 °C are cumulatively summed each day (t). The window of caterpillar availability commences at a threshold value of 80 °C. We assume that the shape of this availability can be approximated as an isosceles triangle and that the total amount of caterpillars (i.e. the area of the triangle, S) is constant between environmental scenarios. On the day that GDD reaches threshold ($t_{\text{threshold}}$), we calculate the base angle of the caterpillar window (θ) to define the rate of increase in caterpillar availability as;

$$\theta = \tan^{-1}\left(A \times \cos\left(t_{\text{threshold}} \times \frac{2\pi}{365} - \frac{\pi}{2}\right) \times \frac{2\pi}{365}\right)$$

The geometry of the resulting caterpillar triangle is determined from the base angles (θ), with the base length l (i.e. caterpillar window duration) and height h derived from the known area (S, set by the total amount of caterpillars) :

$$l = \sqrt{\frac{S}{\tan(\theta)}}, \quad h = \sqrt{\tan(\theta) \times S}$$

From this, we can calculate the total caterpillar availability on any given day (C_t) as follows;

$$C(t) = \frac{2 \times h \times (t - t_{\text{threshold}})}{l} \times \varepsilon \quad \text{for } t_{\text{threshold}} \leq t \leq t_{\text{threshold}} + \frac{l}{2}$$

$$C(t) = \frac{2 \times h \times (t_{\text{threshold}} + l - t)}{l} \times \varepsilon \quad \text{for } t_{\text{threshold}} + \frac{l}{2} < t \leq t_{\text{threshold}} + l$$

$$C(t) = 0 \quad \text{otherwise.}$$

where $t_{\text{threshold}}$ is the day when the caterpillar availability begins (i.e. when the GDD threshold is crossed), $t_{\text{threshold}} + l$ is the day when caterpillar availability ends, h is the height (peak availability) of the triangle, l is the total duration (in days) of caterpillar supply and ε is a scaling factor

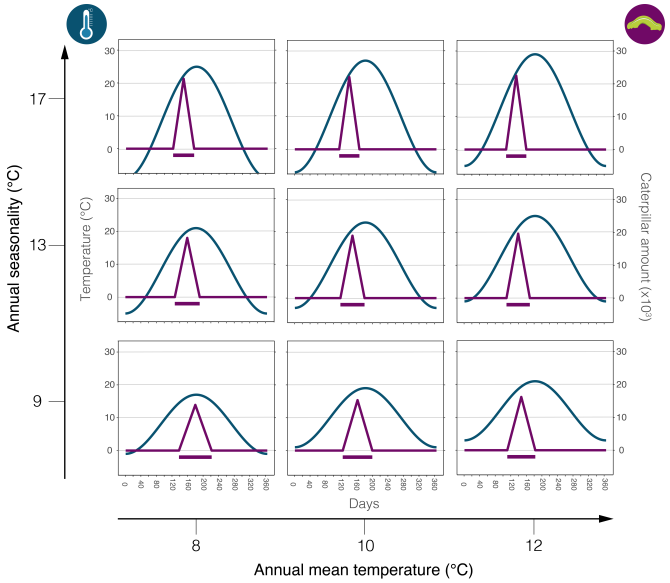


FIGURE 2.1 Graphs showing how caterpillar availability is linked to temperature annual mean and seasonality in the models, illustrating the underlying variation in biotic (caterpillar) and abiotic (temperature) factors influencing the behavioural decisions of simulated birds within each environmental scenario (combination of mean and seasonality) tested. For simplicity, caterpillar availability is modelled as a triangle and we assume that the total caterpillar amount (i.e. area under the triangle) remains constant between environments, linking variation in the width and height of the resource peak. Horizontal purple lines denote the duration of the window of caterpillar availability. In general, windows become shorter with both increasing annual mean and seasonality in temperature. Caterpillar window dates are summarised in appendix A2.

(based on the maximum possible number offspring fledged by all starting individuals' first breeding attempt). When available, parents forage for caterpillars with an intensity between $0 < u_{caterpillar} < 0.99$, selected each day at random.

General food supply

Individuals forage from the general food supply for self-maintenance. The total amount of food available each day (G_t) is given by the sinusoidal function;

$$\text{General food supply}(G_t) = A_{food} \times \sin\left(t \times \frac{2\pi}{365} - \frac{\pi}{2}\right) + H_{food}$$

where A_{food} is the amplitude of food variation (i.e. annual seasonality in food supply), t is the time in days, and H_{food} is the annual mean food supply. We assume that the food supply is constant between environmental scenarios. Individuals forage from the general food supply with an intensity between $0 < u_{food} < u_{max}$, selected each day at random. When laying or incubating eggs, u_{max} is set at 0.9 and 0.8 respectively, and when caring for young $u_{food} = 1 - u_{caterpillar}$, to reflect the need for individuals to split their time between self-maintenance and reproductive behaviours.

We model density dependence in both the general food and caterpillar supply, by allowing individuals to forage from a pool of food/caterpillar supply that is equal to the total amount of food/caterpillar available on a given day (G/C_t) , divided by the number of birds foraging/caring ($N_{foraging/caring}$), respectively. Therefore, the gross food/caterpillar intake ($\gamma_{food/caterpillar}$) is calculated as;

$$\gamma_{food}(u_{food}, t) = G(t) \times \frac{u_{food}}{N_{foraging}}$$
$$\gamma_{caterpillar}(u_{caterpillar}, t) = C(t) \times \frac{u_{caterpillar}}{N_{caring}}$$

2.1.2 | Energy dynamics

Each day, individuals utilise their energy reserves (r) to expend and gain energy based on (1) their reserves from the previous day (r_{t-1}), (2) the costs associated with their action(s) (c) performed on the current day and (3) the amount of food foraged for self-maintenance (γ_{food}), such that their energy reserve at the end of the current day (r_t) is calculated as;

$$r_t = r_{t-1} - c + \gamma_{food} \quad \text{where} \quad 0 \leq r \leq 100$$

2.1.3 | Behavioural classes and relationships

On any given day, individuals have a set of actions to perform (figure 2.2), the availability of which depends on their energy levels at the start of the day. If an individual's starting reserves exceed the basal metabolic rate value (BMR), they can perform one action each of two behavioural classes associated with;

1. self-maintenance
   - 'forage': forage for food from the general food supply, G(t)
2. reproduction
   - 'lay': lay a single egg
   - 'delay': delay incubation for a day
   - 'incubate': incubate eggs for a day
   - 'care': forage for caterpillars to feed chicks
   - 'abandon': abandon the current reproductive attempt

When an individual's reserves fall below BMR, they must focus on self-maintenance and abandon any current breeding attempt - with an exception if the bird is about commence incubation, they can delay incubating (for up to 14 days) while they solely forage from the general food supply. When a bird's reserves fall below 0, they die and any current clutch laid/brood cared for also fails.

Decision logic

LAY

At the beginning of each simulation, individuals are divided uniformly among 15 clutch size classes ('max clutch'), determining the number of eggs they must lay within the same breeding attempt before commencing incubation. Any bird with energy reserves r > BMR can choose to

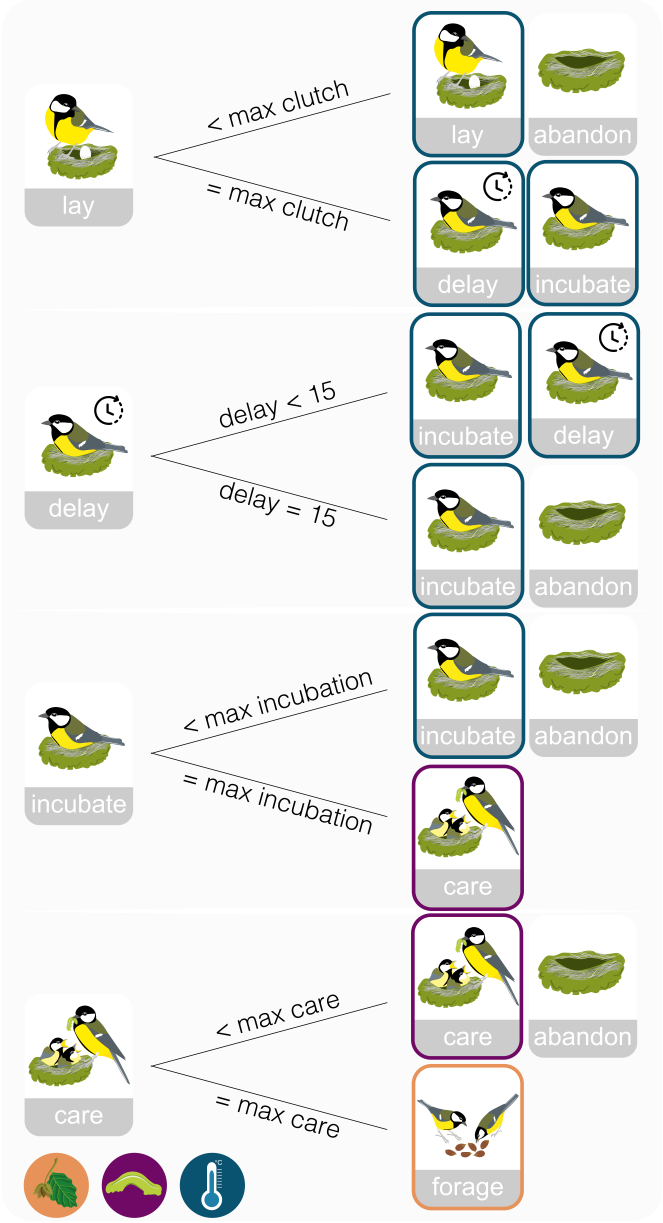


FIGURE 2.2 Simplified flowchart describing how previous actions link to future possible actions if certain conditions are met. Outline colours around each action card show which environmental variable they are influenced by.

start laying, with a probability of choosing to 'lay' vs 'abandon' linked to the mean temperatures over the preceding eight weeks, and calculated from a normal distribution where maximum probability ($P_{\text{lay}} = 0.99$) of choosing to 'lay' is reached at 11.7°C with a standard deviation of 3.3°C. The first day a bird chooses to 'lay' within a breeding attempt sets the lay date for that attempt.

DELAY

Once the number of eggs laid reaches the intended clutch size ('max clutch'), any individual with energy $r > 0$ can choose to delay incubating, for up to 14 days. When a bird chooses to delay, they only pay the costs

TABLE 2.1 Summary of the energetic costs associated with performing different actions

action	cost (c_{action})
forage	BMR $\times$ u_{food}
lay	$0.1 \times$ BMR
incubate	$0.2 \times$ BMR
care	BMR $\times$ $u_{\text{caterpillar}}$

of foraging from the general food supply. After 14 days, they must either 'incubate' or 'abandon' their breeding attempt.

INCUBATE

Birds with reserves $r >$ BMR can begin incubating only after they have laid their full clutch ('max clutch'). The probability that they choose to 'incubate' vs 'delay' or 'abandon' is defined as a sigmoid function;

$$P_{\text{incubate}} = \frac{1}{1 + e^{-0.66 \times (x-16)}}$$

where x is the mean temperature on the current day. Once incubation has commenced, individuals must continue to 'incubate' until they reach the 'max incubation' duration, where they begin caring for hatched nestlings.

CARE

Parents forage from the caterpillar supply (C_t) to provision their young, with the minimum amount of caterpillars to sustain all nestlings in the brood determined by the number of alive offspring (N_{brood}) and their individual critical demand (D_{nestling}) as a function of brood age (a) ;

$$D_{\text{nestling}}(a) \times N_{\text{brood}} = \frac{100}{1 + e^{-1 \times (a-4))}} \times N_{\text{brood}}$$

where maximum demand per nestling (100 caterpillars) is reached at 10 days of age (Perrins (1991)). We simulated brood reduction by having parents allocate caterpillars equally among as many nestlings as possible each day, while the remainder of chicks that do not receive the minimum threshold die. Once the brood reaches maximum care duration ($t_{\text{max}}(\text{care})$), the number of nestlings still alive in the brood (N_{brood}) fledge the nest, and the parent receives the appropriate fitness score to reflect this. Fledged birds start the day with their energy reserves proportional to the amount of caterpillars they received as nestlings and are only allowed to 'forage' for the rest of the year. Parents that successfully fledged chicks must 'forage' for a day before they can start a new breeding attempt.

Action costs

The costs associated with each behavioural action are listed in table 1 above. During reproduction, parents pay both breeding and self-maintenance costs. If an individual tries to breed more than once, they face increased reproductive costs ($\hat{c}_{\text{action}}$) , scaled by the total number of days they previously chose to 'care' for nestlings ($\sum t_{\text{care}}$);

$$\hat{c}_{\text{action}} = c_{\text{action}} \times \frac{1.2}{0.05 \times \sum t_{\text{care}}}$$

Fitness and the optimal strategy

We define fitness as the number of successfully fledged offspring multiplied by their mean quality, where quality is estimated from the the total

TABLE 2.2 Table of constants used to parameterise the ABM.

constant	symbol	value
basal metabolic rate	BMR	25
max lay forage intensity	u_{max}^{lay}	0.9
max incubate forage intensity	$u_{max}^{incubate}$	0.8
max delay	t_{max}^{delay}	14
max incubation	$t_{max}^{incubate}$	12
max care	t_{max}^{care}	21
caterpillar cumulative temperature threshold	$T_{threshold}$	80
caterpillar availability triangle area	S	200
caterpillar development base temperature	T_{base}	10
caterpillar amount scaling factor	k	3000
general food annual mean	H_{food}	75000
general food annual seasonality	A_{food}	3750
temperature at max lay probability	T_{max}^{lay}	11.7 ± 3.3°C
temperature at 50% incubation probability	$T_{50}^{incubate}$	16°C

amount of caterpillars fed to each chick throughout the nestling period. The optimal strategy is then defined as the combination of lay date and clutch size that maximises fitness.

2.1.4 | Simulation set-up

Parameterisation

We aimed to parameterise the ABM with biologically relevant and realistic values (see tables 2 and 3) wherever possible to aid both model performance and interpretability. To achieve this, we used estimates from research articles, and also derived key variables from our long-term research on the breeding biology of wild great tits (*Parus major*) studied since 1947 at Wytham Woods, Oxford, UK. Birds in this population are monitored intensively during the spring breeding season (April-May) by a team of researchers to accurately identify the timing and input effort of key reproductive metrics, such as;

1. the lay date of each breeding pair (the date of first egg laid in the nest),
2. the clutch size (total number of eggs incubated),
3. the hatch date (date of the first nestling hatched),
4. the number of offspring successfully fledged, and
5. their body mass at 14 days of age when they are given a unique identifier metal ring.

We can further estimate;

6. the start of daytime incubation (by recording the date when eggs first feel warm to touch, combined with knowledge of the date of clutch completion, assuming females lay one egg per day),
7. the approximate duration of daytime incubation (defined as the days between the start of daytime incubation and the hatch date), and
8. possible delays in incubation (defined as the number of days between clutch completion and start of daytime incubation).

Model validation

Using this empirical data, we parameterised a baseline ABM to compute daily action patterns of birds simulated under the average (between 1960 and 2021) environmental values of annual temperature mean (H = 10.61°C) and seasonality (A = 12.94 °C), recorded at the Radcliffe Observatory, Oxford, UK, approximately 4km from the great tit study site. To validate that our simulated population behaves in a realistic manner, we ran 100 realisations with a starting population size of 375 birds under this average environment (A = 12.94 °C, H = 10.61°C), and compared the timing of their behavioural actions against those observed in the Wytham Woods population in representative years (1975, 1984, 2001, 2013) with near-average values (within 0.25 SD from the mean) of A and H. Given recorded dates of lay, hatch and incubation as well as clutch sizes and reproductive failures for all breeding pairs in the real-world population, we converted their breeding behaviours into daily actions of 'lay', 'delay', 'incubate', 'care' and 'abandon', and calculated the proportion of individuals performing each action on any given day. We also calculated action proportions from the simulated dataset, and compared peak dates (= day on which max proportion is reached after smoothing the trend line) of the theoretical vs observed behaviours.

The peak dates for when the majority of individuals performed each action class are summarised in figure 2.3 for both the observed and simulated populations at the average temperature annual mean and seasonality values (H = 10.61°C, A = 12.94 °C). In general, the mean timings of when individuals performed different actions matched closely between the observed and simulated datasets (the average difference between all observed-simulated action pairs was 5.6 days). However, there were some discrepancies between when the majority of individuals performed the 'lay' and 'abandon' actions. Peak laying in the simulated dataset occurred 7 days earlier, however its distribution was also much wider. This early laying of simulated individuals likely caused the early peak in abandonment at day 107, which did not occur in the observed population. As a result, the proportion of individuals that reached incubation compared to those that started laying (day 118: 70.5%),

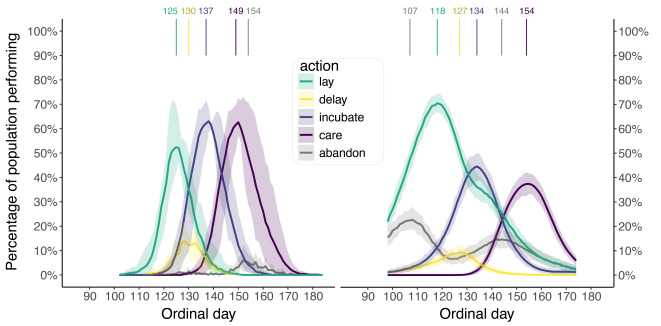


FIGURE 2.3 Distributions showing the proportion of individuals performing each action class on a given day for the observed population (Wytham data, left) and the simulation population (ABM data, right), with the peak date (in ordinal days) of each distribution indicated at the top (showing two distinct peaks of abandonment in the simulated population). Solid lines show the mean, and shaded areas show the 95% confidence intervals estimated across 4 years (left) and 100 realisations (right). Estimates summarised in appendix A1.

TABLE 2.3 Table of variables with value ranges specific to the environmental scenarios tested, and units displayed where appropriate.

variable	**symbol**	**value range** (units)
temperature	T	-0.5 to 29 (°C)
temperature annual mean	H	8, 10, 12 (°C)
temperature annual seasonality	A	9, 13, 17 (°C)
time	t	1 to 365 (days)
length of caterpillar window	l	50 to 82 (days)
caterpillar availability	$C(t)$	0 to 600,000
general food availability	$G(t)$	71,250 to 78,750
foraging intensity	u	0 to 1
energy reserve	r	0 to 100
gross energy intake	γ	0 to 100
action associated cost	c	0 to 100
action probability	P	0 to 1
number of individuals	N	0 to 3000
nestling age	a	1 to 21
nestling demand	D_{nestling}	5 to 100

dropped significantly (day 134: 44.5%) in the simulated population. On the other hand, the second peak in abandonment of the simulated birds was much closer to the observed population's peak. The overall higher variance around the simulated dates illustrates that the simulated population behaves more stochastically, as their actions are probabilistic, whereas the observed natural population's tighter distributions might indicate past selection.

Experimental set-up

We ran simulations with 375 starting individuals allocated a random starting energy reserve ($0 \leq r \leq 100$) and sorted into 15 clutch size categories ($1 \leq$ 'max clutch' ≤ 15), giving a sample size of 25 individuals for each clutch size (as this gave us equal numbers for each clutch size category and 375 was close to the yearly mean of 406 breeding birds between 1960-2021 at Wytham Woods). As we were only interested in fitness in terms of the quality of offspring at the time of fledging, we only modelled daily actions of individuals until the end of the caterpillar availability window in each simulation (after which caring for offspring is not possible), thereby allowing us to significantly reduce the computational load. We tested nine environmental scenarios, each with a unique combination of temperature annual mean (H: 8,10,12) and annual seasonality (A: 9,13,17) values that are slightly outside of the range observed at Wytham Woods (H: 8.9 - 11.8, A: 10.3 - 16.9). We ran simulations for 100 realisations under each environmental scenario (i.e. 900 realisations total). Although this is on the lower end of the number of realisations that studies usually perform, we found that the optimal strategies, as well as the population mean lay dates and clutch sizes, seem to stabilise after c. 50 realisations within each environment (appendix A3 and A4). Therefore, while more realisations would increase the confidence around these estimates, they are unlikely to change the mean trends presented here.

2.2 | Analysis of simulation output

All analyses were performed in R (v4.4.0, R Core Team (2024)). Within each realisation of each environmental scenario (A x H), we identified the optimal strategy as the combination of lay date and clutch size that maximised fitness, where we defined fitness as the *number of fledglings* x *their average quality*, and where average quality was calculated as the mean amount of caterpillars consumed by fledglings in a given nest.

To explore differences in the number of emergent optima between the nine environmental scenarios, we compared optimal strategies and population average strategies between each combination of A and H (summarised in figure 2.4). For each environmental scenario we ran 100 realisations, and from each realisation we extracted the optimal strategy, as well as the average strategy (mean lay date and mean clutch size) of the whole population, after removing any breeding attempts that failed before hatching. Our motivation for removing these failed breeding attempts is that rather than making decisions per se, the simulated population behaves in probabilistic manner, with laying and incubation tied to temperature, and nestling provisioning tied to caterpillar availability. As a result, the 'raw' mean of all breeding attempts is likely a function of the parameter values we seeded the population with, resulting in a mean clutch size of 8 and lay dates near the date when temperatures reach the maximum probability laying temperature encoded in the model. This would bias the mean estimates towards these (though empirically-guided but still) somewhat arbitrary parameter values. Instead, by considering only those breeding attempts that have successfully hatched young, we allow some degree of selection to occur, making the range of emergent strategies more representative of what a bird making behavioural 'decisions' would do (i.e. behaving more realistically).

After excluding these attempts that failed before hatching, we calculated the mean and standard deviation of optimal lay dates and clutch sizes, as well as of the population average lay dates and clutch sizes, across the 100 realisations within each environmental scenario. Additionally, we calculated the difference between the mean optimal and the mean population average lay dates and clutch sizes, separately for each environment. Lastly, we performed one-way ANOVA comparisons with Tukey's post-hoc tests (in base R) on these standard deviations and mean differences between values of A (for each level of A, we had three data points corresponding to the three levels of H) and H separately (three data points in each group, as for A) to test whether certain environments allowed for more variable optimal strategies to emerge and how optimal strategies differed compared to the behaviour of the rest of the population, respectively.

To explore whether some environments are more conducive to breeding, we ran linear models (in base R), with *number of successful breeders* (individuals that fledged at least one offspring, averaged per environment) or *number of fledglings* (total number of fledglings in the population, averaged per environment) as response, and annual mean (H) and seasonality (A) as predictors.

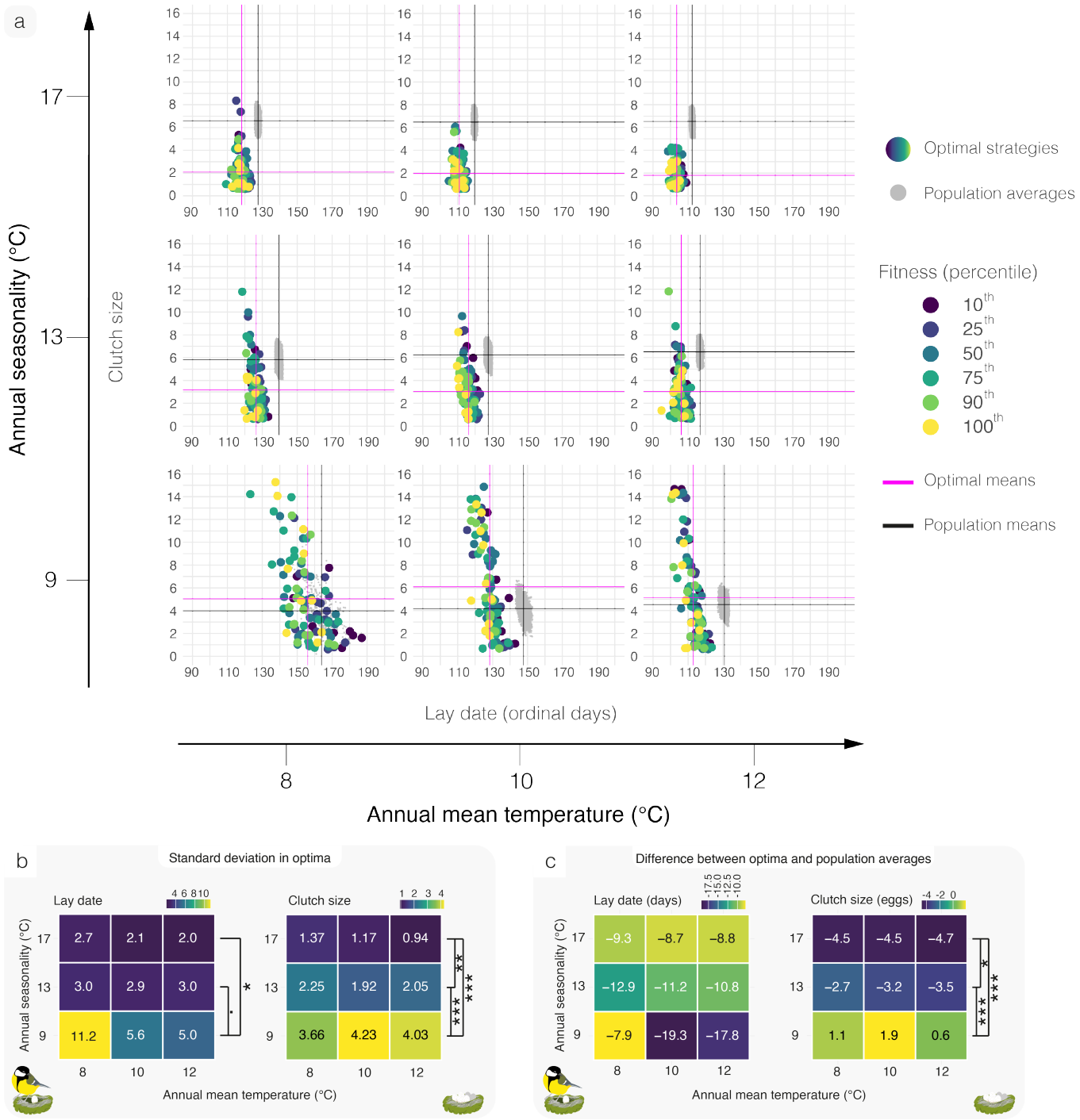


FIGURE 2.4 Summary plots illustrating the distribution of optimal strategies across environmental scenarios. (a) Scatterplots showing the population average strategies (i.e. combinations of lay date and clutch size) in grey, and the optimal strategies coloured according to fitness (= number fledglings x mean number of caterpillars eaten) broken down into percentiles. Each realisation (of 100 total) is represented by two points; one for the population average strategy in grey (after removing breeding attempts that failed prior to hatching), and another one for the optimal strategy from that realisation in colour. Horizontal lines show the mean clutch sizes, while vertical lines show the mean lay dates for the population averages (in black) and the optimal strategies (in magenta). (b) Heatmap showing the standard deviations in optimal lay dates (left) and clutch sizes (right) with significant Tukey's test comparisons (where . = p < 0.1, * = p < 0.05, ** = p < 0.01, *** = p < 0.001) between values of temperature seasonality (A) indicated. These standard deviations are calculated from the optimal strategies, shown as coloured points in plot (a). (c) Heatmap showing the difference between the optimal minus the population mean lay dates (left) and clutch sizes (right) with significant Tukey's test comparisons between values of A indicated. These differences are calculated from the vertical and horizontal lines, respectively. Model output values for (a) are summarised in appendix A5, and for (b) and (c) in appendix A6. Summary plots of mean lay dates and clutch sizes including all breeding attempts (including those failed to hatch young) are shown in appendix A7.

To understand how the optimal strategies might vary with annual mean (H) and seasonality (A) in temperature, we ran separate linear models (in base R), with *optimal lay date* or *optimal clutch size* as response, and the interaction between A and H as predictor, and used the 'marginaleffects' package (v0.20.1, Arel-Bundock (2024)) to calculate predicted mean and 95% confidence intervals.

Finally, to compare how patterns observed from the long-term Wytham Tits dataset align with the theoretical optima predicted from the ABM, we used breeding data excluding second broods (< 2% of the total number of breeding attempts since 1960) between 1960 - 2021 (as monitoring techniques became more standardised in 1960) and ran linear mixed models using the 'lme4' package (v1.1.35.3, Bates et al. (2015)), with *clutch size* or *lay date* as response, the interaction of A and H as fixed effects, and *year* as random effect. We tested values of A between 11 and 15, and values of H between 9 and 12, as these ensured we had adequate sample size of corresponding representative years in the long-term population for these environmental combinations. We z-scored the response variables in both the theoretical and observed dataset to allow for comparison between the two.

3 | RESULTS

3.1 | Patterns in optimal strategies

3.1.1 | Low temperature seasonality allows for a wider range of optimal strategies to emerge

Optimal strategies (combinations of lay date and clutch size) varied markedly between environmental scenarios (summarised in figure 2.4). Firstly, the standard deviation (SD) in both lay dates and clutch sizes varied significantly between different levels of annual seasonality in temperature (A) (ANOVA of SD in lay dates: $p = 4.30 \times 10^{-2}$ and clutch sizes: $p = 1.50 \times 10^{-5}$, figure 2.4a,b). Under increasing temperature seasonality, the range of optimal lay dates that resulted in the highest fitness within each realisation became more restricted (mean difference [95% CI] in SD of lay dates at A=9 vs A=17 was -5.0 SD [-10.0, -0.02], p = 0.0493 ; figure 2.4a,b). Similarly, lower seasonality allowed for a wider range of clutch sizes to maximise fitness (mean difference [95% CI] in SD of clutch sizes at A=9 vs A=17 was -2.8 SD [-3.4, -2.2], $p = 1.27 \times 10^{-5}$; figure 2.4a,b), resulting in a higher mean optimal clutch size (5.4 at A=9 and 2.0 at A=17; figure 2.4a). On the other hand, standard deviations in optimal lay dates and clutch sizes remained similar between different levels of annual mean in temperature (H) (ANOVA of SD in lay dates: p = 0.629, and clutch sizes: p = 0.996; figure 2.4a,b).

3.1.2 | High temperature seasonality causes population average and optimal strategies to diverge

The difference between population average lay dates versus those that resulted in the highest fitness was similar between all levels of temperature seasonality (A) and annual mean (H) (2.4a,c), although optimal lay dates were earlier than the population average in all environments (figure 2.4a). On the other hand, optimal clutch sizes became more distinct from the population average with increasing levels of A, but not H (ANOVA of difference in clutch sizes for A: $p = 1.12 \times 10^{-5}$, for H: p = 0.968; figure 2.4a,c). Specifically, optimal clutch sizes were larger than the population mean at A=9 (mean optimal - population difference = 1.2 eggs, figure 2.4a), however at mid and high levels of A, optimal clutch sizes dropped further and further below the population average (mean optimal - population difference at A=13 was -3.1 eggs and at A=17 was -4.6 eggs; figure 2.4a,c)

3.1.3 | Lower seasonality limits the number of breeding birds and fledglings

The number of breeders that reached hatching stage (summarised in appendix A8) increased with higher levels of seasonality in temperature (slope mean + 95% CI of average number of breeding attempts vs A = 17.8 [11.9, 23.7], $p = 2.8 \times 10^{-4}$), as well as under increasing annual mean temperatures, though not significantly so (slope vs H = 11.2 [-0.3, 22.8], p = 0.0545). The total number of fledged young within populations (summarised in appendix A8) also increased under higher seasonality (slope mean + 95% CI of average number of fledged young vs A = 36.3 [16.7, 56.0], $p = 3.95 \times 10^{-3}$), but did not differ between values of annual mean (slope mean + 95% CI of average number of fledged young vs H = 26.3 [-13.0, 65.4], p = 0.152).

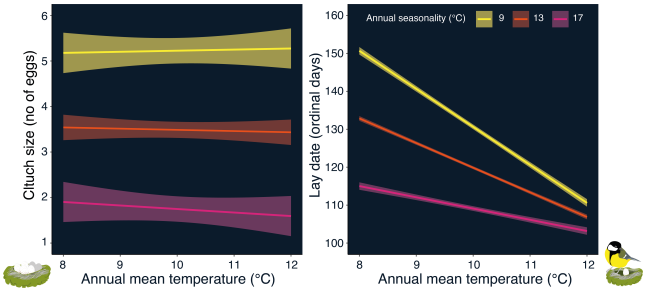


FIGURE 2.5 Interaction plots showing how the optimal clutch sizes (left) and lay dates (right) vary in response to changes in annual mean temperatures at different levels of annual seasonality (difference between annual max and min temperature). Trend lines show predicted mean and 95% confidence intervals, derived from linear models with an interaction between temperature mean and seasonality.

3.1.4 | Optimal clutch size is driven by seasonality, whereas optimal lay dates also respond to changes in annual mean temperatures

Variation in optimal clutch sizes between the environmental scenarios was driven by differences in annual seasonality in temperature (A) only (mean[95% CI] slope of the additive effect of A = -0.43 [-0.49, -0.38]), with an increase in seasonality selecting for smaller clutch sizes (mean [95% CI] of clutch sizes at A=9: 5.4 [5.1, 5.7], at A=13: 3.1 [2.8, 3.4], at A=17: 2.0 [1.7, 2.2]; figure 2.5). On the other hand, lay dates were influenced by variation in both annual temperature mean and seasonality, whereby higher annual mean temperatures caused a shift towards earlier optimal laying (mean [95% CI] slope of H = -17.97 [-18.97, -16.97]), however the rate of this advancement diminished under increasing seasonality (mean [95% CI] slope of A = -11.52 [-12.27, -10.76]). Under the environmental scenarios tested, this resulted in a trend towards more similar lay dates under higher annual mean temperatures (difference in lay dates between A=9 and A=17 at H=8: 38 days, at H=10: 17 days, at H=12: 9 days; figure 2.5).

3.2 | Comparison of predicted optima against patterns in a wild population

When comparing predicted optimal lay dates against those observed in the long-term wild tit population, we found that both expected and observed values showed a negative relationship with annual mean temperature across all levels of seasonality (mean [95% CI] slope of standardised lay date against H for expected: -1.14 [-1.21, -1.08], and observed: -2.30 [-3.74, -0.86]; figure 2.6). However, observed lay dates did not always align with the expected optimal values. In general, observed slopes tended to show a steeper, stronger response from birds to advance their lay dates when faced with increasing annual mean temperatures, compared to a level of response that would be deemed 'enough' to reach optimality. The strength of both observed and expected responses tended to weaken at higher seasonality, though more so for observed trends (mean [95% CI] slope of standardised lay date against A x H for expected: 0.056 [0.051, 0.061], and observed: 0.12 [0.015, 0.23]; figure 2.6) . This means that under low levels of seasonality, observed values diverged from the expected optima with increasing temperature, whereas at high seasonality observed values overlapped with the predicted optima as temperature increased. At mid-level seasonality however, moving away from the mean temperature in either direction resulted in divergence between observed and expected lay dates (figure 2.6).

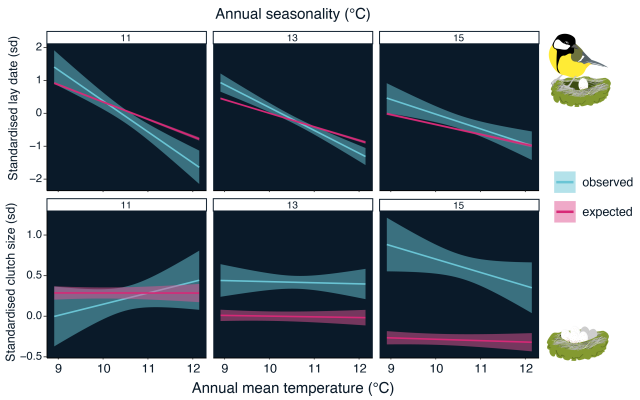


FIGURE 2.6 Comparison of life-history responses to environmental variation in the observed (Wytham Woods data) versus expected (ABM results) population. Estimates come from models with an interaction between seasonality levels (top number in each panel) and annual mean (x axis). Responses are standardised within variable (clutch size or lay date separately) to allow for comparison between observed and predicted values. Observed trends are estimated using all breeding attempts and therefore correspond to population average responses. On the other hand, expected predictions are estimated from the optimal strategies only. Trend lines show predicted mean and 95% confidence intervals.

Comparing observed and predicted optimal clutch sizes showed contrasting patterns. In general, predicted optimal clutch sizes did not respond to changes in annual mean temperatures (mean [95% CI] slope of standardised clutch size against the additive effect of H: 0.0088 [-0.044, 0.027] p = 0.628; figure 2.6), but were overall smaller under higher levels of seasonality (mean [95% CI] slope of standardised clutch size against the additive effect of A: -0.14 [-0.16, -0.12] p = 1.71 x 10^{-49}; figure 2.6). However, observed patterns showed a slope reversal with increasing seasonality, whereby clutch sizes tended to decrease in response to rising temperatures under high seasonality. At low seasonality, patterns in expected and observed clutch sizes overlapped significantly, meaning that wild birds closely tracked the optimum under these conditions. However, at both mid and high levels of seasonality (A = 13,15), wild birds laid more eggs than predicted to be optimal, diverging most from the optimal predicted clutch sizes under low annual mean temperatures coupled with high seasonality (figure 2.6).

4 | DISCUSSION

Our study demonstrates the complex nature of life-history responses to environmental variation, using a simulated population of birds living in a temperate seasonal ecosystem, relying on an ephemeral food source for reproduction. In particular, we showed that changes in both annual mean and seasonality (difference between annual minimum and maximum) in temperature caused significant shifts in the optimal strategies that maximise fitness (number x quality of offspring) under the different environmental scenarios. Optimal lay dates (date of first egg laid in a clutch) advanced strongly in response to simulated changes in both temperature mean and seasonality, while variation in optimal clutch sizes (total number of eggs in a clutch) seemed to be primarily driven by selection towards smaller clutches under increasing seasonality. We found similar patterns in the long-term Wytham Tit dataset, showing strong

selection on lay dates to match the theoretically predicted optima, however, we found that real-world birds laid larger clutches in general than what is predicted to maximise their fitness. Below we discuss the biological implications of different strategies drawing from empirical evidence in the literature, but we also highlight the limitations of our model and how it may be expanded in future to address remaining questions around the co-optimisation of multiple life-history traits in response to environmental change.

4.1 | The early bird catches the worm

Across all the environmental scenarios (combinations of annual mean and seasonality in temperature) we tested, individuals that bred earlier than the rest of the population reached higher fitness scores. Breeding earlier than conspecifics is generally considered beneficial for several reasons, including lower nest predation rates, lower competition for resources, more time for development, and more opportunities to establish a territory post-fledging (Perrins (1970), Sutton and Freeman (2023),Götmark (2002)) - all leading to higher fitness by increasing fledging success and recruitment rates (Verboven and Visser (1998)). In accordance, studies often report a seasonal decline in breeding success, both in terms of the number and quality of offspring produced (Verboven and Visser (1998)). We also observed this seasonal decline in fitness even among the optimal strategies, as birds reaching the highest fitness (top 10% of optimal strategies; figure 2.4a yellow points) were consistently earlier on average than the lowest percentile (bottom 10%; figure 2.4a dark purple points) birds.

4.2 | High seasonality limits the range of optimal strategies

In our models, high temperature seasonality caused a shortening of the window of caterpillar availability, thereby severely restricting the timing and duration that breeding activities could occur. In general, higher seasonality enabled larger clutches to be initiated in the population as a whole, however, these larger clutches performed worse (with regards to total fitness contributions) than birds laying fewer eggs. This could potentially be explained by the temperature - caterpillar dynamics defined in our model, specifically that the total caterpillar amount (area under the distribution) did not change between environments and therefore the height of the resource distribution was coupled to its duration (as described in section 2.1.1.2 and figure 2.1). This means that under high temperature seasonality, there was a steep increase in caterpillar availability, resulting in a higher abundance of caterpillars on any given day, albeit for a shorter duration of time. This allowed parents to care for larger clutches as there was a greater potential that they could meet the higher food demands of the nest. However, a shorter breeding season resulted in higher nest failures among the larger clutches, as these required a longer time frame to be laid (due to the inherent link between lay date and clutch size with birds laying one egg per day) and any active nests had to be automatically abandoned once caterpillar availability ceased, hence why the most successful birds laid smaller clutches. In reality, parents are able to provide a wider selection of (less nutritious) food items to sustain their brood (Naef-Daenzer et al. (2000)), however, this is beyond the scope of our model.

In comparison, environments with lower seasonality allowed for a wider range of lay dates and clutch sizes to maximise fitness. As we explained above, due to the total caterpillar amount being fixed between environments, a lower seasonality resulted in a longer breeding season, especially under low annual mean temperatures. We hypothesise that this allowed for more flexibility in life-history trait values by reducing competition in two ways; (1) on the one hand, a longer breeding season enabled birds to initiate reproduction on a wider range of lay dates thereby minimising the number of concurrent breeding attempts, and (2) on the other hand, a lower caterpillar availability on any given day decreased the carrying capacity, and increased the likelihood of parents failing to forage enough food to meet the brood demand. Indeed, we observed fewer successful breeding attempts (appendix A6) and smaller population average clutch sizes (figure 2.4a) under low seasonality, however those that prevailed benefited from the reduced competition and thus were able to raise larger broods than the rest (shown by the larger clutch sizes of optimal birds in figure 2.4a).

4.3 | Stronger effect of temperature on timing of breeding than on clutch size

While we found that variation in both annual mean and seasonality of temperature significantly influenced the emergent values of optimal life-history traits, responses in lay date (timing of breeding) appeared to be greater than in clutch size (reproductive effort). Increasing annual mean temperatures caused optimal lay dates to advance, consistent with a shift towards earlier breeding observed in response to warmer springs in the wild great tit population of Wytham Woods (figure 2.6), and indeed across numerous populations of tits (Bauer et al. (2010), Andreasson et al. (2023)), as well as other taxa (Thackeray et al. (2016)). However, our models also showed that the relationship between both expected (from the ABM) and observed (from the long-term data) lay dates and annual mean temperatures became less pronounced with increasing seasonality, suggesting that, while seasonality itself already selects for earlier laying, it may also set limits on its absolute values.

In contrast, shifts in response to variations in temperature in both expected and observed clutch sizes were much more subtle than in lay dates (figure 2.6). Predicted optimal clutch sizes were unaffected by increases in annual mean temperatures, but lower clutch sizes reached higher fitness under increasing seasonality. This was in contrast to the patterns observed in the Wytham great tits, where population average clutch sizes tended to increase slightly under higher seasonality and this led to significant discrepancies between the observed and predicted clutch sizes under these environmental scenarios.

Possible biological explanations for the larger clutch sizes observed in the real-world population might reflect differences in how they balance life-history trade-offs. As a short-lived species, great tits exhibit higher reproductive efforts early in life at the expense of future survival (Fokkema et al. (2016)), which might explain why Wytham birds laid larger clutches than predicted to be optimal when years are considered in isolation. In nature, parents may also lay larger clutches than optimal as a form of reproductive insurance strategy against starvation during times of scarcity or against partial predation of the nest (Forbes (1991)). Although brood reductions were allowed in our simulations, the end of the caterpillar availability set a hard limit on nestling provisioning, as opposed to tit parents in the wild often bringing lower quality food items to the nest in times of scarcity, thereby allowing them greater flexibility to utilise adaptive brood reduction strategies (Naef-Daenzer et al. (2000), Lee and Chung (2020)). This would tip the scale towards the number as opposed to the quality of offspring, whereas in our simulations fitness was scored based on keeping a balance between the two sides of this trade-off.

Lower predicted clutch sizes might also have resulted from our model specifications. Firstly, we tested a uniform distribution of clutch sizes, as opposed to the normal distribution observed in nature. We justify this decision as we wanted to test which clutch sizes emerged optimal, while minimising inbuilt biases by having equal sample sizes representing each clutch size category. Moreover, the mean starting clutch size of our simulation is slightly lower (8 eggs) compared to the mean numbers observed in the Wytham Tit dataset (mean of 8.18 between 1960 - 2021), therefore we argue that these two are comparable. Nevertheless, a uniform distribution of clutch sizes in our models meant that a greater portion of the population faced an increased likelihood of not being able to forage enough caterpillars to meet the higher brood demands. The increased probability of nest failures at higher clutches (also present in natural populations: Boyce and Perrins (1987)) was likely exacerbated by the model specification that birds could only forage for caterpillars at a randomly selected intensity each day (again, to allow optimal strategies to emerge more organically), preventing them from adjusting their provisioning rates in response to the brood demand. Moreover, our model validation (in figure 2.3) showed that our simulated populations experienced two peaks in abandonment; during laying and incubation. This is opposed to the patterns observed at Wytham Woods, where birds exhibit the highest rates of abandonment during nestling provisioning, perhaps indicating that the parameterised energetic costs associated with egg laying and incubation may be unrealistic in our simulations. As larger clutches carry higher egg laying costs, this could further increase the failure rates of the larger clutch size categories.

4.4 | Applications of our modelling framework

In the ABM presented in this study, we explored how variations in annual mean and seasonality in temperature influence the optimal combinations of lay dates and clutch sizes of birds, using great tits as a model system. Comparison of the simulated patterns against behaviours observed in natural populations can offer insights into how future climate change might affect the ability of individuals to track predicted shifts in life history optima. For instance, under 'business-as-usual' RCP8.5 emission scenario projections (Met Office (2018)), the UK is expected to experience an increase in both annual mean and seasonality in temperature (with summers warming more rapidly than winters). Our model suggests that optimal lay dates will continue to advance in response to rising mean temperatures, though this advance will likely be less pronounced under high seasonality. Great tits at Wytham Woods exhibit similar responses in lay dates, though they tend to advance more rapidly than predicted to be optimal. This suggests that, under moderate increases in temperature annual mean and seasonality, they are likely to closely track these predicted shifts in the optima (as has been predicted previously for this population: Vedder et al. (2013), for UK passerines; Phillimore et al. (2016b)), but under a more severe rise in temperature, birds in this population may advance beyond the optima predicted from our models (as has been predicted for other tit populations: Gienapp et al. (2013)), potentially resulting in increased mismatches with their food source.

It is important to note, however, that any inferences drawn from the model presented here assumes that the temperature sensitivity of both prey (caterpillars) and consumers (birds) remains constant over time, therefore ignoring the contributions of evolutionary change, which could be beneficial in minimising the phenological mismatches in our tit-caterpillar system (Nussey et al. (2007)). Due to the modular nature of ABMs, our model could be expanded to simulate responses to temperature variation across multiple years, testing different magnitudes of change in temperature sensitivity between prey and consumers to understand their potential effects on future predicted phenological mismatches (Both et al. (2009), Burgess et al. (2018)). Parameterising our model with estimates from other long-term studies, such as the individual-based data on the breeding behaviour of over 200 populations of temperate passerines across Europe, hosted by the SPI-Birds Database (Culina et al. (2021)), would provide an opportunity to evaluate how optimal strategies of life-history decisions might vary between populations experiencing different rates of environmental change. Moreover, a multi-year approach would allow us to tease apart how trade-offs between current and future reproduction might alter the predicted optimal life-history traits in a given year, perhaps shedding light on the discrepancies between predicted and observed clutch sizes in our study. Furthermore, following the survival and reproduction of offspring beyond fledging would enable a more comprehensive look at the trade-offs between offspring quantity and quality experienced by individuals in the face of environmental variation.

CONCLUSION

Overall, our agent-based model provides a valuable framework for exploring the complex interplay between environmental factors in shaping life history strategies in seasonal ecosystems. By simulating daily decision-making of an iteroparous bird population, modelled after great tits, we were able to demonstrate how changes in environmental conditions affect the optimal balance between the seasonal timing and effort of reproduction, though the conclusions that can be drawn about patterns in clutch sizes may be limited by the model specifications. Furthermore, we discussed how future work could expand on this model to predict expected shifts in life history optima under continued environmental change. Although our models were parameterised using the great tit - winter moth caterpillar system, this framework could be applied to a range of other species where individuals rely on an ephemeral food source for reproduction, providing important insights into how multiple life-history traits may be co-optimised in response to environmental variation.

AUTHOR CONTRIBUTIONS

A.K. conceived and planned the project with the help and supervision of J.P., C.R., E.C. and B.S. A.K. wrote the code with contribution from J.P. A.K. performed the simulations and analysed the results. A.K. and B.S.interpreted the results. A.K. produced the figures, and wrote the chapter with feedback from B.S.

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ETHICAL NOTE

The long-term monitoring of the Wytham tit population was approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/TitBreedingEcology).

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APPENDIX A

Action	Observed Peak Date	Observed Peak Proportion [CI] (%)	Simulated Peak Date	Simulated Peak Proportion [CI] (%)
Lay	125	52.4 [42.4, 69.6]	118	70.5 [66.9, 74.0]
Delay	130	12.4 [9.7, 15.9]	127	9.1 [6.1, 11.8]
Incubate	137	62.1 [51.7, 71.5]	134	44.5 [38.6, 50.0]
Care	149	61.6 [50.9, 70.0]	154	37.4 [32.4, 42.2]
Abandon	154	5.1 [1.2, 8.4]	107 and 144	22.4 [18.7, 27.0] and 14.7 [11.3, 17.9]

Table 1: Peak dates and proportions of individuals performing each action class, summarised for the observed and simulated populations, relating to Chapter 2 figure 3.

A	H	start	peak	end	length
9	8	139	180	221	82
9	10	125	162	199	74
9	12	112	147	182	70
13	8	129	160	191	62
13	10	119	149	179	60
13	12	110	139	168	58
17	8	123	149	175	52
17	10	116	141	166	50
17	12	109	134	159	50

Table 2: Dates (in ordinal days) of the caterpillar windows in Chapter 2 figure 1.

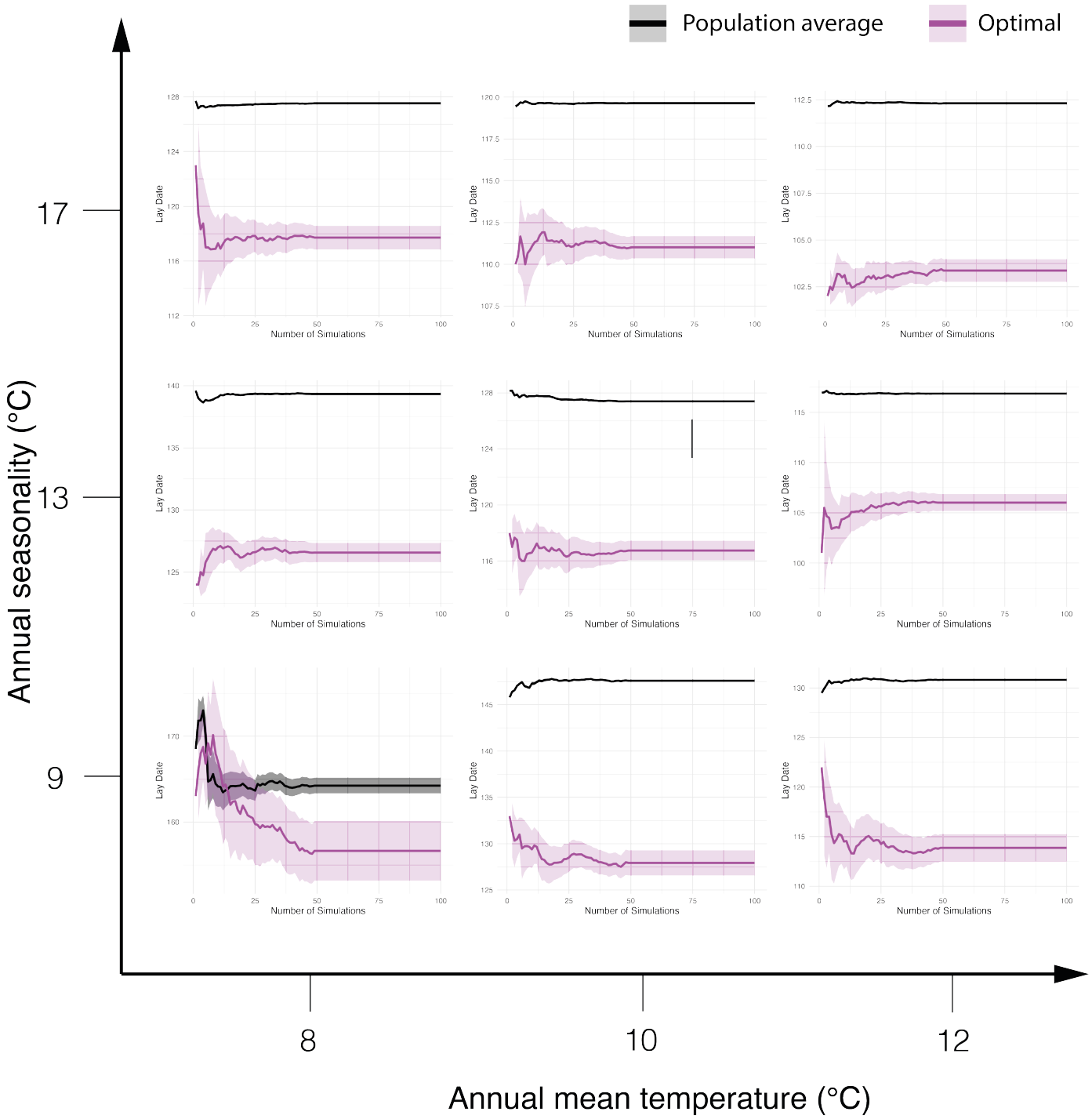


Figure 3: Graphs showing how population average and optimal lay date estimates stabilise after 50 realisations in each environment. Lines show the mean and shaded areas denote the 95% confidence intervals.

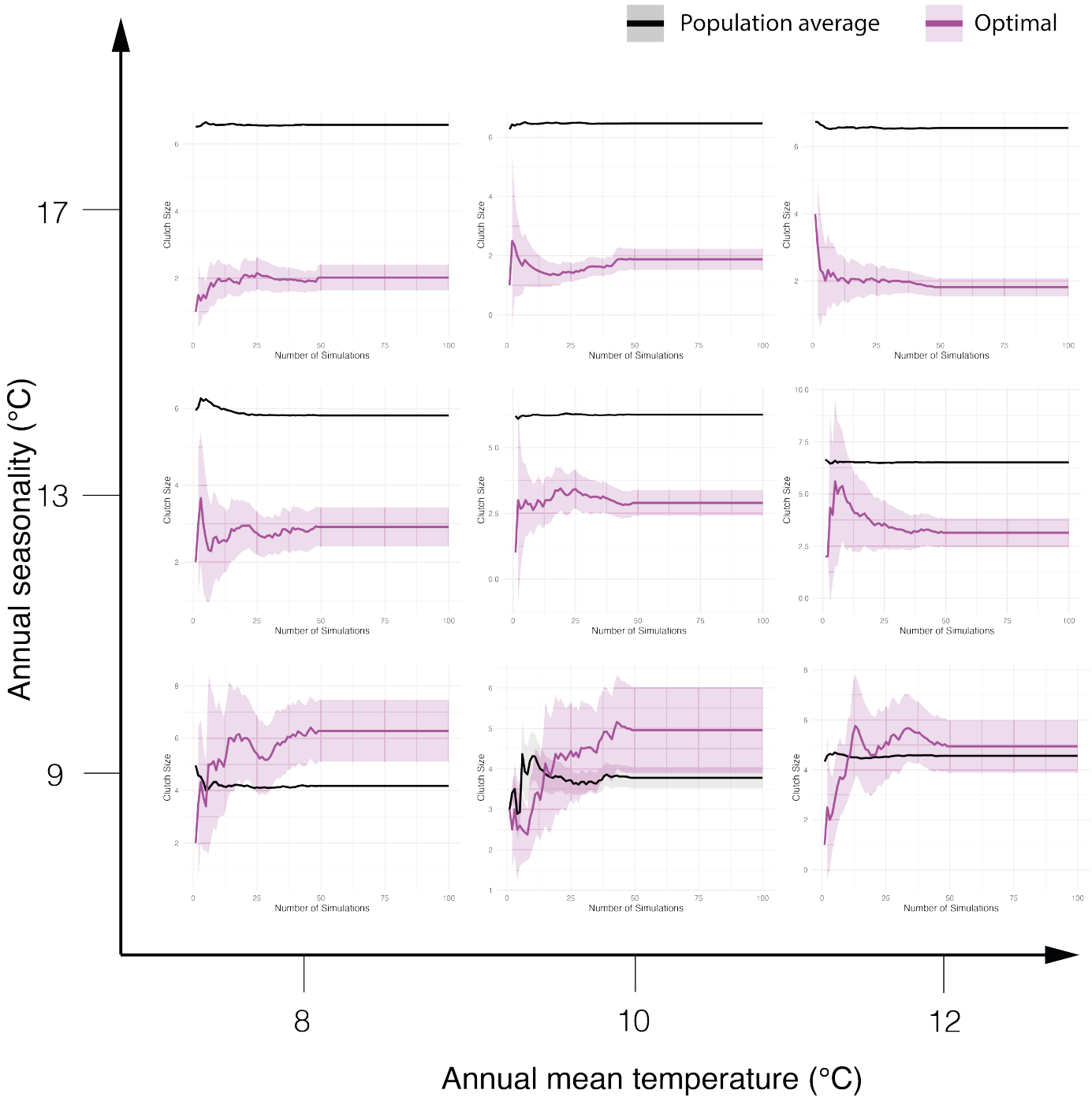


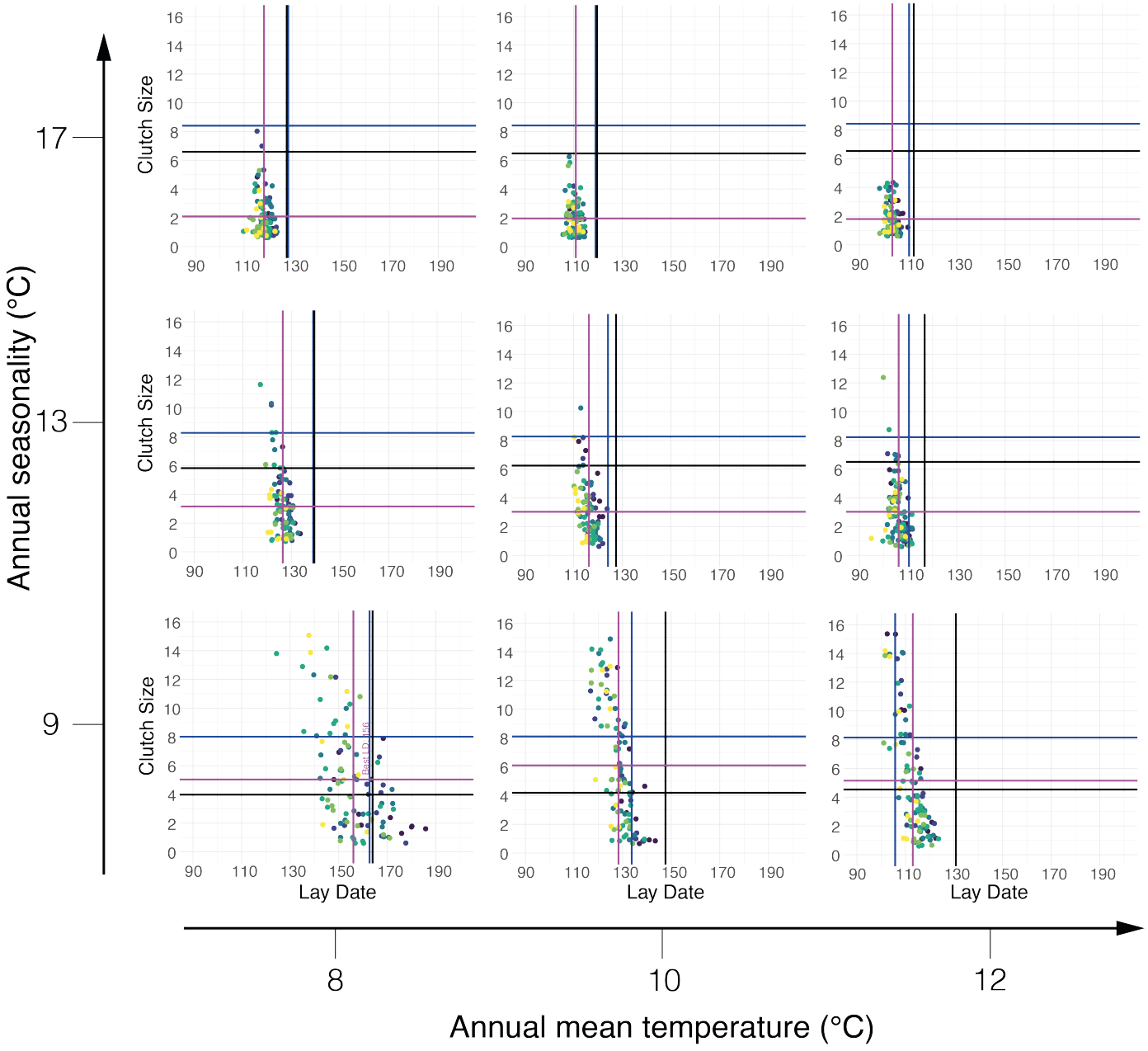
Figure 4: Graphs showing how population average (black) and optimal (magenta) clutch size estimates stabilise after 50 realisations in each environment. Lines show the mean and shaded areas denote the 95% confidence intervals.

A	H	lay date (population average)	lay date (optimal)	clutch size (population average)	clutch size (optimal)
9	8	164	156	4.0	5.0
9	10	148	128	4.2	6.1
9	12	131	113	4.5	5.2
13	8	139	126	5.8	3.2
13	10	127	116	6.2	3.0
13	12	117	106	6.5	3.0
17	8	128	118	6.6	2.1
17	10	120	111	6.5	2.0
17	12	112	104	6.5	1.8

Table 5: Summary table relating to Chapter 2 figure 4a showing how different environmental scenarios (annual seasonality A and mean H) select for different population average and optimal mean lay dates and clutch sizes, plotted as black and pink lines in Chapter 2 figure 4a, respectively.

variable	trait	comparison	difference	lower CI	upper CI	p-value (Tukey's)	p-value (ANOVA)
SD	CS	a13-a9	-1.90	-2.47	-1.33	0.000128	1.50E-05
SD	CS	a17-a9	-2.81	-3.39	-2.24	0.000013	1.50E-05
SD	CS	a17-a13	-0.91	-1.49	-0.34	0.006455	1.50E-05
SD	CS	h10-h8	0.02	-3.62	3.66	0.999894	0.996
SD	CS	h12-h8	-0.09	-3.72	3.55	0.997138	0.996
SD	CS	h12-h10	-0.10	-3.74	3.54	0.995933	0.996
SD	LD	a13-a9	-4.31	-9.30	0.68	0.084495	4.30E-02
SD	LD	a17-a9	-5.01	-10.00	-0.02	0.049329	4.30E-02
SD	LD	a17-a13	-0.70	-5.69	4.29	0.904458	4.30E-02
SD	LD	h10-h8	-2.12	-9.92	5.68	0.697384	0.629
SD	LD	h12-h8	-2.28	-10.08	5.52	0.661349	0.629
SD	LD	h12-h10	-0.16	-7.96	7.64	0.997785	0.629
difference	CS	a13-a9	-4.31	-5.45	-3.17	0.000060	1.12E-05
difference	CS	a17-a9	-5.78	-6.91	-4.64	0.000011	1.12E-05
difference	CS	a17-a13	-1.46	-2.60	-0.32	0.017896	1.12E-05
difference	CS	h10-h8	0.09	-7.47	7.66	0.999217	0.968
difference	CS	h12-h8	-0.50	-8.06	7.07	0.977968	0.968
difference	CS	h12-h10	-0.59	-8.16	6.98	0.969135	0.968
difference	LD	a13-a9	3.41	-5.73	12.55	0.524553	0.207
difference	LD	a17-a9	6.05	-3.09	15.20	0.185402	0.207
difference	LD	a17-a13	2.64	-6.50	11.79	0.667212	0.207
difference	LD	h10-h8	-3.01	-14.21	8.19	0.703121	0.699
difference	LD	h12-h8	-2.41	-13.61	8.79	0.793575	0.699
difference	LD	h12-h10	0.60	-10.61	11.80	0.985425	0.699

Table 6: Summary statistics relating to Chapter 2 figure 4 comparing the standard deviation (SD) in optimal clutch sizes (CS) and lay dates (LD) (figure 4b) as well as the difference between optimal and population averages (figure 4c) across the different values of annual temperature mean (comparison:h) and seasonality (comparison:a), listing the mean difference, lower and upper 95% confidence intervals, as well as the p-values estimated for each pairwise comparison from Tukey's post-hoc tests, as well as the overall one-way ANOVA estimated p-values.



A	H	LD (all)	CS (all)	LD (successful)	CS (successful)	LD (optimal)	CS (optimal)
9	8	163	8.0	164	4.0	156	5.0
9	10	134	8.1	148	4.2	128	6.1
9	12	106	8.1	131	4.5	113	5.2
13	8	139	8.3	139	5.8	126	3.2
13	10	124	8.3	127	6.2	116	3.0
13	12	110	8.2	117	6.5	106	3.0
17	8	128	8.4	128	6.6	118	2.1
17	10	119	8.4	120	6.5	111	2.0
17	12	110	8.4	112	6.5	104	1.8

Figure 7: Summary plots illustrating the distribution of optimal strategies across environmental scenarios. Scatterplots show the optimal strategies coloured according to fitness (= number fledglings x mean number of caterpillars eaten) broken down into percentiles. Horizontal lines show the mean clutch sizes (CS), while vertical lines show the mean lay dates (LD) for all breeding attempts (in blue), for successful breeding attempts (that hatched young, in black) and the optimal strategies (in magenta). The exact values of these are listed in the table below.

A	H	mean number of successful breeders	mean number of fledglings
9	8	3.63	14.36
9	10	35.76	120.26
9	12	68.37	206.31
13	8	111.46	319.91
13	10	146.87	390.18
13	12	167.60	426.70
17	8	172.78	402.72
17	10	175.58	391.49
17	12	186.86	418.99

Table 8: Table summarising the mean (across 100 realisations) number of successful (with hatched young) breeders and the mean number of fledglings under each environmental scenario (temperature A: seasonality, H: annual mean)

Chapter 3

featheRscan: a field method and R package for collecting feather quality metrics



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ABSTRACT

Feathers play an essential role in all aspects of avian performance, from physiology to reproduction and fitness. However, research focused on understanding the drivers and consequences of variation in feather quality has been slowed by limited data collection opportunities due to ethical considerations, as well as time-consuming manual data processing techniques. Here we present protocol and best practices of a novel, non-invasive and time-efficient data collection technique to extract feather structural quality information from photographs of feathers taken in vivo using an affordable portable digital microscope. We also introduce `featheRscan`, an R package that provides automated processing of feather images to derive commonly used metrics of feather quality such as rachis and barb width, as well as barb and barbule densities. Using a dataset of c. 1500 images from 500 individual birds of 4 species (blue tit *Cyanistes caeruleus*, great tit *Parus major*, zebra finch *Taeniopygia guttata* and mute swan *Cygnus olor*), we demonstrate that our protocol can be applied effectively to different species of varying sizes and colouration. We also show example uses of this approach by describing some general demographic patterns across our study species, as well as exploring the potential for feather quality to mediate life-history trade-offs. We show that `featheRscan` is able to retrieve feather quality information from images of various species, and argue that using it in combination with a digital microscope in the field greatly improves affordability and efficiency of both data collection and analysis, as well as enabling an overall more ethical research practice.

1 | INTRODUCTION

Feathers fulfil several essential functions, including social communication, protection, insulation, and flight Jenni and Winkler (2020a). The different structural and visual properties of the plumage can be a key determinant of how well these roles are performed, potentially impacting an individual's fitness. There is evidence that feather properties are determined by intrinsic (e.g., genetic Gienapp and Merilä (2010)) and extrinsic factors (e.g., temperature Broggi et al. (2011), Osváth et al. (2018)) during their growth and renewal (moult), potentially creating variation both within a bird's lifetime as well as between individuals. This variation has been shown to influence key fitness-related traits involved in reproduction and survival Bortolotti et al. (2002), and can therefore be used as an indicator of individual quality in birds.

The majority of research on individual variation in feather properties so far has mainly focused on their visual characteristics (e.g., colouration Berzins and Dawson (2019), Charmantier et al. (2017), García-Navas et al. (2012)), leaving the variation in their structural properties rather unexplored (but see Gamero et al. (2015), Vágási et al. (2012)). This may in part be due to the availability of standardised data collection protocols and analysis techniques, including established specialised software (e.g. 'Avicol': Gomez (2006), 'pavo': Maia et al. (2019)) to facilitate data processing.

Studies characterising plumage structural quality have traditionally used plucking to obtain samples (Vágási et al. (2012)). Feather removal does not only require ethical justification, especially with regards to more critical feather types (eg. flight feathers), but may itself hinder investigation into certain types of research questions, such as drawing direct links between feather structure and future performance. Subsequent feather analysis usually involves manual counting of structures either directly under a microscope or from images taken with a camera attachment, which is still labour-intensive and time-consuming.

Though popular image analysis tools exist, these have been mostly developed for very specialised applications within the biomedical fields primarily (e.g. 'EBImage'; Pau et al. (2010)). Some of the more general image processing libraries have been written in languages such as Python and C+, whereas most ecological research is carried out using R (though some advancements have been made to adapt them for R language users, eg. 'Rvision'; Garnier and Muschelli (2024)). This means that research on feather structure currently lacks the tools to easily obtain and analyse large amounts of data in a time-efficient manner.

Here, we present a novel research framework encompassing both a data collection protocol and an analysis tool to facilitate research on feather macrostructure. We advocate the use of an inexpensive portable digital microscope and an automated image analysis pipeline, offering a non-invasive and time-efficient approach to enable research into a wide range of questions around the ecology and evolution of feathers. We establish best practices for obtaining optimal photos for image analysis and describe the key functions of `featheRscan`, as well as drawing attention to its limitations. As an example, we explore key demographic correlates of variation in plumage structure and test its potential role as a mediator of life-history trade-offs in birds, using a dataset of c. 1500 feather images obtained from c. 500 individuals of four species.

2 | DESCRIPTION OF THE RESEARCH FRAMEWORK

2.1 | Image collection

The following protocol provides a novel technique for collecting feather macrostructure information from individual birds, without the need for feather plucking. We used a digital microscope (model AM7915MZT) and stand (RK-06A) by Dino-Lite, fitted with a 5MP camera allowing magnification of up to x200 and a cross-polarisation dial to reduce glare. The microscope is powered from a laptop via USB connection and live feedback of the image as well as image capture are controlled using the DinoXcope 2.4 software for Mac (or DinoCapture 2.0 for Windows).

Prior to commencing any data collection, it is important to set up a table of calibration profiles to obtain the correct scale at the desired magnifications (ie. the number of pixels that equate to a fixed distance at the different magnification levels that will be used during data collection). This can be achieved through the DinoScope 'Calibration Manager' or other image processing software like Fiji (Schindelin et al. (2012)). Depending on the feathers being imaged, we recommend covering the background surface with a contrasting colour to aid later detection of the feather structures (eg. covering the stand base with a white sheet of paper for dark feather structures). An individual bird is then held in the ringer's hold position (where appropriate) with its feather stretched out flat against the base of the microscope stand for image capture.

For the purposes of visualising smaller feather mactrostructural elements (figure 3.1), such as the barbules, magnification should be set to x100. We suggest taking images of multiple feathers per individual to estimate repeatability, as well as taking replicate photos of each feather to avoid motion blur.

To use the feather structure detection functions of `featheRscan`, it is crucial that each picture exhibits the following features; (1) only a single feather is visible, (2) the image is in focus with low to moderate glare and (3) there are no other objects in view around or on top of the feather.

2.2 | Image analysis

2.2.1 | Dependencies

`featheRscan` was written in R 4.4.0 (R Core Team (2024)), and utilises image processing functions primarily from the 'imager' (v1.0.1, Barthelme (2024)) and 'Rvision' (v0.8.0, Garnier and Muschelli (2024)) libraries, which are loaded automatically during installation together with other external packages.

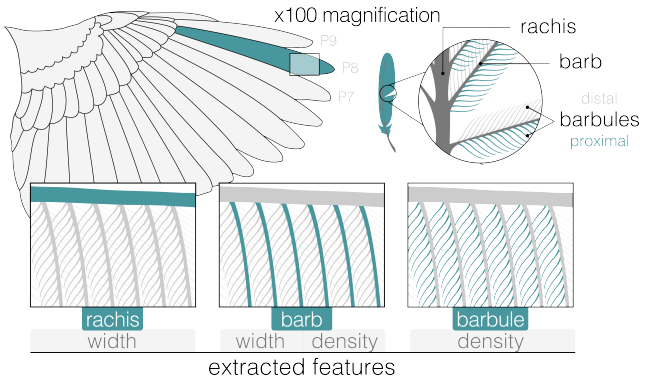


FIGURE 3.1 Schematic of the feather structural elements visible at x100 magnification and the feather quality features that can be extracted using `featheRscan`.

2.2.2 | Package description

Photographs of feathers can be analysed with the `featheRscan` R package introduced here, which provides an automated process for extracting key macrostructure metrics commonly used to describe feather quality, such as rachis and barb width, as well as barb and barbule density, using the following core functions summarised in figure 3.2 :

- `transform_image()`: Pre-processes the image to aid subsequent detection of key features, using contrast enhancement and adaptive thresholding. (figure 3.2:1)
- `find_rachis()`: If present, finds the location of the rachis, returns the coefficients of the best-fit line through its central axis and calculates rachis width (in pixels and mm). (figure 3.2:2)
- `get_orientation()`: If the rachis is not present, finds the orientation of the feather in the image and returns the coefficients of the longest perpendicular line crossing the barbs, subsequently used for cropping the image. (figure 3.2:2*)
- `crop_image()`: Crops the image at a specified crop_dist distance away from the rachis, if present (figure 3.2:3), otherwise crops the image at the perpendicular line returned by the `get_orientation()` function. (figure 3.2:3*)
- `count_barbs()`: Finds the coordinates of the barbs along the top of the cropped image (figure 3.2:4a), from which it calculates barb density (no. of barbs / mm of rachis). It further extracts barb widths (in pixels and mm) and fits a polynomial curve through each barb (figure 3.2:4b), subsequently used by the `count_barbules()` function.
- `count_barbules()`: Counts barbules along each barb on the proximal side (figure 3.1) within sections parallel to the central axis curves returned by `count_barbs()` function, subsequently calculating barbule density (no. of barbules / mm of barb).

`featheRscan` also provides utility functions for setting up directories, batch processing images and writing output files for future analysis. Users are presented some built-in options to optimise the analysis of their images and extract feather quality features for their specific needs. These include (1) specifying the feather's colour (ie. dark or light), (2) defining the cropping distance relative to the position of the rachis, and (3) choosing to output analysed images with the detected features highlighted for subsequent data quality control.

At its current stage, running `featheRscan` with its full functional repertoire (figure 3.2), including extraction of all four key features (figure 3.1) and saving of analysed images, takes around 2 minutes / image (on an 8GB MacBook Air), providing a considerably faster and hands-off alternative compared to manual measurement techniques. This processing speed can be further enhanced through parallelization techniques and/or higher computational power.

2.2.3 | Limitations and alternatives

The fully automated pipeline does not yet have inbuilt filtering to remove erroneous measurements without the need for manual checking of the analysed image outputs. Common issues that lead to erroneous measurements are highlighted in figure 3.3. In particular, rachis width estimates rely on detecting the largest continuous dark region in the image, however, light reflections and shadows can sometimes hinder the accurate delineation of this region correctly. Using the polarisation filter on the Dino-Lite microscope should minimise the occurrence of this, as well as testing techniques, such as gamma correction, could reduce glare and lighten dark shadows in the image. Similarly, uneven lighting or blur in the image can hinder accurate barb detection, as this step relies on barb pixels forming clear peaks in intensity (i.e. being darker than other regions). As before, image polarisation helps keep an even lighting across the image, however, testing different local contrast enhancement techniques could further improve barb detection. For feathers without a rachis, improvements for a reliable orientation detection method are needed, using edge detection and Hough transformation techniques to identify the orientation of the most prominent lines (i.e. barbs) in the image. A simpler solution is to ensure that feathers without a rachis are aligned to the top horizontal line of the image frame, such that this already forms the perpendicular line for barb detection.

Inevitably, feathers can show signs of wear or have dirt particles on them, which can disrupt accurate counting of barbules. As barbules are counted parallel to all barbs (of at least 1 mm length) in the image, excluding erroneous barbule density values is unlikely to significantly affect the overall feather-level average estimate. If the normal range of barbule densities for the species in question in known, this information can be used to simply filter out erroneous counts post-automation. However, a more general solution is needed to identify and remove erroneous estimates within the automated analysis itself. For instance, calculating Laplacian variances could help identify if the image region is out of focus, and estimating average distances between barbules (i.e. peaks in pixel intensity) could be used to indicate regions with damage or dirt shown as irregularities in spacing.

However, a semi-automated approach provides a good alternative that is still faster than the fully manual counting of individual barbules.

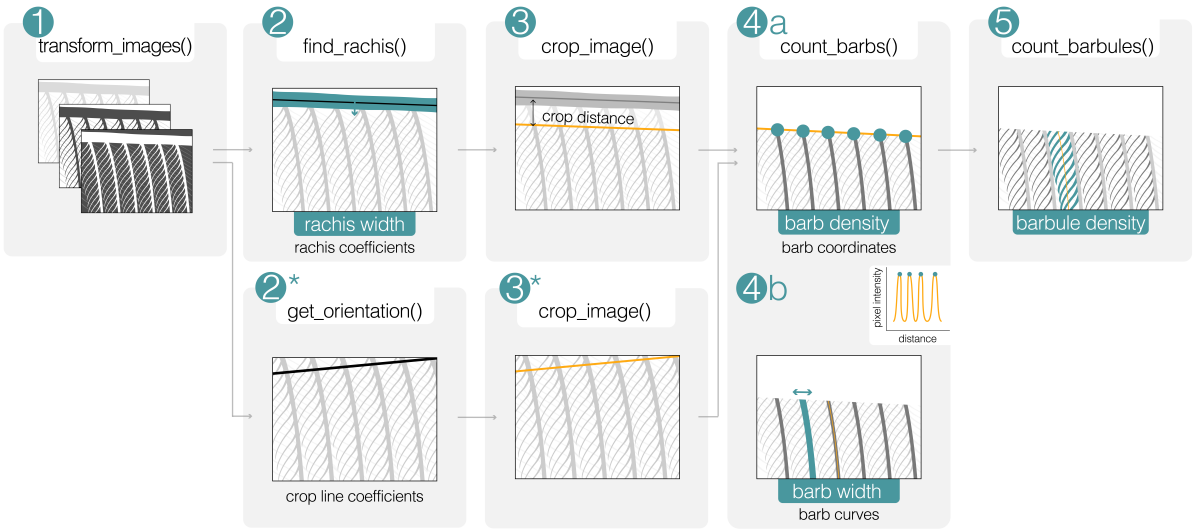


FIGURE 3.2 Steps of the image analysis pipeline illustrating the output (highlighted in teal) of each function call (top of each panel). Asterisks mark steps or adjustments to functions only implemented in the case of no rachis present in the image. Steps (a) and (b) of panel 4 are both carried out within the `count_barbs()` function. An image is first (1) transformed (e.g. grayscale, contrast enhance), followed by (2) locating the rachis to measure its width, or (*) drawing a line perpendicular to the barbs. (3) The image is cropped at a specified distance from the rachis, or (*) along the perpendicular line. Along this crop line, (4a) the locations of the barbs are identified as peaks in pixel intensity, and counted to give the barb density estimate, as well as (4b) measuring their individual widths. (5) Proximal barbules are counted as peaks in pixel intensity parallel to each barb to estimate barbule density.

The cropped images returned by the `crop_image()` function can be used to manually draw the lines (in software like Fiji; Schindelin et al. (2012)) over which to count barbules. By exporting the pixel intensity profiles of each line and importing these into `featheRscan`, the `count_barbules()` function can now identify barbules from regions unobstructed by feather damage or irregularities, essentially skipping step 3 of the automation pipeline (figure 3.2). To evaluate the accuracy and consistency of barbule density estimates obtained from this semi-automated approach, we analysed a subset of 30 images of zebra finches (*Taeniopygia guttata*) that did not display any major irregularities (which would lead to erroneous counts, as detailed above). Comparing a fully automated, a semi-automated, as well as a manual counting approach, we found that all pairwise combinations of results form these techniques showed moderately strong correlations (with Pearson's $r \geq 0.78$), most notably for the correlation between manual and semi-automated counts ($r = 0.88$, appendix B1). As estimating barbule densities is downstream of all other functions in the automation, issues with barbule densities often co-occur with erroneously measured features upstream, therefore, we only focused on validating this feather structure feature for now.

`featheRscan` was developed using flight feathers, primarily of the wing, with a prominent central rachis and neatly arranged rigid barbs and barbules, and is therefore optimised for detecting structural elements in feathers that exhibit these characteristics.

3 | IMPLEMENTATION OF THE RESEARCH FRAMEWORK

The following examples illustrate how large amounts of data on feather macrostructure can be collected in the field and analysed in a time-efficient manner using the above described combination of digital microscope images analysed with `featheRscan`.

3.1 | Example 1: Demographic patterns in feather structure across species

3.1.1 | Introduction

Feathers fulfill many key functions, including flight, insulation, water repellency, and social communication (Jenni and Winkler (2020a)). They can vary in morphology between species due to differences in size (Worcester (1996)). Feathers can also vary within species depending on the developmental stage (Moreno and Soler (2011)), such as changing from the fluffy plumage nestlings into adult-type feathers that provide flight and protection. Many species also show sexual dimorphism in plumage colour (Roeder et al. (2019)). Although a lot of research has focused on individual variation in feather colouration, few have investigated how the structural makeup might differ between key demographic parameters.

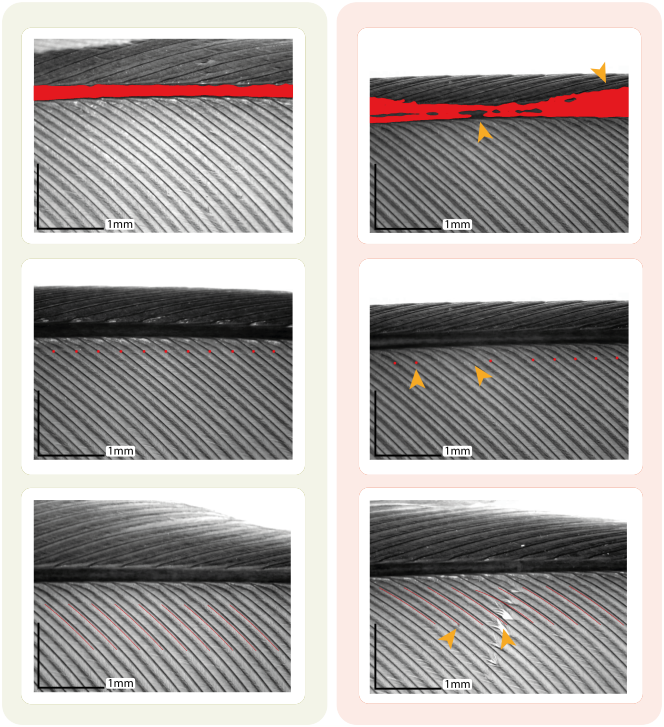


FIGURE 3.3 Images of zebra finch primary feathers at x100 magnification, showing examples where the automation occurred correctly (left) and where irregularities in the feather caused erroneous measurements (right). Extracted features are shown in red, with top panels measuring barb width, middle row counting barb density, and bottom panels estimating barbule density. Orange arrows highlight the most commonly encountered issues, such as glare on the rachis (top: this is the same image as below it), uneven lighting (middle), and damage or dust particles in the feather structure (bottom).

3.1.2 | Methods

To study the links between feather quality and key demographic parameters such as age and sex, we obtained c. 1500 images of three primary feathers (P7, P8, P9, figure 3.1) from four species; with records (including recaptures) of 252 blue tits (*Cyanistes caeruleus*), 64 great tits (*Parus major*), 160 zebra finches (*Taeniopygia guttata*) and 30 mute swans (*Cygnus olor*). Wild blue and great tits were captured during the non-breeding seasons (July - March) between 2021-2023, using mist netting techniques (Karr (1981)), their age and sex identified by trained bird ringers from plumage colouration and through breeding and natal information provided by the long-term monitoring of nest boxes at Wytham Woods, Oxford (UK) by the Wytham Tit Project (since 1947, Lack (1964)). Captive zebra finches were imaged in March of 2023 and 2024, housed at University of Groningen (NL) as part of a long-term foraging cost manipulation study (Koetsier and Verhulst (2011)) providing information on their exact birth dates and sexes. Wild mute swans were captured in September of 2023 at Abbotsbury Swannery in Dorset, England (UK) as part of ongoing monitoring and conservation efforts (Perrins and Ogilvie (1981)), with birth records kept of locally hatched birds allowing us to obtain age and sex information. All four species undergo annual complete post-breeding moults, resulting in a new set of feathers every year (Jenni and Winkler (2020a), Newton (2009)).

We used `featheRscan` to extract four key feather quality metrics from each image; rachis width (mm), barb width (mm), barb density (no. of barbs / mm) and barbule density (no. of barbules / mm) measured 0.43 mm away from the rachis (i.e. crop_dist = 0.43, following protocol by Broggi et al. (2011)). Because mute swan feathers lacked a central rachis and had inconsistent orientations, we could only estimate barbule densities in a semi-automated approach in this dataset. Moreover, we measured semi-automated barbule densities for blue tits and zebra finches to serve as comparison against the automated counts.

To explore associations between feather quality and the selected demographic traits, we fitted linear mixed models in R (v4.4.0, R Core Team (2024)), using the 'lme4' package (v1.1.35.3, Bates et al. (2015)). We ran simple additive models separately for each species with *feather structural element* as response, and *sex* and *age* included as fixed predictors, estimating mean and 95% confidence intervals. In models with swan and zebra finch feathers, we included *age* as a continuous variable, as we knew the exact age in years for these birds. In models with blue tit and great tit feathers, we included *age* as a categorical variable grouped by juveniles (i.e. independent birds less than a year old) and adults (older than one year). For all species, we also included year as a fixed effect (included as a fixed term instead of random because estimating variance components with only two levels is unreliable, **?**), except in swans where we only had one data collection event. Additionally, as zebra finches had been under long-term foraging cost manipulations (Koetsier and Verhulst (2011)), we included *treatment* as a fixed covariate. In models with barb width or barbule density, we included random terms of *image ID* nested within *bird ID* to account for repeated measures of these variables within images. For rachis width and barb density, we only included *bird ID* as random effect.

3.1.3 | Results and Discussion

There were apparent differences in the average values of each feather quality metric between the four species. In general, both the width and density of feather structural elements tended to increase with species size (blue tit < great tit < zebra finch < mute swan), except in the case of barb widths which were considerably lower in zebra finches (mean of BT: 32.45, GT: 32.78, ZF: 26.86 x 10^{-3} mm). We also observed sex differences in most features, with males exhibiting feathers with a wider rachis in great tits (mean = 31.76 x 10^{-3} mm, p = 0.0516), wider barbs across all species, as well as a higher density of barbs in great tits (mean = 0.31 no/mm, p = 0.0341), and a higher density of barbules in zebra finches (mean = 1.00, p = 0.100). Furthermore, we found consistently negative relationships with age regarding barbule density across all species, using both automated and semi-automated methods, although the magnitude of these effects tended to be smaller when using the latter approach.

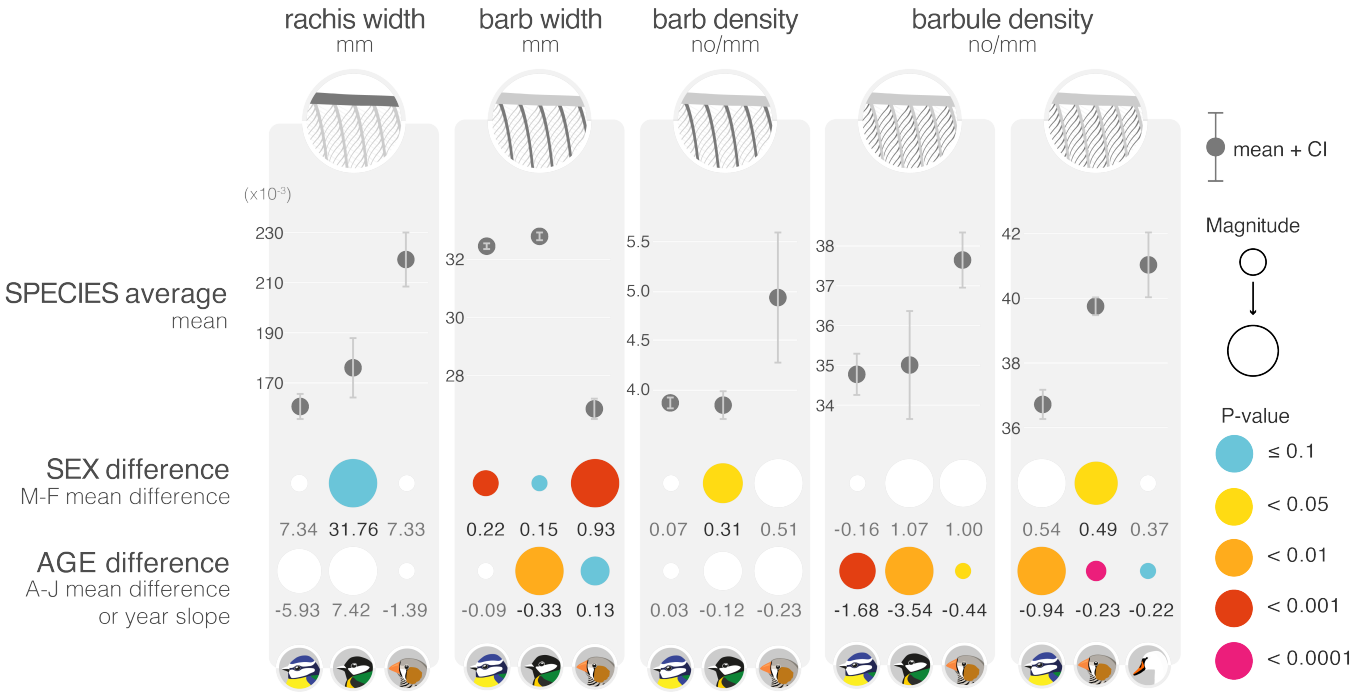


FIGURE 3.4 Summary of the relationships between variation in feather structural elements and key demographic variables. Top row compares the mean and 95% confidence interval estimates of the species-level average values. The bottom two rows show sex and age differences, with point size indicating the relative magnitude (z-scored within feather metric and demographic variable to allow comparison between species), and the mean difference written below. Sex values are shown as the mean difference between males (M) relative to females (F). For blue tits (BT) and great tits (GT), mean age differences are shown in terms of adults (>1 year, A) relative to juveniles (<1, J). For zebra finches (ZF) and mute swans (MS), the coefficient for age corresponds to the slope of age in years as a continuous predictor. The number of individual-level observations with both age and sex recorded are as follows: BT = 135 (auto), 57 (semi-auto), GT = 36 (auto), ZF = 155 (auto), 160 (semi-auto), MS = 30 (semi-auto). Rachis width, barb width, and barb density are counted with an automated approach. The left panel of barbule density is counted using an automated approach, while the right panel is quantified using a semi-automated approach.

3.2 | Example 2: Feather quality as potential mediator of life-history trade-offs

3.2.1 | Introduction

Optimal life-history traits are shaped by trade-offs due to energy and/or time constraints, for instance between the quantity and quality of offspring, or between current and future reproduction (Stearns (1989)). However, the mechanisms underlying these trade-offs are not well known. As plumage production is costly, both in terms of time and energy, it provides a good candidate mechanism potentially linking competing life-history traits. Indeed, feather quality has been shown to be influenced by diet composition (Pap et al. (2008)) as well as moult duration (Dawson et al. (2000)), demonstrating both its energy- and time-demanding nature, respectively. As such, variation in feather quality might explain how the survival and reproductive consequences of life- history trade-offs arise. Therefore, we explored whether key feather quality metrics (rachis width, barb width, barb density, barbule density) vary with:

1. reproductive effort (i.e. clutch size) of the parents in yearlings; testing the offspring quantity vs quality trade-off, and
2. timing of breeding (i.e. first egg date) in adults; testing a current vs future reproduction trade-off.

Given the high energetic demands of feather growth, we hypothesised that (1) juvenile birds hatched in larger broods would face higher competition for resources and therefore might develop feathers of lower quality in terms of fewer and thinner structural elements. With regards to adult birds, we hypothesised that (2) reproducing later in the season would limit the time available for the completion of moult, thereby forcing individuals to increase feather growth rate, which may negatively impact the structural quality of newly moulted feathers.

3.2.2 | Methods

During the non-breeding seasons (i.e. July - March) of 2021, 2022 and 2023, we captured 140 blue tits at Wytham Woods, Oxford, using mist netting techniques (Karr (1981)), and photographed three primary

feathers of their right wing (P7, P8, P9; figure 3.1). 112 of these individuals were yearlings that had been born in nest boxes monitored by the Wytham Tit Project (Lack (1964)), allowing us to obtain information about the clutch size (number of eggs laid) in their natal brood. The remaining 28 birds photographed were older than a year and had been observed breeding in monitored nest boxes, allowing us to estimate first egg dates directly preceding the current moult season. In this species, individuals in their first year of life complete a partial post-juvenile moult over the summer, replacing their juvenile-type contour feathers on the body and some parts of the wing, but retaining most of their remiges (flight feathers) that were grown in the nest, including the primaries imaged here, until their first post-breeding moult (Jenni and Winkler (2020b)). This means that adult birds older than one year undergo an annual complete post-breeding moult, during which they replace all their feathers including the primaries (Jenni and Winkler (2020b)).

We analysed the collected images with `featheRscan` to extract four key feather quality metrics; rachis width (mm), barb width (mm), barb density (no. of barbs / mm) and barbule density (no. of barbules / mm) measured 0.43 mm away from the rachis (i.e. crop_dist = 0.43, following protocol by Broggi et al. (2011)). Additionally, we analysed a subset of feather samples to estimate barbule densities using a semi-automated approach for comparison. To explore the relationships between feather quality metrics and the aforementioned life-history traits, we fitted linear mixed models in R using the 'lme4' package. We modelled adult and yearling birds separately to estimate mean and 95% confidence interval (CI) estimates for the slopes of (1) yearling feather metrics regressed on natal clutch size and (2) adult feather metrics regressed on lay date. For models where the response variable was rachis or barb width, we included *bird ID* as random term to account for the three repeated measures obtained from three feathers per bird. Whereas in models where the response variable was barb or barbule density, we included *image ID* nested within *bird ID* as random effects to control for pseudoreplication of repeat measures within images of the same individual. As fixed effects, we included in model (1) the natal clutch size (laid by the parent) of each yearling bird, and in model (2) the date of first egg (in ordinal days) laid by each adult during the breeding season preceding image collection. To allow comparison of the effect size between feather metrics, we calculated the percent change as the slope coefficient divided by the intercept (x100).

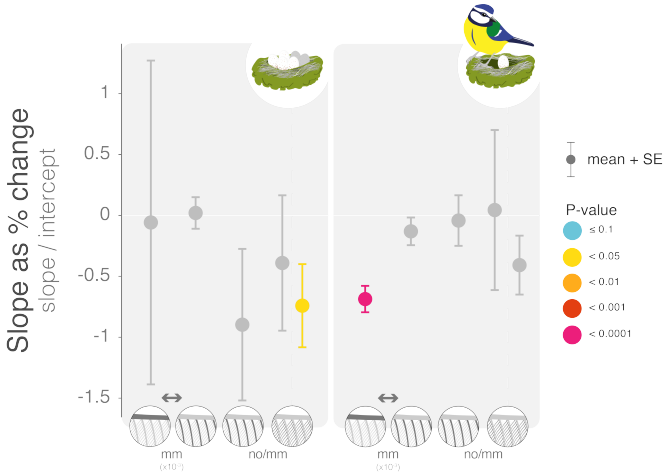


FIGURE 3.5 Summary of the relationships between variation in feather structural elements (in order from left: rachis width, barb width, barb density, barbule density) and the life-history traits of natal clutch size in yearlings (<1 year old, left) and lay date in adults (>1 year, right). Results from automated analyses are shown to the left of the dashed line, and semi-automated estimates on the right. The response estimates show the percentage change in mean slope values relative to the overall mean (i.e. intercept), allowing us to compare the relative magnitude of effects between feather metrics. Points denote the mean change and error bars show the standard errors. The number of yearlings with information about natal clutch size were 112 (auto) and 27 (semi-auto), and the number of adults with lay date information were 28 (auto) and 12 (semi-auto). Rachis width, barb width, and barb density are counted with an automated approach. Barbule density is counted using both an automated approach (to the left of the white dashed line), and a semi-automated approach (to the right of the white dashed line).

3.2.3 | Results and discussion

When comparing the number of offspring in a nest with their individual feather qualities, we found a negative relationship with barbule density using the semi-automated method, whereby birds that fledged from a larger clutch size grew lower barbule density feathers (figure3.5a). This translates to a -0.7% reduction in barbule density relative to the overall mean, or a decrease of 0.29 barbules / mm with every extra egg laid by the parent (p = 0.0397). This could be explained by chicks receiving a smaller share of resources for feather growth when they have more siblings. On the other hand, we found no associations between natal clutch size and juvenile feathers in terms of the other extracted quality metrics, potentially due to selective pressures maintaining the structural integrity of the feather by keeping the rachis and barbs at an optimal baseline. However, many of the features also exhibited larger variance estimates likely due to the inclusion of erroneous counts in the automated method (as described earlier).

Similarly, we found that adult birds commencing egg laying later in spring tended to (though not significantly so) develop feathers with lower barbule density estimates in the following autumn (figure3.5b). This meant an average of -0.4% reduction in barbule density relative to the overall mean, or a decrease of 0.16 barbules / mm with every day of delay in breeding (p = 0.123). Contrary to our findings in juveniles, we found that rachis width also decreased in association with later breeding, on average by -0.7% compared to the mean ($p = 1.33 \times 10^{-9}$). Interestingly, automated estimates showed less variation in adults than in juveniles. Overall, these patterns might indicate a trade-off between the two costly activities of reproduction and moult, whereby late breeding individuals might have limited time / energy to complete their feather growth before resource availability deteriorates in autumn. However,

we need to validate the accuracy of all automated feather metrics before drawing any conclusions from either life history comparison.

4 | DISCUSSION AND FUTURE DIRECTIONS

4.1 | Advantages of adopting this research framework

We have demonstrated the ease and efficiency of collecting extensive feather quality data using an affordable digital microscope setup, which facilitates in vivo measurements in the field for a more ethical approach to studying the structural properties of feathers. Additionally, we introduced the `featheRscan` package, an automated pipeline for extracting key feather quality features commonly used in this field. Our proposed framework should enable researchers to collect and process large amounts of feather data with a significantly faster turnaround compared to manual methods. Our examples demonstrate the potential for this method to work across species with diverse sizes and feather colourations. We also show how the feather structural features extracted by `featheRscan` could provide biologically relevant measures of individual quality associated with key demographic and life-history traits.

4.2 | Future applications: feather structure needs more research

Despite some of the associations shown here, there remains much to be uncovered about the mechanistic link between the genetic, ecological and physiological drivers and consequences of variation in feather structural composition. For instance, disentangling the relative contributions of genetic and environmental determinants of feather production could shed light on the adaptability of this trait to both periodic and directional changes in the environment. In particular, responses to elevated heat stress, experienced during nestling growth or moult, and higher frequency of extreme climatic events, especially sudden temperature drops in the winter in temperate regions, are of key importance when predicting population survival trends under expected climate projections. Although there is some evidence that certain feather structures may be selected for in different habitats (Broggi et al. (2011), Sándor et al. (2021)), we still do not yet have a clear understanding of the level of plasticity in these traits to respond to ecological pressures over shorter timescales. We also do not yet fully understand the direct consequences of variation in feather quality metrics. For instance, the extent to which conditions in early development determine performance later in life, such as illustrated in our example of offspring quantity and quality trade-off dynamics. With regards to temperature responses, we need to clearly define how feather structural elements contribute to thermoregulation, and what the downstream survival impacts may be.

CONCLUSION

Given the potential for fast collection and analysis of large amounts of data, combined with the array of unanswered questions in this important avian trait, we hope that others may in future adopt our research framework presented here. However, further work is needed to refine the functions of `featheRscan`, especially with regards to filtering out erroneous estimates, before we can rely on a fully automated approach. We developed `featheRscan` to be accessible and easy to implement, with clear documentation to allow (and indeed encourage) anyone to contribute to its improvement once published open-source.

AUTHOR CONTRIBUTIONS

A.K. conceived and planned the project with the help and supervision of C.R., E.C. and B.S. A.K. collected the data under the guidance of B.S. regarding the population of great and blue tits, S.V. regarding the population of zebra finches, and C.P. regarding the population of mute swans. S.V. helped catch zebra finches and C.P. helped catch mute swans for feather imaging. A.K. wrote and tested the code. A.K. analysed the example data, and all authors contributed to the interpretation of these results. A.K. created illustrations and wrote the chapter, with feedback from B.S.

ACKNOWLEDGEMENTS

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ETHICAL NOTE

All activities involving bird handling and experimentation were conducted by trained fieldworkers holding appropriate licences (personal BTO-issued permit number: C/6982) and carried out under appropriate ethical review, while adhering to agreed guidelines. The long-term monitoring of the Wytham tit population was approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/TitBreedingEcology). All methods and experiments using zebra finches were performed under approval of the Central Committee for Animal Experiments (Centrale Commissie Dierproeven) of the Netherlands, under license AVD1050020174344.

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APPENDIX B

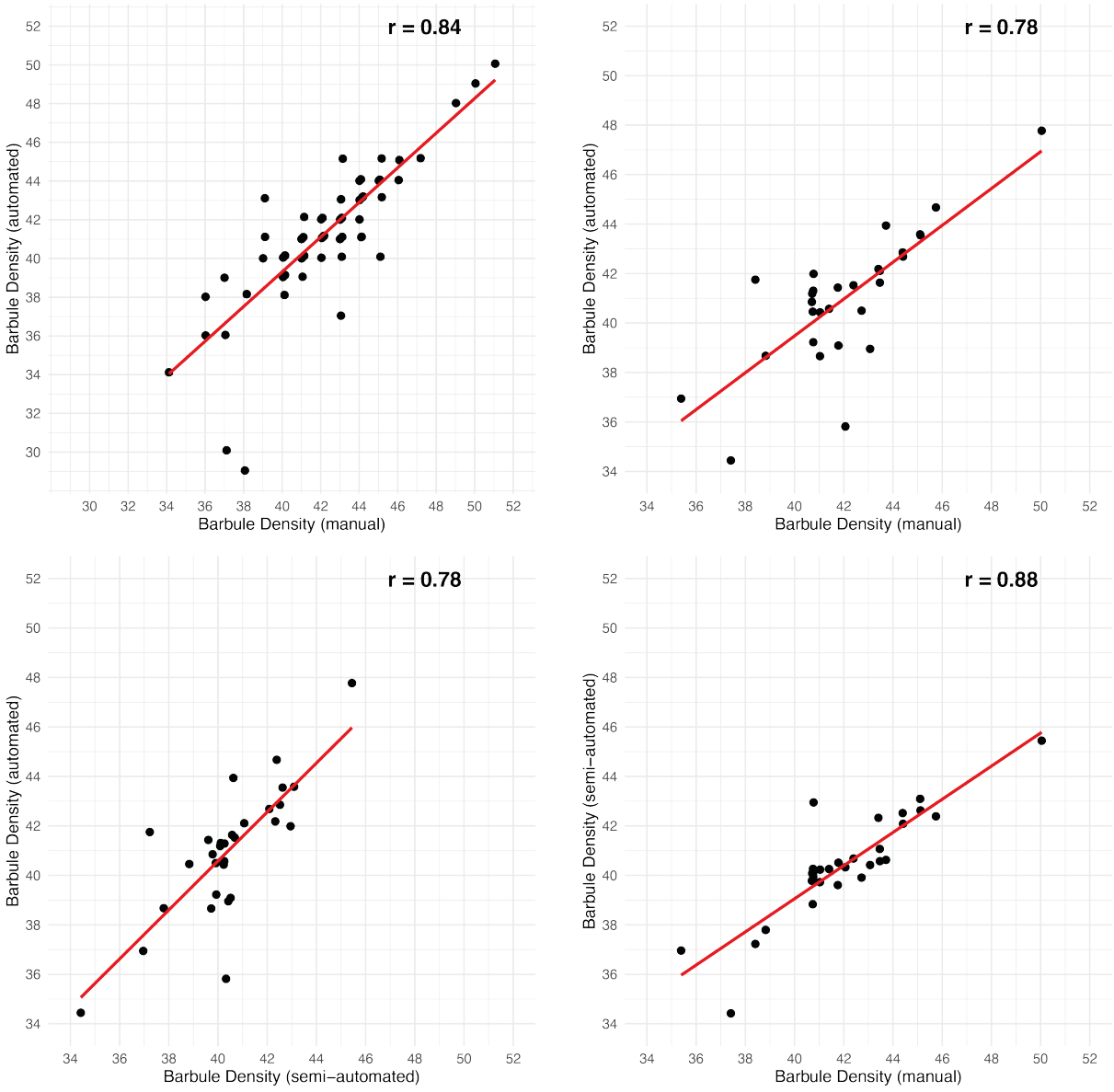


Figure 1: Comparison of automated, semi-automated and manual estimates of barbule density from 30 zebra finch feather images. Top left panel includes three repeat measures within a feather, where the same barb was counted both manually and in an automated manner. In the rest of the plots, each point shows a feather-level average. Pearson's r correlation coefficients are written in the top right corners.

Chapter 4

Plumage structure as a mediator of trade-offs between offspring quantity and quality: an experimental study of blue tits, *Cyanistes caeruleus*



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ABSTRACT

Resource and time limitations force organisms to face trade-offs when optimising competing life-history traits, such as between the number and quality of offspring. Evidence across taxa shows that raising more young may decrease their immediate condition and influence long-term survival and reproductive success. However, mechanisms that can explain both short- and long-term effects of trade-offs are scarce. In birds, variation in feather quality may provide an easily quantifiable link. In this study, we manipulated the brood sizes of wild blue tits (*Cyanistes caeruleus*) to understand how early developmental conditions affect nestling feather structure. We found that increased brood sizes resulted in lighter chicks with shorter wings and higher barbule density feathers. We discuss possible hypotheses that could suggest chicks under resource limitation prioritise feather structure over body growth, but highlight methodological caveats that may hinder our interpretation. These findings provide insights into how early developmental constraints shape feather morphology, with potential long-term consequences for survival.

1 | INTRODUCTION

Evolutionary theory suggests that fitness is maximised when organisms live long and reproduce prolifically (Stearns (1992)). In reality, however, individuals do not have unlimited time and resources to simultaneously increase all their aspects of fitness, thus preventing the existence of these so-called 'Darwinian demons' (Smith (1978)). Instead, individuals living in time and/or energy restricted environments will face trade-offs between competing evolutionary targets, and exactly how they strike an optimum balance between key fitness-related traits has long intrigued life history theorists.

Trade-offs between life-history traits can present in several ways (Stearns (1989)), of which the problem of balancing both the number and quality of offspring is key to maximising lifetime reproductive success - a definition of fitness particularly relevant to ecology and the study of natural populations (Clutton-Brock (1988)). Support for the quantity-quality trade-off across various taxa is presented in several foundational papers (including in plants: Smith and Fretwell (1974), mammals: Clutton-Brock et al. (1982), insects: Roff (1993), fish: Hutchings (1991) and birds: Lack (1947), Perrins (1965)), as well as in more recent studies (eg. in fish: Morrongiello et al. (2012)). Evidence comes from both observational and experimental studies, the latter of which often uses techniques to manipulate the 'quantity' side of the scale to look at the observed effects on the 'quality' of offspring (Smith et al. (1989)). Describing the condition of these young at the phenotypic level, however, can be approached from many different perspectives, such as measuring physiological (eg. immune response Verhulst et al. (2005), Sinervo and Svensson (1998)) or morphological (eg. body mass De Kogel (1997)) traits, and looking for any links with differences in future survival and reproductive success.

In birds, plumage production poses serious demands, both in terms of time and energy, thus providing a good candidate trait to study the consequences of avian life-history trade-offs (Kiat and Sapir (2018)). Various aspects of plumage quality have been shown to be influenced by food availability and diet composition (Pap et al. (2008), Swaddle and Witter (1994)) as well as feather growth duration (Dawson et al. (2000), Griggio et al. (2009), Møller and Nielsen (2018)) demonstrating both its energy- and time-demanding nature, respectively. Though feathers are periodically replaced, their structure remains fixed for extended periods of time in-between these moult bouts, during which they can have profound impacts on several functions essential for survival (eg. flight (Møller and Nielsen (2018)) and thermoregulation (Osváth et al. (2018)), reviewed by Jenni and Winkler (2020a)). This dual nature makes feathers ideal candidates to study life-history trade-offs over different timescales; allowing us to examine both the immediate effects of conditions during growth, as well as the carry-over impacts on future performance, thereby linking past resource allocation to future fitness outcomes.

However, we still know little about how early developmental effects, including the relative influence of genetic and environmental factors, may shape feather morphology and impact future survival. Most studies investigating variation in plumage quality have focused on coloration (eg. Hadfield and Owens (2006), Walker et al. (2013)), while those examining feather structure tend to study the processes during moult (eg. Bortolotti et al. (2002), Vagasi et al. (2012), reviewed in Jenni and Winkler (2020a)). To our knowledge, no studies have specifically explored how early conditions experienced in the nest might influence the structural makeup of the first flight feathers produced (but see research on body feathers: Callan et al. (2019), Taff et al. (2022)). This is important, because in many passerine species, the juvenile flight feathers of the wing (specifically the primaries and secondaries) are kept for the first year of life, until they are shed in the first post-breeding moult cycle (Jenni and Winkler (2020b)). Therefore, the quality of these feather types produced in the nest could have profound effects on first year survival (often very low amongst passerines; Cox et al. (2014)), and may influence the success of their first breeding attempt (also a critical element of fitness among these generally short-lived species; Lindström (1999)).

In this study, we seek to understand how nestlings balance resource allocation towards feather growth during early development by manipulating the brood sizes of wild blue tits (*Cyanistes caeruleus*), thereby inducing resource constraints via increased sibling competition and/or altered parental provisioning (Verhulst and Tinbergen (1997)). By exploring the impact of altering the number of offspring, we aim to shed light on the mechanisms that link short-term developmental trade-offs with potential long-term fitness outcomes in birds, using feather structure as a key indicator of offspring quality.

2 | METHODS

We performed a series of brood size manipulations with a partial cross-fostering design (under ethical approval) across two years (2022, 2023) in a population of wild blue tits (*Cyanistes caeruleus*) breeding at the 385 ha mixed deciduous forest of Wytham Woods, Oxford (UK).

2.1 | Brood size manipulation protocol

Following standardised procedures for the long-term monitoring of tit breeding activity (Perrins (1965)), nests were visited weekly between late March and early June to determine the laying date (LD; date of first egg laid) and clutch size (CS: number of eggs laid), then every two days around the expected end of the incubation period to determine the hatch date (HD; date of first egg hatched). Once chicks were present in a nest, they were paired up with another nest with the same clutch size and hatch date. Each nest was then randomly allocated to either the increased brood (IN) or decreased brood (DE) treatment.

On day 3 (where hatch day is day 1), chicks were exchanged between pairs of nests such that IN nests received two extra chicks, whereas DE nests ended up with two chicks fewer relative to their original brood size (BS: number of chicks alive in the nest), while keeping the proportion of own vs foreign young approximately 1:1. To achieve this, the number of chicks that each nest donated was calculated as:

$$\text{Number of chicks donated by IN} = \frac{\text{BS}_{\text{original}} - \text{manipulation}}{2}$$

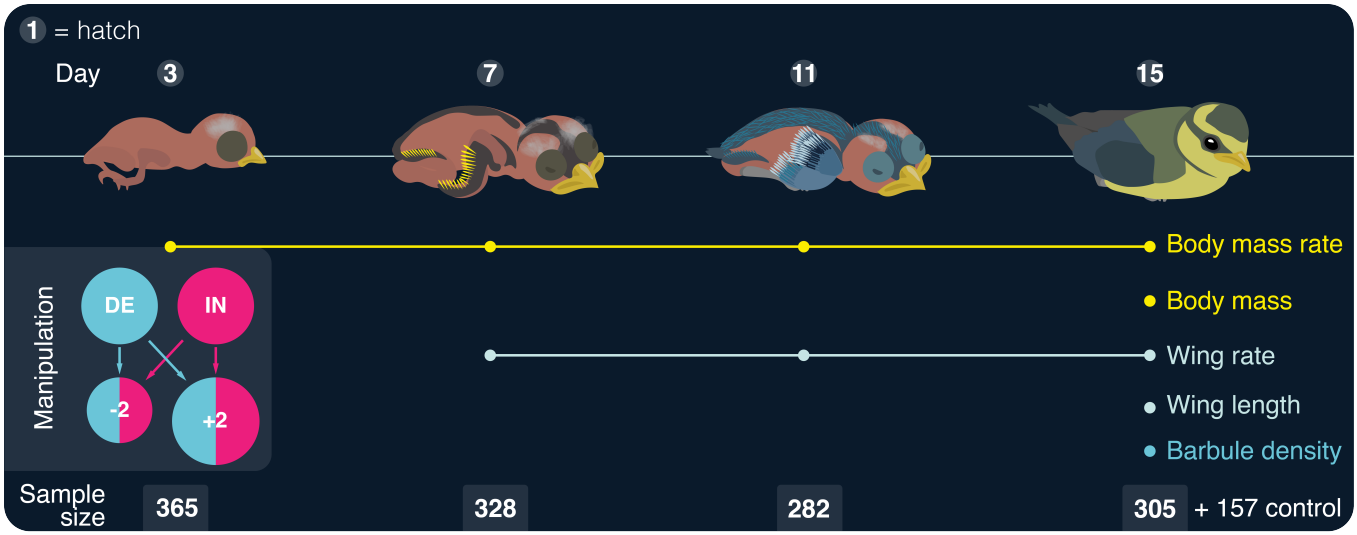


FIGURE 4.1 Timeline of the experimental protocol and summary of the key variables measured at each stage. Chicks are cross-fostered on day 3 between pairs of nests to manipulate their brood sizes by an increase (IN) or a decrease (DE) of two nestlings. Growth rate in body mass is calculated from body mass measurements taken on days 3, 7, 11, and 15, while wing growth rate is estimated from wing lengths measured on days 7, 11, and 15. On day 15, besides the final body mass and wing metrics, we also photograph wing feathers to characterise their macrostructure in terms of barbule density values derived from image analysis.

$$\text{Number of chicks donated by DE} = \frac{\text{BS}_{\text{original}} + \text{manipulation}}{2}$$

where manipulation = 2, and the obtained number of chicks to donate was rounded down. We exchanged offspring between pairs of nests always in the same order; moving donated chicks first from IN treatment nest to DE nest, then from DE back to the IN nest. This was done in order to minimise the risks of of parental desertion and hypothermia (as altricial nestlings at this age cannot regulate their own body temperature; Perrins (1979)) by never leaving fewer than four chicks in a nest while completing the brood swapping. Prior to cross-fostering, each nestling was weighed to the nearest 0.1g and marked for individual identification by painting a unique combination of claws with permanent marker (similar to previous studies using nail varnish; Biard et al. (2007), Nilsson et al. (2009)). After marking, we randomly assigned each chick to either the 'home' (i.e. staying in their nest of origin) or the 'away' (i.e. being transferred to their nest of rearing') group. The 'home' nestlings were placed back inside their nest of origin, while the selected 'away' young were transported to the matching DE nest in a moss and wool padded insulated bag, heated from the bottom and sides with reusable gel hand warmers (by LifeSystems). We repeated the same procedure to weigh, mark, select and transport donated chicks from the DE to the IN nest. Handling time at a nest box took c. 10-15 minutes, while transfer of experimental chicks took no more than c. 20-25 minutes. Therefore, the total time that experimental chicks remained unfed did not exceed 30-40 minutes, during which we estimated that they could potentially miss less than two feeding visits each (estimates based on provisioning rates reported in Banbura et al. (2001), García-Navas et al. (2013) and Keller and Van Noordwijk (1994)). On day 5, we visited all nests again to repaint the claw patterns with permanent marker.

On day 7, the nails of nestlings were large enough to safely perform claw-clipping in order to further ensure their individual identification patterns (a technique previously used by Evans and Sheldon (2012), Shapiro et al. (2006) among others). The translucence of the developing claws at this age allowed for easy identification of the bone core underneath, and therefore enabled safe removal of the outer keratinous tips without causing harm (with the occasional slight bleeding stopped using styptic powder). Claw-clipping ensured that chicks remained individually identifiable until their legs grew big enough to have metal identification rings fitted (detailed below). At this age, individuals usually begin feather development, therefore we could measure wing length (to the nearest 1mm) using a metal wing ruler (from the carpal joint to the tip of the longest primary) besides weighing each nestling to the nearest 0.1g.

On day 11, all chicks were weighed and their wing length measured once more. Nestlings that were large enough, received uniquely numbered metal leg rings (issued by the British Trust for Ornithology), whereas smaller nestlings' claws were re-clipped where necessary.

On day 15, all chicks were weighed and their wing length measured for the final time. Any unringed nestlings received metal leg rings. We additionally photographed three primary feathers of the wing (p7, p8 and p9) using a digital microscope (Dino-Lite AM7915MZT with 5MP camera) at x100 magnification. These images were later analysed to estimate barbule density (as number of barbules / mm of barb) (appendix figure C1). After day 25, all nests were visited to confirm fledging and record the identity of any dead nestlings.

In total, for each nestling we had life history information such as their nest of origin, their nest of rearing, the hatch date, the clutch and brood size of their nest of rearing prior to manipulation and the brood size of their nest of rearing after manipulation. In addition, we had morphometric measurements such as their body mass, wing length and feather structure on day 15, as well as their body mass growth rate (in g / 4 days calculated from four time points) and wing growth rate (in mm/ 4 days calculated from three time points)(experimental protocol summarised in figure 1).

Throughout the breeding season, we also monitored nearby blue tit nests that did not have a matching experimental pair, from which we obtained day 15 morphometrics and used these as control (CO) nests in our analyses.

2.2 | Data preparation

We successfully manipulated the brood sizes of 62 nests with a total of 550 individual chicks and monitored an additional 43 unmanipulated nests with 316 chicks. However, some of these nests exhibited sub-optimal hatching success rates, where the brood size at the time of manipulation did not match the original clutch size. As we were interested in understanding how parents balance the trade-offs between offspring quantity and quality under controlled experimental conditions, we decided to remove these instances from our analyses. Our reason is that the effort required (to raise a certain brood size) versus effort intended (i.e. the clutch size) was already at an imbalance outside of our experimental control, and this could hinder our ability to attribute any changes in nestling condition to the effects of the brood size manipulation alone. Therefore, we performed statistical tests on a subset of 522 chicks from 60 nests; 40 manipulated nests with 365 chicks, and 20 unmanipulated nests with 157 chicks to serve as control.

Clutch sizes of manipulated broods ranged between 8 and 11 (mean = 9.2 , sd = 0.8), while those of control nests ranged between 5 and 12 (mean = 8.4, sd = 1.6)(appendix figure C3). Manipulations occurred between ordinal days 124 and 139 (mean = 132, sd = 4), and we monitored control nests with an even distribution across very similar dates (123-139, mean = 131, sd = 4) (appendix figure C3). One concern with selecting chicks at random for transfer during cross-fostering is that the body mass distributions within a nest might change and potentially influence the developmental trajectories of the nestlings. However, we found no significant differences between nest mean chick body mass before and after manipulation, apart from the slight increase in body mass post-manipulation across the 2023 decreased treatment nests (mean difference = 0.17 g, paired t-test p = 0.0493, appendix figure C2).

We used the 'featheRscan' R package (thesis Chapter 3) to extract barbule density estimates as the number of barbules / 1 mm section of barb, measured from a standardised point of 0.43 mm away from the rachis (following protocol by Broggi et al. (2011)), repeated for a total of three barbs per feather image. We used a semi-automated approach of manually drawing the lines (in Fiji, Schindelin et al. (2012)) over which to then automatically count barbules with the featheRscan package, as the feathers at this stage of growth were often incompletely unfurled and the algorithm had difficulties detecting the areas in the image that had clear and visible barbules present.

2.3 | Data analysis

All statistical analyses were carried out using the R statistical software (v4.4.0, R Core Team (2024)). To estimate wing and body mass growth rates, we used the 'lme4' package (v1.1.35.3, Bates et al. (2015)) to run linear mixed models with *wing length* or *body mass* measurement as response, *day* (as number of days since hatching, where hatch day = day 1) as fixed linear or quadratic predictor, and random terms including *nest of origin*, *nest of rearing* and *individual ID*. We expected a linear pattern in wing growth (reviewed in Jenni and Winkler (2020a), pp76-88), therefore fitting day as a linear predictor in these models. On the other hand, we considered fitting a logistic or sigmoidal curve (Ricklefs (1967)) for the rate of change in body mass. However, due to the low sampling rate (only 3-4 time points) and lack of records for the early (prior to day 3) and late (after day 15) stages of growth, our data structure was only adequate for simple quadratic models with *day* squared to be tested. Where this resulted in a significant quadratic effect, we used the linear slope coefficient to describe the overall growth rate, though in most cases, a simple linear regression (without quadratic term) was a better fit.

To calculate the degree to which our five morphometric measures (wing rate, body mass rate, day 15 wing length, day 15 body mass, day 15 barbule density) were correlated, we used the 'Hmisc' package (v5.1.3, Harrell Jr (2024)) to compute Pearson's R for each pair of variables (using a single mean value of barbule density across the 9 repeat measures per individual) without controlling for any covariates (which we refer to as 'full' correlations in figure 2).

We also computed 'partial' correlations (figure 2) using the 'ppcor' package (v1.1, Kim (2015)) between each pair of morphometrics while also controlling for *manipulation* (as a continuous numerical predictor given as the number of chicks added or removed), *clutch size* (i.e. number of eggs laid; identical between the nest of origin and rearing), *hatch date* (i.e. the ordinal day of the first chick hatched in the nest; also identical between the nest of origin and rearing) and *year*, all of which may affect the response variables separately, thereby altering the correlations between them.

To understand how these covariates might influence our five morphometrics overall, we ran a series of linear mixed models using 'lme4' and fit each *morphometric* as response separately. As fixed effects, we included *manipulation* (as a continuous variable), *clutch size*, *hatch date*, and *year* (included as a fixed term instead of random because estimating variance components with only two levels is unreliable, Gelman (2007)). Finally, as random effects we included *nest of origin* and *nest of rearing*.

To further disentangle the relationship between brood size (i.e. offspring quantity) and nestling morphometrics (i.e. offspring quality), we examined how the effects of the manipulation may vary relative to the starting clutch size laid by parents. For this, we tested models with an interaction between *manipulation* (this time as a factor with levels 'decreased', 'control' and 'increased') and *clutch size* (z-scored against its

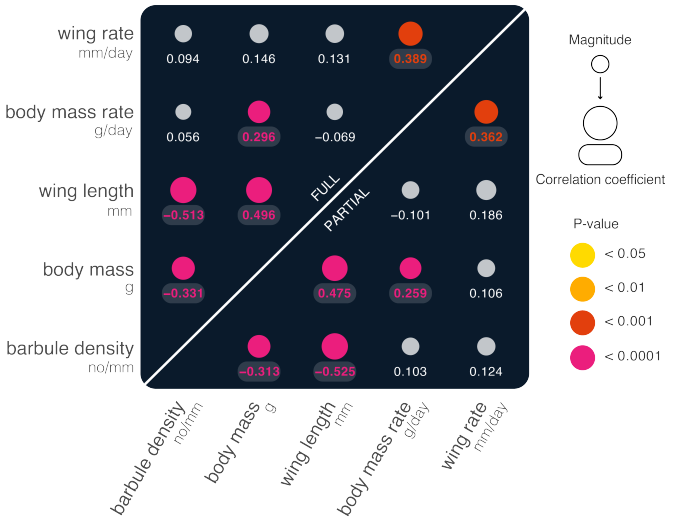


FIGURE 4.2 Summary of the 'full' and 'partial' correlation estimates between each pair of morphometric variables. Point size illustrates the magnitude of correlation, equal to the correlation coefficient reported underneath, and is standardised across each row to allow for comparison between a given variable and all the correlation test regarding it. Points are coloured by significance of the correlation, and greyed out for p > 0.05.

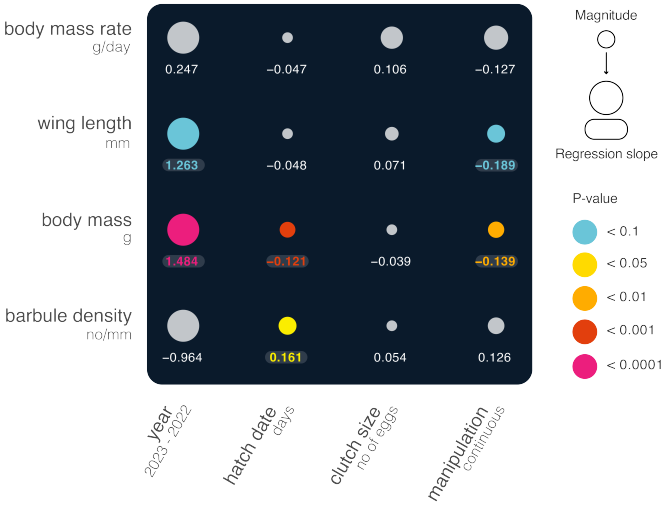


FIGURE 4.3 Summary of mixed model regression estimates between each offspring morphometric and the fixed predictors. Point sizes illustrate the magnitude of the regression slope, with the exact estimate written underneath each, and are standardised within each row to allow for comparison between the effects of the different predictors on the given response variable. Points are coloured by p-value, including those approaching significance, and are greyed out for values of p > 0.1.

mean in each year separately). As before, we included *hatch date* (z-scored) and *year* as fixed effects, and *nest of origin* and *nest of rearing* as random terms.

We used the 'spdep' package (v1.3.5, Bivand (2008)) to test for any evidence of spatial autocorrelation in our model residuals by calculating spatial weights matrices based on the Euclidean distances between nest box coordinates (as there was little variation in altitude across nest boxes, mean = 87.8 m, sd = 20.8) . The distance between our nest boxes ranged between 16 m and 1433 m (mean = 587 m, sd = 346 m), therefore we tested thresholds of neighbour distances between 50 m and 1000 m (at increments of 50 metres) for each year separately. As we found no evidence of spatial autocorrelation (Moran's I < -0.00337 and p > 0.397 for all tests), we did not include any terms specifically accounting for this in our models to avoid overfitting.

The cross-fostering design of our experiments allowed us to partition the total variation in our morphometrics (after accounting for variation caused by the fixed effects) into contributions by the *nest of origin* (which includes genetic and early developmental effects) versus the *nest of rearing* (which accounts for environmental effects during growth), and also estimate any residual variation capturing measurement error and the potential effects of other variables not considered in our models. Given our nine replicate measures for barbule density (three measurements per each of three feathers of an individual), we could further estimate the variation between individuals that is not due to the fixed effects (by including nestling ID as a random term), the variation within an individual between its feathers (by including a random term for feather image nested in chick ID), as well as the residual variation capturing differences between barbule counts within feathers. In this model, we also accounted for consistent differences observed between feather positions (reported also by Dawson (2005)), by including primary feather position as a fixed effect.

Finally, to see if the brood size manipulation caused any changes in nestling survival rates, and whether the effects differed depending on starting clutch size, we used 'lme4' to fit a generalised linear model with a binomial distribution and logit link function of *fledgling proportion* (a nest level measure of the number of chicks fledged relative to the manipulated brood size) as the response, standardised *hatch date*, *year* and an interaction between standardised *clutch size* and *manipulation* (a factor) as the fixed effects.

3 | RESULTS

3.1 | Correlations between morphological traits

We found weak-to-moderate correlations between several of our morphometric traits, and their magnitudes did not change significantly even after controlling for the covariates of *manipulation*, *clutch size*, *hatch date* and *year*. In particular, we found the strongest correlation between wing length and barbule density (r_partial = -0.525, p = 4.57 x 10^{-32}, N = 437), followed by wing length and body mass (r_partial = 0.475, p = 5.67 x 10^{-56}, N = 440). We also observed a weak correlation between barbule density and body mass (r_partial = -0.313, p = 1.72 x 10^{-11}, N = 445),

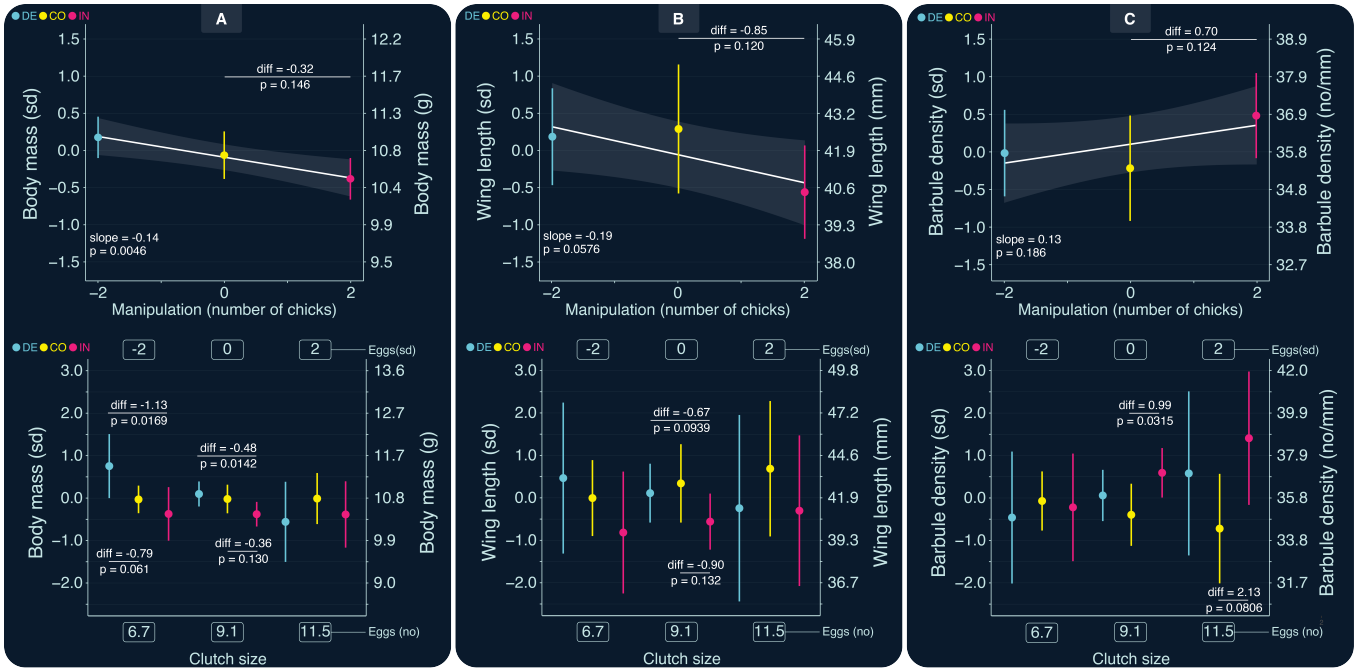


FIGURE 4.4 Summary of the effects of brood size manipulation on (a) body mass, (b) wing length and (c) barbule density. Top panels show results from additive models testing for an overall effect of the manipulation; solid lines correspond to the predicted mean regression slope with shaded areas denoting 95% confidence intervals ((a) shows a significant trend line, (b) and (c) are representative for visualisation purposes). Points represent the predicted mean values of the given response variable for each treatment group separately, with error bars showing 95% confidence intervals. Bottom panels illustrate results from models that included an interaction between manipulation and clutch size, detailing the effects of the manipulation at the mean clutch size, as well as 2 standard deviations on either side of it. Points and error bars show predicted mean and 95% confidence intervals. The same comparisons, but with controlling for wing length, are shown in appendix figure C6.

however this completely disappears once we account for wing length as a covariate (*r*_partial = -0.0594, p = 0.226, N = 422). There was very little difference in wing rate between individuals (mean = 14.5 mm / 4 days, sd = 0.61 mm / 4 days, coefficient of variation (CV) = 4.21%, appendix figure C4) and this was reflected in the lack of correlations between wing rate and the other morphometrics. The weak positive correlation between body mass rate and wing rate (*r*_partial = 0.362, p = 0.0018, N = 76) illustrated that chicks grow their body and wings at the same time. Day 15 body mass also correlated weakly with body mass rate in a positive manner (*r*_partial = 0.259, p = 1.77 x 10^{-6}, N = 335) showing that nestlings had to grow faster to reach a higher body mass over their two-week growth (appendix figure C5).

3.2 | Overall effects of the brood size manipulation

Overall, mixed model results highlighted body mass to be the most strongly associated with several of the fixed effects, followed by barbule density and wing length to certain degree, however, variation in body mass rate could not be explained by any of the modelled predictors. Chicks' morphometric values showed consistent differences between years, growing on average 1.48 g heavier in 2023 (standard error (se) = 0.25, p = 1.33 x 10-7), with 1.26 mm longer wings (se = 0.72, p = 0.0854), and -0.96 fewer barbules / mm in their feathers (se = 0.58, p = 0.103). Their morphometrics also varied depending on their hatch dates, with each day's delay in hatch date resulting in chicks growing -0.121 g lighter (se = 0.03, p = 1.39 x 10 - 4), with 0.161 more barbules / mm (se = 0.07, p = 0.0232) in their feathers. There were no significant relationships observed between clutch size and any of these variables of body condition. On the other hand, brood size manipulation was associated with shifts in body mass and, to a certain degree, wing length and barbule density, with addition of each extra chick causing a -0.139 g reduction in body mass (se = 0.05, p = 4.58 x 10 -3), a -0.189 mm reduction in wing length (se = 0.09, p = 0.0576) and 0.126 barbules / mm increase in barbule density (se = 0.09, p = 0.186).

3.3 | Treatment effects depend on brood size

Looking at the consequences of brood size manipulation in more detail, we found that observed responses in nestling morphometrics depended on the starting clutch size in several cases. Analyses testing for an interaction between manipulation (as factor) and clutch size revealed that the

apparent negative slope of body mass against manipulation (as a continuous variable of number of chicks added, slope = -0.14 g, p = 0.0046) was primarily driven by the effects on low (i.e. mean - 2 sd) and medium (i.e. mean) sized nests. At low clutch sizes, the difference between DE and IN treatment nests (diff = -1.13 g, p = 0.0017) was more than double that of the difference between treatment groups at the mean clutch size (diff = -0.48 g, p = 0.0142), whereas neither addition nor removal of chicks to large clutch sizes resulted in significant differences in body mass between the treatment groups. The effects on wing length showed similar patterns, with the overall negative relationship being driven by changes at low-to-mid clutch sizes. On the other hand, the positive slope of barbule density in response to an experimental increase in brood sizes (slope = 0.13 barbules/mm, p = 0.186) was only apparent at mid-to-high starting clutch sizes and, in particular, as a result of the IN treatment. Addition of chicks to an already large nest caused shifts towards higher barbule densities compared to the CO group (diff = 2.13 barbules / mm, p = 0.0806), with this difference being almost twice as large as the change observed at medium starting clutches (diff = 0.99 barbules / mm, p = 0.0315). These effects on barbule density are significantly diminished once we account for the strong correlation between barbule density and wing length (slope = 0.045, p = 0.588; IN-CO diff_medium = 0.75, p = 0.0972; IN-CO diff_high = 1.86, p = 0.0496). However, the effects on body mass remained similar even after controlling for its correlations with wing length (slope = -0.11, p = 0.0075; DE-IN diff_low = -0.93, p = 0.0133; DE-IN diff_medium = -0.36, p = 0.0222).

3.4 | Genetic and environmental contributions to morphology

Variation in wing length was more strongly explained by the nest of origin (i.e. genetic and pre-day 3 early developmental effects), with genetic effects contributing more than twice as much to the total variance (17.3%) compared to the influence of conditions experienced in the nest of rearing (8.3%). On the other hand, variation in both body mass and barbule density could be attributed to rearing conditions to a much greater extent compared to the influence of the birth nest. The ratio of environmental to genetic effects was roughly 3:1 in body mass (36.0% : 12.1%) and c. 2:1 in barbule density (20.0% : 10.1%), which remained similar even after accounting for wing length in these models (36.2% : 10.6% in body mass, 23.1% : 12.0% in barbule density). Having replicate measures for barbule density allowed us to further partition the variance, revealing that although the ratio of environment:genes remained roughly 2:1 (11.3% : 6.1%), their total contribution was diminished by a half (from 30.1% to 17.4%), as between- and within-individual differences accounted for about a third of the variance each (between: 29.8%, within: 25.5%). Controlling for wing length in these models revealed that differences in barbule density between individuals could in large be explained by differences in wing length (reducing the between individual component from 29.8% to 19.9%). Variation in all morphometrics included large portions of residual variance (wing: 74.5%, body mass: 51.9%, barbule density: 70.0% or 27.1% depending on the model), accounting for measurement error as well as the effects of any other variables not considered in our models (e.g. sex: Jacot and Kempenaers (2007)).

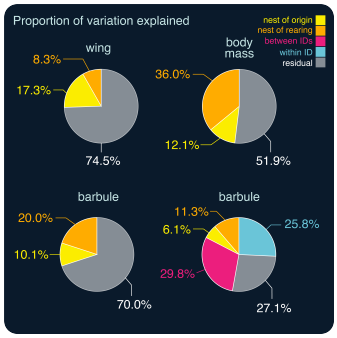


FIGURE 4.5 Pie charts showing the estimated contributions of genetic (nest of origin) and environmental (nest of rearing) effects towards variation in day 15 wing length, body mass and barbule density. Using nine replicate measurements per individual, variation in barbule density is further partitioned into differences between individuals, as well as variation within an individual due to differences between their feathers. Here the residual variance also includes differences between barbule estimates within the same feather.

3.5 | Treatment effects on fledging success

Brood size manipulation also caused significant changes to nestling survival patterns, calculated as the proportion of chicks fledged from a nest relative to the experimental brood size. Increasing nests by two chicks decreased their probability to successfully fledge the nest by about 5% across all clutch sizes (CO - IN at clutch 8 : 96.5% - 93.1%, clutch 9 : 97.0% - 91.5%, clutch 10 : 97.4% - 89.5%). Decreasing nests by two chicks had positive effects on fledging rates at clutch sizes below the

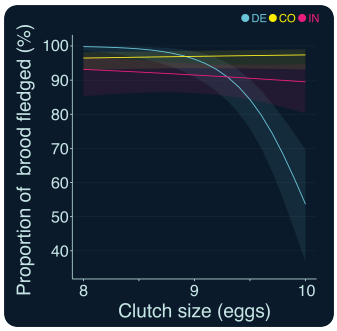


FIGURE 4.6 Nestling survival, calculated as the proportion of chicks fledged from a given nest, averaged for each treatment group, with lines and shaded areas showing predicted mean and 95% confidence interval estimates. Only clutch sizes between 8 and 10 had relevant fledging information for all three manipulation groups.

mean (DE - CO at clutch 8 : 99.8% - 96.5%), however, we saw a significant drop in survival at larger clutches (CO - DE at clutch 10 : 97.4% - 53.7%).

4 | DISCUSSION

In general, increasing the brood size caused chicks to reach lower body mass by day 15, along with shorter wing lengths and higher barbule density feathers, leading to reduced fledging success rates. These findings agree with trends observed across several previous studies on tits, showing that addition of hatchlings to a nest may result in chicks with lower body mass (Hõrak (2003), Jacot and Kempenaers (2007), Pettifor et al. (2001)), as well as shorter tarsi (another proxy for skeletal size; Hõrak (2003), Jacot and Kempenaers (2007)), and lower fledging rates (Sanz and Tinbergen (1999)). Although to our knowledge no research has previously looked at the effect of brood size on feather macrostructure, such as barbule density, studies have reported effects on other feather characteristics such as plumage coloration (Jacot and Kempenaers (2007)).

The effects of brood size manipulation on body mass were more apparent at lower starting clutch sizes, perhaps indicating a weaker ability of parents to adjust to changes in provisioning requirements. Indeed, a recent review of 78 studies of birds (including 58 brood size manipulation experiments) revealed that trade-offs may often be masked by variation in parental quality, where higher quality parents are able to care for larger clutches without compromising their own survival (Winder et al. (2023)). Moreover, the 'individual optimisation hypothesis' (IOH) proposes that variation in clutch sizes exists due to differences in individual quality selecting for different optimal clutch sizes that maximise offspring recruitment (Lack (1947), Perrins and Moss (1975)), thereby balancing offspring quantity and quality. An appropriate test for the IOH requires the manipulation of brood sizes and a measure of resulting fitness scores based on offspring recruitment rates primarily.

Although we did not follow up on offspring recruitment rates, our results showed that chicks raised in control nests had similar morphometric values (body mass, wing length, barbule density) and fledging rates across all starting clutch sizes, which may support the IOH, as recruitment rates are generally correlated with fledging mass (Perrins (1965)). However, we found an overall negative relationship between manipulation and nestling body mass, as well as slightly higher fledging success rates of reduced broods compared to both control and increased nests, potentially suggesting that there was selection for smaller clutch sizes in our dataset. Though many studies have demonstrated strong support for the IOH and a balancing selection on clutch size across different species (e.g. collared flycatcher: Gustafsson and Sutherland (1988), great tit: Pettifor et al. (1988 2001), common kestrel: Daan et al. (1990)), some studies even on the same species have also found selection for both smaller (Verhulst (1995)) and larger (Tinbergen and Sanz (2004)) clutch sizes compared to the numbers originally laid by individuals. This discrepancy in clutch size patterns even between populations of the same species may be explained by individual optimisation being modulated by environmental constraints, such that evidence for IOH may not always be present (Tinbergen and Sanz (2004)). For instance, between-year differences in overall food abundance or predictability in the temporal food peak may render individual plastic responses in clutch size sub-optimal. Indeed, we observed strong year effects on body mass and wing length, potentially highlighting marked difference in environmental contexts between the two years of manipulation.

On the other hand, the effects of brood size manipulation on barbule density were greater at mid-to-large starting clutch sizes, perhaps indicating the presence of resource allocation trade-offs (Van Noordwijk and De Jong (1986)), specifically a prioritised investment towards structural, long-lasting traits like feathers (which are kept for the whole first year of life in this species), as opposed to a more transient trait like body mass (Nilsson and Gårdmark (2001), Mainwaring and Hartley (2012)). After controlling for the correlations between wing length (as a proxy for overall size) and body mass, we still found a significant effect of the manipulation, indicating that chicks under increased treatment grew not only smaller, but thinner. On the other hand, wing length accounted for most of the observed changes in barbule density, suggesting increased treatment chicks did not grow more barbules relative to their overall wing size.

However, due to limitations in our methodology, we cannot infer for certain whether the negative relationship between wing length and barbule density is a result of differential amounts of feather material production. This is because (1) our last wing length measurement was taken on day 15 (as customary to avoid for force fledging), however chicks' feathers usually keep growing until at least fledging and often beyond, therefore, we cannot be certain whether our wing length measurement taken on day 15 is representative of the individual's final wing length. The inferences we can draw are limited also because (2) we measured incremental growth of the wing, rather than of individual feathers. Since wing length is measured from the carpal joint to the tip of the longest primary, it combines values of both bone length and actual feather length. Therefore, we cannot say for certain whether individuals that have longer wings by day 15 have longer feathers, are of larger skeletal size, or both.

Given that the rate of wing growth was strongly conserved between birds in our study (and indeed also shown in other studies; Jenni and Winkler (2020a) p86), we describe two hypothetical (non-mutually exclusive) scenarios of feather growth patterns (figure 7) to help us draw inferences about the potential differences in resource allocation patterns towards feather material production.

Scenario A describes a case where both IN and CO birds have the same skeletal size, but differ in the timing of when they begin feather growth. Individuals under diet restrictions (eg. from increased competition in the nest or from inadequate provisioning rates by parents) might take longer to reach the threshold body size and/or condition to begin plumage development. This means that by the time of the wing measurement taken on day 15, CO chicks would have been growing their feathers for a longer time, therefore having completed a potentially larger portion of their final feather length.

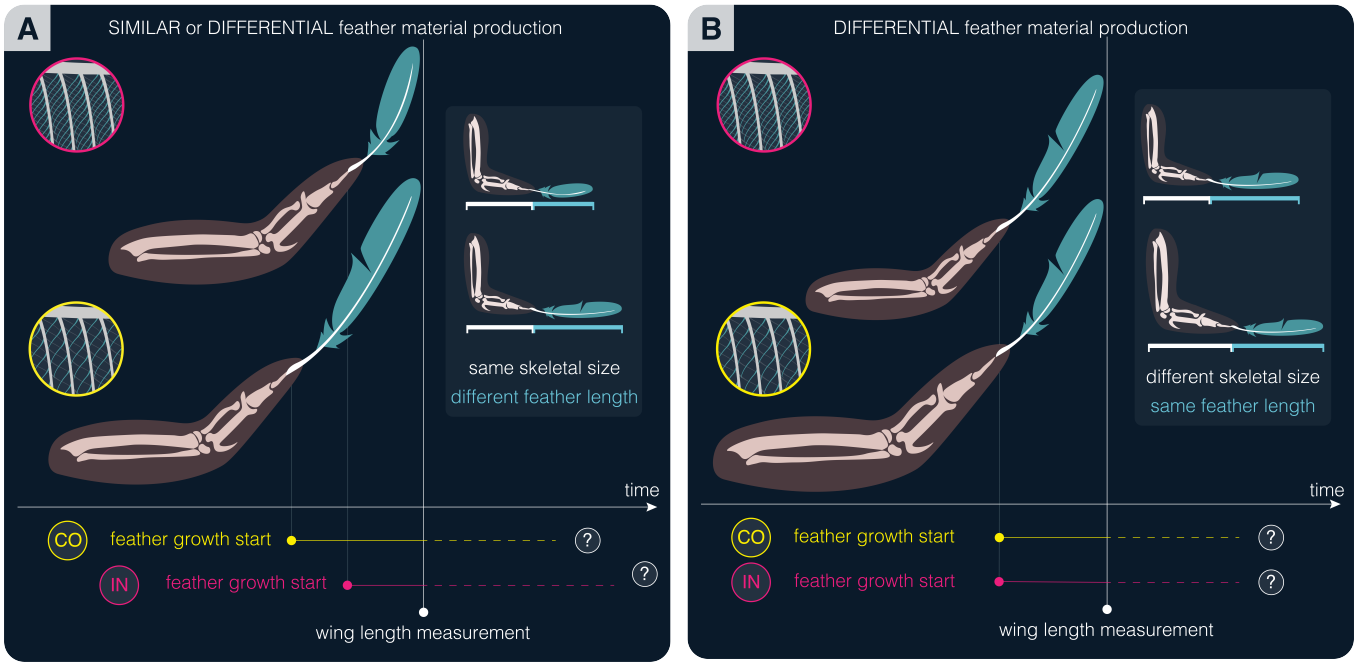


FIGURE 4.7 Illustrations describing the different scenarios that could lead to shorter wing lengths in the increased treatment (IN) compared to the control (CO) birds. (A) Both groups have the same skeletal size, but IN treatment birds start growing their feathers later. Given a fixed rate of growth in wing length, they would end up with shorter feathers by the measurement day. (B) Both groups have the same feather length, because they started growing them at the same time. However, increased treatment birds grow overall smaller with a small skeletal size, resulting in a shorter wing length measurement on day 15.

In this scenario, if the wing lengths measured on day 15 represent the final wing lengths of these birds (i.e. CO birds ending up with longer wings and IN birds with shorter), then having observed higher barbule densities in the IN treatment birds would not necessarily mean that they have produced more barbules relative to their overall wing size. For instance, if both treatment groups had similar amounts of resources available for plumage production, then growing larger feathers would require feather material to be distributed more sparsely over a larger surface, compared to the shorter wings of IN treatment birds.

Under the same scenario, if day 15 measurements instead do not directly represent final wing lengths, and individuals from IN broods reach similar final wing sizes as CO birds (only later, beyond our final time point) but with higher barbule densities, then this would indicate differential resource allocation patterns between the treatment groups; those with higher barbule densities would have had to deposit more feather material over the same feather length compared to lower barbule density individuals.

Scenario B describes the possibility where CO and IN treatment birds both begin feather development at the same time, however the overall wing length measurements differ due to differences in bone lengths rather than differences in individual feather lengths. Under this scenario, the observed higher barbule densities relative to a similar sized feather of IN treatment birds would suggest a differential resource allocation pattern of larger amounts of feather material deposited per unit of time, compared to the more sparsely structured feathers of control birds.

Research shows that feather follicle size is directly proportional to the amount of feather material deposited per unit of time (Jenni et al. (2020)). If follicle sizes and feather growth rates by length are similar between individuals (as seen in our study), then observing longer wings with lower barbule densities would simply be the result of the same amount of deposited feather material being stretched over a larger portion of the feather, thereby supporting scenario A. However, there is indication that follicle sizes can vary between feathers as well as within a feather during its growth period (Jenni et al. (2020)).

From among the nestlings of this study, 50 individuals were recaptured using mist-nets in the following autumn or spring (i.e. before their first moult) and their wing length measured. We found a weak positive correlation between day 15 wing length and final wing length (Pearson's R = 0.296, p = 0.0413), with smaller wings having grown more since fledging compared to those that were already large on day 15 (regression slope of final vs day 15 wing length = 0.23, p = 0.0413), supporting the idea that chicks whose wings are shorter on day 15 are likely underdeveloped, and not necessarily overall smaller, thereby supporting Scenario A. Similarly, a brood size manipulation of captive zebra finches showed marked differences in the wing lengths between treatment groups early on, but by 3-6 months' of age, the originally shorter wing length chicks from the

increased treatment group caught up with the rest of the population (De Kogel (1997)).

On the other hand, comparative studies on flat skin museum specimens found that birds with larger wing lengths tended to have longer bones (humerus, ulna, and manus) which contributed proportionally more to the overall wing length than the length of their primary feathers Nudds (2007), Nudds et al. (2011)). This suggests that individuals with longer wing lengths might have disproportionately longer bones rather than longer feathers, thereby supporting Scenario B.

4.1 | Concluding remarks and future directions

In reality, the answer probably lies between these two extreme hypothetical scenarios. Our results indicated a larger genetic component in wing length, whereas variation in barbule density was better explained by environmental factors, suggesting that individuals are more flexible to vary their barbule density compared to their wing length. However, due to the methodological constraints, we are unable to draw direct conclusions about the exact resource allocation patterns in feather growth between individuals of different barbule densities. Future research could benefit from quantifying the amount of feather material deposited per unit of time (as emphasised by Jenni et al. (2020)) as a metric of differential investment. As this would however require feathers to be plucked (Dawson (2003)), we recommend a less-invasive approach to measure both feather length and feather width (as a proxy for overall size) to disentangle the relationship between overall feather size and barbule density.

AUTHOR CONTRIBUTIONS

A.K. conceived and planned the project with the help and supervision of C.R., E.C. and B.S. A.K. performed the manipulations, collected, and analysed the data. All authors contributed to the interpretation of the results. A.K. produced the figures, and wrote the chapter with feedback from B.S.

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ETHICAL NOTE

All activities involving bird handling and experimentation were conducted by trained fieldworkers holding appropriate licences (personal BTO-issued permit number: C/6982) and carried out under appropriate ethical review, while adhering to agreed guidelines. The long-term monitoring of the Wytham tit population was approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/TitBreedingEcology). Brood size manipulation experiments involving blue tits were also approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Board (approval reference number: APA/1/5/ZOO/NASPA/Sheldon/BlueTitBreedingManipulation). Additionally, Special Methods endorsement for the use of claw-clipping for the individual identification of nestlings was provided by the BTO (under permit: C/6982, project code: 12131).

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APPENDIX C

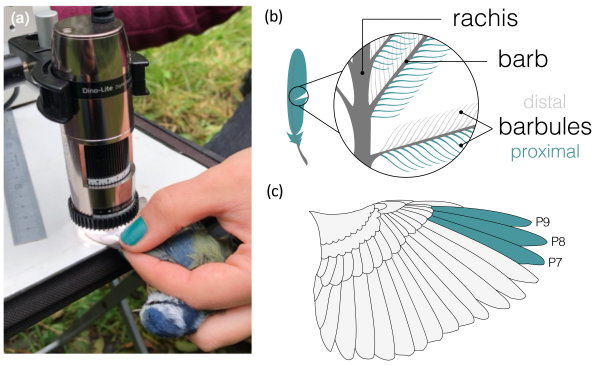


FIGURE 1 (a) Photograph of the sample collection technique using the DinoLite digital microscope, (b) schematic of the feather macrostructural elements of interest, (c) diagram showing the positions of the three primary (P) feathers imaged on an individual.

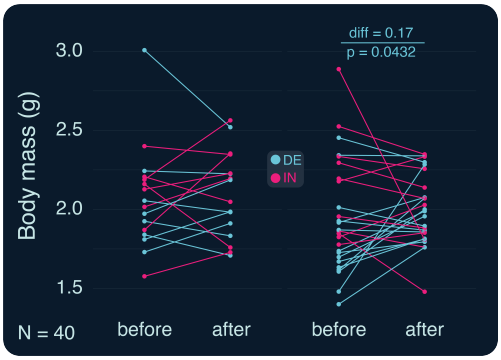


FIGURE 2 Pairwise comparisons of the average body mass in a nest before and after cross-fostering. Each point represents a single nest.

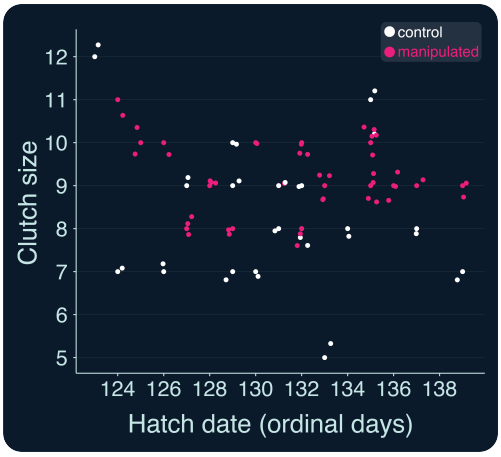


FIGURE 3 Distribution of hatch dates and clutch sizes of the manipulated and control nests. Each point represents a single nest.

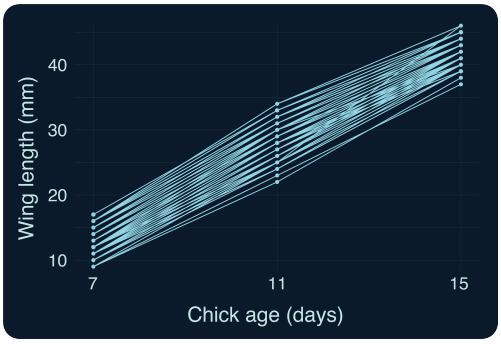


FIGURE 4 Plot showing the linear nature of wing length growth across the three time points measured, where day 1 corresponds to hatching. Each point is a single measurement, with lines connecting measurements on the same individuals.

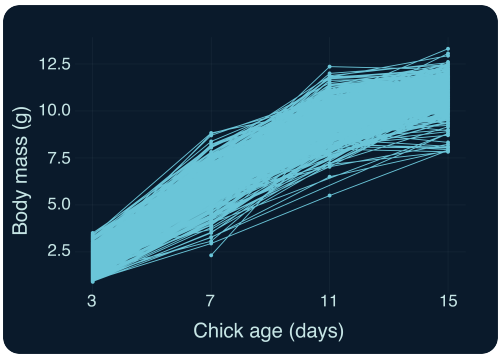


FIGURE 5 Plot showing the mostly linear, but somewhat asymptotic nature of body mass growth across the four time points measured, where day 1 corresponds to hatching. Each point is a single measurement, with lines connecting measurements on the same individuals.

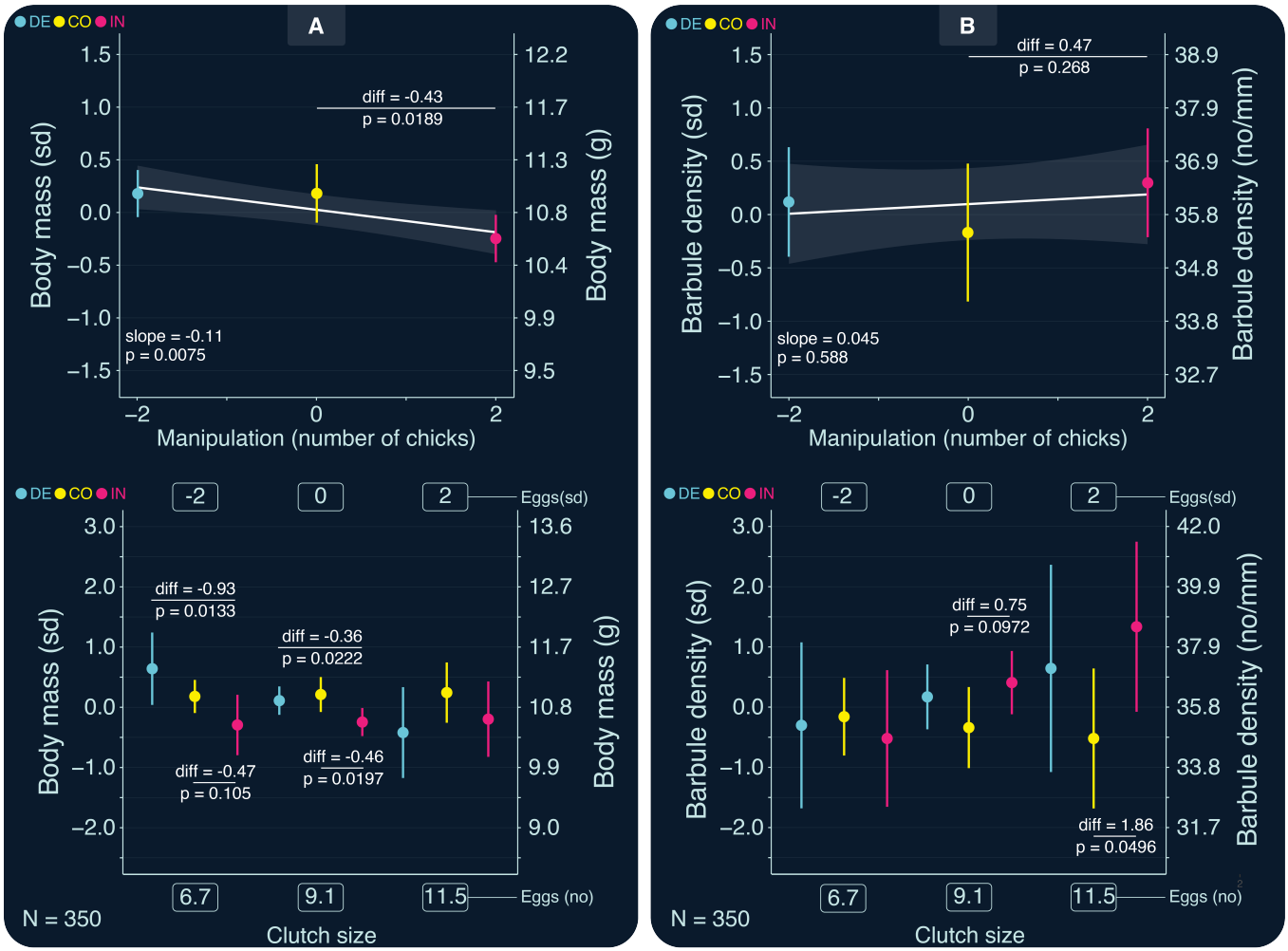
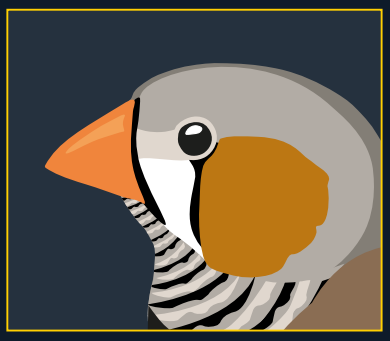


FIGURE 6 Summary of the effects of brood size manipulation on (a) body mass and (b) barbule density, after controlling for wing length as an additional fixed effect. Top panels show results from additive models testing for an overall effect of the manipulation; solid lines correspond to the predicted mean regression slope with the shaded areas denoting 95% confidence intervals ((a) shows a significant trend line, while (b) is only for visualisation purposes), points represent the predicted mean values of the given response variable for each treatment group separately, with error bars showing 95% confidence intervals. Bottom panels illustrate results from models that included an interaction between manipulation and clutch size, detailing the effects at the mean clutch size as well as 2 standard deviations on either side of it. Points and error bars show predicted mean and 95% confidence intervals

Chapter 5

Manipulated foraging costs alter individual morphology and thermoregulation in a small passerine: linking feather structure and insulation efficiency



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ABSTRACT

Plastic responses to environmental variation are important for survival across taxa. In birds, evidence for plastic adjustments to feather structure and its consequences for thermoregulation are limited. Here we tested the effects of manipulated foraging costs on individual body and feather morphology, and explored how they influence thermoinsulative capacity in zebra finches (*Taeniopygia guttata*). Using a combination of in vivo techniques, we quantified four key feather structural elements from images, estimated heat loss through the plumage via infrared thermography, and measured metabolic rates. We found that manipulated resource availability significantly altered the morphology and physiology of birds, though these changes did not lead to detectable impacts on survival in captivity. Notably, we identified a quadratic relationship between barbule density and insulation efficiency, suggesting that optimal heat retention is maximised at intermediate barbule densities. Our study highlights the complex interplay between morphological and thermoinsulative adjustments to environmental stressors, and we discuss potential energy allocation trade-offs between these competing responses. While we addressed some of the missing links between feather structure and insulation, further work is needed to understand how changes in plumage condition across a broader ranger of feather types might influence the energetic trade-offs between different thermoregulatory mechanisms and their long-term effects on fitness and survival.

1 | INTRODUCTION

The ability of organisms to respond to changes in the environment is crucial for their survival. Phenotypic plasticity forms an essential component of this survival toolkit, enabling individuals to alter aspects of their morphology, physiology, or behavior in response to environmental changes without the need for genetic adaptation (West-Eberhard (1989)). This flexibility allows them to take advantage of beneficial conditions and avoid the effects of detrimental ones. For many temperate species, this involves tracking fluctuations in resource availability and adjusting to seasonal temperature variations in order to survive.

Examples of individual adaptation to these challenges via phenotypic plasticity are widespread across taxa. In response to variation in resource availability, behavioural adaptations include strategies such as food hoarding to ensure access to resources when they are scarce (e.g. mammals: Sklepkovych and Montevecchi (1996), reviewed in: Vander Wall (1990)), and migration to track spatiotemporal changes in food supply (e.g. mammals: Bohrer et al. (2014), birds: Thorup et al. (2017), reviewed in: Dingle and Drake (2007)). Many animals exhibit physiological adjustments such as increasing fat storage during periods of unpredictability in food supply (e.g. birds: Gosler (1996), even humans: Higginson and McNamara (2016)). Morphological changes can also help take advantage of available resources, for instance by modifications of head shape or size in response to diet (eg. fish: Robinson and Wilson (1995) , insects: Thompson (1992)). Similarly, temperature fluctuations elicit a wide range of plastic responses, which can also be categorised into these broad themes. Behavioural adaptations include huddling for warmth in endotherms (Gilbert et al. (2010)) and sun basking in ectotherms (Otten et al. (2024)). Physiological adjustments often involve changes in metabolic rates (Broggi et al. (2007)), especially in organisms that undergo torpor or hibernation (Geiser (2004)). Finally, morphological adaptations, such as growing thicker coats or denser plumage to increase insulation in response to cold, are also common (Beltran et al. (2018)).

In birds, feathers fulfil many functions essential for survival, including flight and thermoregulation, thereby forming an integral component of how individuals cope with environmental challenges (Jenni and Winkler (2020)). In particular, we see evidence that feather structure varies between species depending on habitat-specific demands for flight (Pap et al. (2015)) and insulation (Barve et al. (2021), D'alba et al. (2017)). Since feather production is energetically demanding, plumage quality may vary depending on the time (Dawson et al. (2000), Griggio et al. (2009), Møller and Nielsen (2018), Horák et al. (2022)) and resources (Swaddle and Witter (1994), Pap et al. (2008), DesRochers et al. (2009)) invested in feather growth. Variation in the structural integrity of feathers has in turn been linked to differences in individual survival and fitness (Merilä and Hemborg (2000)), through its potential effects on flight efficiency (Swaddle et al. (1996), Møller and Nielsen (2018)) and insulation (Broggi et al. (2011)). Due to the continuous wear and tear, coupled with high survival demands, feathers must be periodically replaced to maintain their functionality (Jenni and Winkler (2020)). This periodic nature of feather moult and regrowth could enable individuals to respond to changing conditions.

While the role of phenotypic plasticity in feather coloration is well-documented (Järvistö et al. (2016), Hegyi et al. (2008), Moreno et al. (2019)), much less is known about how birds adjust feather structure in response to environmental variation, particularly in relation to thermoregulation and survival. Several studies have suggested the importance of feather quality for insulation and overwinter survival (Nilsson and Svensson (1996), Broggi et al. (2011), Dawson et al. (2000)). However, the links between feather structure and its thermal properties have not been well established. In recent years, a number of methods have been developed to quantify heat loss through avian plumage. These approaches can be divided into two broad categories; on the one hand, those that utilise samples of feathers (D'alba et al. (2017)) and flat skin specimens (Graveley et al. (2020)) to derive direct estimates of heat transfer from a source of known temperature through the feathers, building upon industry methods to measure down and pelt insulative power. On the other hand, the few studies focussed on avian thermoregulation from a more ecological perspective have made use of thermal imaging technology (Stone et al. (2021)) and respirometry (Nilsson and Svensson (1996)) to study these processes in live birds.

In this study, we used a combination of experimental and observational approaches to address the missing links between environment-induced variation in feather structure and its consequences for thermoregulation and survival in birds. We explored how resource availability may impact individual condition, with particular focus on feather quality, by experimentally manipulating the foraging costs of a captive population of zebra finches (*Taeniopygia guttata*). We quantified individual differences in four key feather structural elements using images of feathers photographed in vivo using a digital microscope and automated image analysis pipeline. In addition to information about feather quality, we also measured individual variation in thermoinsulative traits, using infrared thermography to derive a proxy for insulation via the plumage, as well as recording resting metabolic rates. In combination, we investigated which aspects of individual morphology had the strongest influence on thermoregulation, bridging the gap between variation in feather structure and insulation. Finally, we explored whether changes in morphology and physiology in response to food availability could predict differences in survival in this population.

2 | METHODS

2.1 | Study population

We studied a captive population of 103 individual zebra finches (*Taeniopygia guttata*) housed in outdoor aviaries (of size 310L x 210H x 150W in cm) at the Groningen Institute for Evolutionary Life Sciences (NL) across two data collection periods (March of 2023 and 2024).

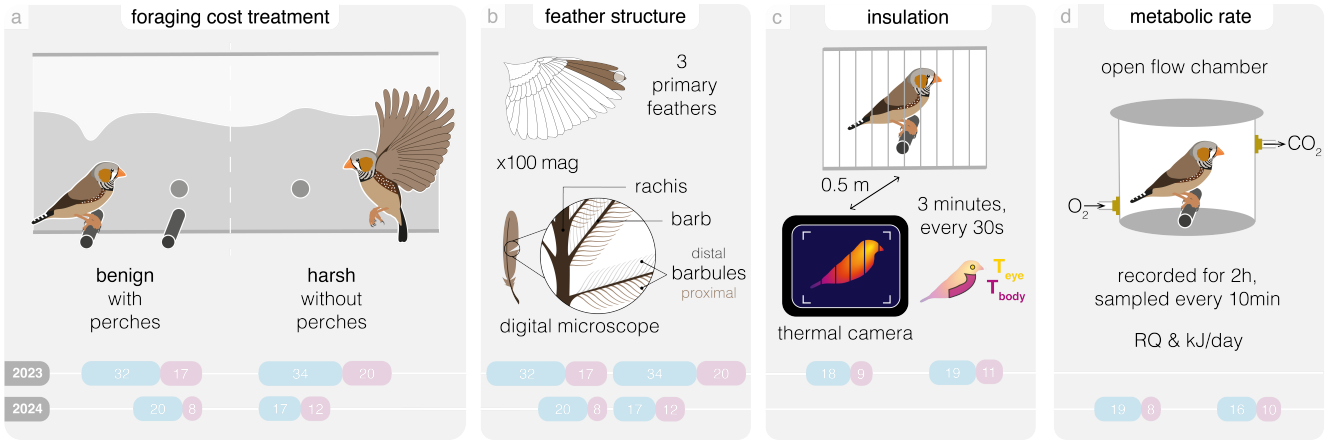


FIGURE 5.1 Summary of data collection protocol. (a) Foraging costs were manipulated by adding or removing perches to feeders, creating 'benign' and 'harsh' food treatments, respectively. (b) Feather structural quality was estimated from images of three primary feathers (P7, P8, P9) taken at 100x magnification using a digital microscope to measure the width of the rachis and barbs, and count the density of barbs and barbules. (c) Insulation efficiency was estimated from thermal videos of birds recorded for three minutes, with individual frames selected every 30 seconds for analysis, from which to measure the temperature of the eye (T_{eye}) and body (T_{body}). (d) Metabolic rate of birds at rest was measured in open flow chambers recorded for two hours, with samples of the amount of oxygen (O_2) consumption and carbon dioxide (CO_2) production sampled every 10 minutes, from which the respiratory quotient (RQ) and daily energy expenditure (in kJ/day) could be calculated. Numbers along the bottom show sample sizes at each data collection step. Within each panel, the left two numbers correspond to numbers of 'benign' treatment birds, while the right two denote 'harsh' treatment individuals. Each grouping is further broken down into males represented by blue, and females indicated in pink.

2.1.1 | Food treatment

All individuals were part of a long-term experiment running since 2007 (under ethical approval), where the birds' foraging costs were manipulated in a way that mimics variation in food availability under natural settings. As described in detail by Koetsier and Verhulst (2011), rather than restricting their diet, individuals under both treatment conditions were given the same food, however, while birds in the 'benign' group could sit on perches attached to the feeders (i.e. experiencing low foraging costs), those in the 'harsh' treatment had to fly back and forth to obtain resources (i.e. experiencing high foraging costs) (summarised in figure 5.1 a). This gave us an opportunity to study how difference in resource availability might affect individual condition through key morphological measures of the body and feather, as described below.

2.2 | Data collection

2.2.1 | Feather imaging and morphometrics

We brought birds indoors from their outdoor aviaries in small groups (of c. 7 individuals) to temperature controlled rooms kept at a constant 22°C (somewhat below the thermoneutral zone of zebra finches; 29.5 – 40°C, Calder (1964)), to minimise the effect of variation in air temperature on the thermal readings detailed below. We followed the protocol described in **Chapter 3** to image three primary feathers of their wing (P7, P8 and P9) using a digital microscope (Dino-Lite AM7915MZT with 5MP camera) at x100 magnification. We used the 'featheRscan' R package to extract estimates of four key feather structural elements to characterise feather quality as detailed in **Chapter 3**, such as; the width of the rachis (mm) and barbs (mm), as well as the density of barbs (no/mm of rachis) and barbules (no/mm of barb) (feather structural elements illustrated in figure 5.1 b). At the time of analysis, the featheRscan functions were limited to fully automated quantification of the rachis and barbs only, therefore we measured barbule density in a semi-automated manner by manually defining the regions over which to count barbules using featheRscan. Additionally, we measured body mass (to the nearest 0.1g) and wing length (to the nearest mm).

We decided to photograph primary feathers instead of plucking body feathers, which are considered to be more important for insulation, as this provided a faster and non-invasive option. Therefore, we assume that feather quality estimated from the primaries are a proxy for the overall plumage quality of an individual. Birds in this population typically begin moult of their primary feathers from April onwards and peaking over the summer (van der Heide, unpublished), therefore we scheduled data collection for March in both years to avoid confounding our feather structure estimates with any potential differences between old and newly moulted feathers (indeed, no birds were observed to be in active moult at the time of data collection).

2.2.2 | Infrared thermography

After collecting an individual's morphometrics, we placed each bird in a separate wire-gridded cage (of size 80L x 40H x 40D cm) with food and water provided ad libitum. We modified the perches inside the cages, using card board to block a section of the perch to make it shorter, thereby encouraging birds to sit close to the cage grid (appendix figure D1). This ensured that the area of the pixels comprising the bird's body was larger compared to the area corresponding to cage grid pixels, and therefore the warm temperature readings taken from the body were less influenced by the cool values of the cage grid. Birds perched close to the cage grid also ensured that the distance and orientation of the birds relative to the thermal camera remained constant between individuals (Tabh et al. (2021)).

We used a thermal camera mounted on a tripod (FLIR A65, f=13mm, spatial resolution 1.31 mrad, thermal sensitivity <0.05 °C @+30 °C, frame rate = 7.5 Hz, FLIR Systems, Wilsonville, Oregon) to record the surface temperatures of each individual in turn for 3 minutes from a fixed distance of 50 cm from the cage (appendix figure D1). During filming, we left the room to minimise the possibility that acute stress responses to our presence may influence the eye temperature readings (Edgar et al. (2013), Jerem et al. (2015)), as the degree of this response is likely to vary between individuals (Cockrem (2007)). A 3-minute recording period was chosen to maximise the chances of the bird entering the field of view on the perch with its whole body visible. Once thermal readings on an individual were completed, we moved the camera to the next cage and repeated the process until all birds in the room were recorded.

From these thermal videos, we sampled a frame every 30 seconds for every individual (= 6 frames per individual). We performed manual segmentation of the images in Fiji (Schindelin et al. (2012)) to remove any cage grids covering the bird and outline two regions of interest; (1) the bird's eye, and (2) the bird's ventral side, comprised of the breast and abdomen between the neck and thighs (figure 5.1c). We used the 'Thermimage' R package (v4.1.3, Tattersall, GJ (2021)) to convert thermal readings into temperature values within these two regions of interest, after setting emissivity to 0.95 for feathers (Graveley et al. (2020), Cossins (2012)) and subject distance to 0.5m.

From each individual frame, we calculated (1) the temperature of the unexposed skin around the eye (T_{eye}) as the maximum value recorded within the eye region (following protocol by Jerem et al. (2015)), and (2) the temperature of the front of the body covered in feathers (T_{body}) as the mean of all temperature values recorded within the delineated ventral region. As the skin around the eye is not covered by feathers, surface temperature readings of this region have been shown to track changes in core temperatures in birds (eg. when responding to a stress signal, Jerem et al. (2018)), therefore we used T_{eye} as a non-invasively obtained proxy for internal body temperature. On the other hand, a bird's ventral side (as outlined above) is covered in feathers, therefore surface temperatures across this region of the body (T_{body}) can be used as a measure of heat loss through the plumage. We used this area of the body to measure heat loss as it was the largest region visible from a side view of the bird that contained feathers from a single feather tract (ie. feathers of similar type and function). Using these temperatures recorded from an exposed vs an insulated region, we could define a proxy for insulation efficiency as the estimated amount of temperature trapped by the plumage, calculated as T_{eye} - T_{body}.

2.2.3 | Respirometry

Following thermal imaging, birds were moved to another temperature-controlled room (~22°C) kept in the dark, and placed in separate cylindrical respirometry chambers of c. 2L in size with a transparent lid, a perch at the bottom, and no food or water provided (illustrated in figure 5.1d). We used a respirometry set-up as described in Briga and Verhulst (2021), allowing up to 8 individuals to be measured simultaneously. In brief, air flow entering the chambers was set to c. 20L/h using mass-flow controllers (5850S; Brooks) calibrated with a bubble flow meter, and dried using a molecular sieve (3 Å Merck). Dried samples of air exiting the chambers were analysed using a paramagnetic analyser (Servomex Xentra 4100) to record the volume of oxygen consumed (VO_2) and carbon dioxide produced (VCO_2), from which we calculated respiratory quotients (RQ = VCO_2 / VO_2, Schmidt-Nielsen (1997)) and resting metabolic rates (MR in kJ/day) using Weir's equation (Weir (1949)):

$$\text{MR (kJ/day)} = (3.9 \times \text{VO}_2\,\text{(mL/h)} + 1.1 \times \text{VCO}_2\,\text{(mL/h)}) \times \frac{24\,\text{(h)}}{1000} \times 4.184$$

Metabolic rates were measured for 2 hours, between 12:00 and 17:30, measuring two groups each day consisting of 7-8 individuals at a time. Air through the chambers, as well as reference readings from the outside air, were sampled every 10 minutes for 60s, with sample and reference air measured on the same analysers. We discarded the first hour of recording to allow sufficient air mixing and stable readings. We further removed any suspected erroneous samples with an RQ outside the normal range of 0.7-1.0 (excluding 27 samples out of 340, Schmidt-Nielsen (1997)), before calculating mean daily metabolic rates at the individual level (averaged across c. 6 readings per individual).

2.3 | Statistical analysis

All statistical analyses were performed in R (v4.4.0, R Core Team (2024)).

2.3.1 | Correlations between morphometric indices of individual condition

To establish the baseline associations between our measures of individual morphology (body mass, wing length, and the four feather structure traits), we used the 'Hmisc' package (v5.1.3, Harrell Jr (2024)) to calculate Pearson's R for each pair of variables (using individual-level mean values for each feather structure trait due to having repeat observations), without controlling for any covariates (which we refer to as 'full' correlations in figure 5.2a). We also computed 'partial' correlations (5.2a)

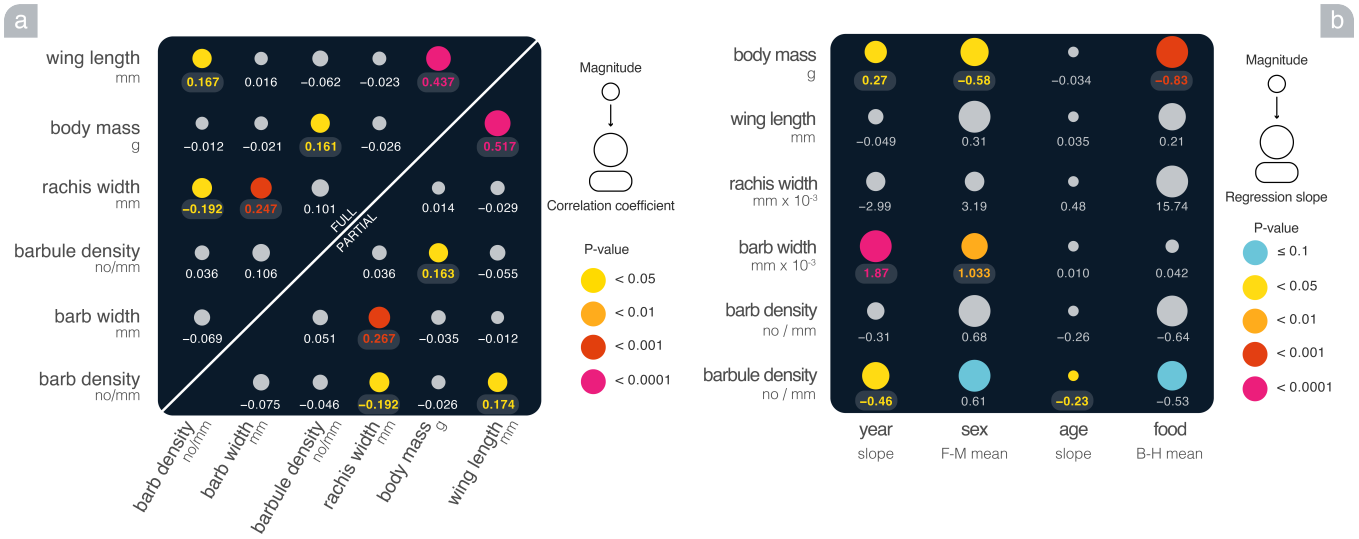


FIGURE 5.2 Summary of (a) the correlations between morphological measures of individual condition. Coefficients shown in the top left represent 'full' correlations between the raw individual-level values of each morphometric, whereas coefficients in the bottom right indicate 'partial' correlations between each pair of traits, after controlling for covariates that are likely to influence morphometrics values directly. The linear relationships between these covariates and each morphometric trait are summarised in panel (b), with regression slopes given for continuous variables like year and age, and mean differences for categorical variables like sex (M: male, F: female) and the food treatment (B: benign foraging costs, H: harsh foraging costs). Within each panel, coefficients are z-scored row-wise to allow visual comparison of the effect sizes.

using the 'ppcor' package (v1.1, ?) between each pair of morphometrics while controlling for covariates we suspected might influence their relationships, such as the *food treatment* ('benign' or 'harsh'), *sex*, *age* (in years) and *year*.

2.3.2 | Sources of variation in morphology and thermoregulation

To see whether the manipulated foraging costs caused any differences in individual morphology, we used the 'lme4' package (v1.1.35.3, Bates et al. (2015)) to test a series of mixed models, separately fitting each *morphometric* (body mass, wing length, rachis width, barb width, barb density, barbule density) as response and *food treatment* as fixed predictor. We also controlled for fixed effects of *sex*, *age* and *year* (included as fixed term for having only two levels, Gelman (2007)), and included a random term for *individual ID* to prevent pseudoreplication of individuals measured across both years. In models testing the effects on feather structural elements, we fitted the response as the mean of all replicate estimates from that individual in a given year. We ran models both with the raw response values, as well as their z-scored equivalents to be able to compare the effect sizes between variables that were on different scales (Schielzeth (2010)).

We measured insulation efficiency in both years, while metabolic rate in only 2024. Due to the high percentage of birds never observed in frame during the 3-minute recording period in 2023 (45% or 46/103 individuals), we attempted to adapt the thermal imaging protocol in 2024. However, this resulted in inconsistent practices between the two years, such as changes to the distance between camera and subject, as well as changes to the orientation of the birds relative to the camera lens, both of which can result in inaccurate temperature readings (Tabh et al. (2021)). Therefore, the following models were run using thermography data collected in the first year only.

To test for any impacts of the food treatment on the birds' ability to thermoregulate, we ran mixed models with similar fixed effects as before (*food treatment*, *sex*, *age*), but this time fitting either *insulation efficiency* (T_{eye} - T_{body}) or *metabolic rate* (resting MR) as response. As the two thermoinsulative variables were measured in separate years, we did not need to control for *year*, nor *individual ID* in this case. We ran models with both the raw and z-scored values to allow comparison between variables.

2.3.3 | Relationships between individual morphology and thermoregulation

To understand how body condition (body mass and feather structure) may influence an individual's thermoregulatory mechanisms, we ran mixed models with either *insulation efficiency* or *metabolic rate* as response, and testing combinations of body mass with each of the feather structure estimates in turn (rachis width, barb width, barb density, barbule density) fitted as fixed effects. We also tested models with body mass and/or feather structure fitted as quadratic predictors, as a non-linear relationship between body mass and metabolic rate is well-documented (Nagy et al. (1999)). We ranked models based on their Akaike information criterion (AIC; Akaike et al. (1973)), and selected

'best fit' models with the lowest associated AIC scores separated by at least 2 values in difference (Burnham et al. (2011)) to identify which morphometric trait/s (and whether linear or quadratic) would best explain the patterns in thermoregulation.

Since body mass is a composite measure of body fat and lean mass, we also calculated a standardised mass index (as described in Peig and Green (2009)) using wing length as a proxy for skeletal size (Gosler et al. (1998)) to further disentangle the relationships between body condition (after controlling for overall size) and thermoinsulative performance. Just as before, we looked at the effects of manipulated foraging costs (models with response: *SMI* | fixed effects: *sex*, *age*, *year* | random effect: *ID*) before testing how well body condition may explain differences in thermoregulation (models with response: *insulation efficiency* or *metabolic rate* | fixed effects: *SMI* and *feather elements*, as well as their quadratic form, *food treatment*, *sex*, *age*)

2.3.4 | Variation in morphology and insulation as predictors of survival

Finally, to see if survival differences between individuals could be predicted by key morphological or thermoinsulative traits, we ran a series of generalised linear models (in base R) with a binomial distribution to fit *survival status* (survived to 2024, or died before 2024) as response, testing each morphometric (*body mass*, *wing length*, and the four *feather structure* traits) or thermoregulatory variable (*insulation efficiency* only, as metabolic rate was not measured in 2023) as a fixed effect in separate models, as well as including *food treatment*, *age* and *sex*. For visualisation purposes, we also compared the 2023 metrics of birds that survived and those that died between the first and second year of data collection, using linear models with z-scored *morphometric* or *insulation* as response, and *survival status*, *food treatment*, *age* and *sex* as fixed predictors.

3 | RESULTS

We found few but negligible associations between most morphometric variables (Pearson's r < 0.3 for all but one correlation). However, there was a moderate positive correlation between body mass and wing length (r_{full} = 0.437, p = 7.61 x. 10^{-9}), which became slightly stronger once we controlled for the covariates of year, sex, age and food treatment ($r_{partial}$ = 0.517, p = 4.90 x. 10^{-12}) (correlations summarised in figure 5.2a).

3.0.1 | Sources of variation in morphology and thermoregulation

There were significant differences in both body and feather metrics of birds that were recorded in both years (figure 5.2b); with individuals in the second year weighing more (on average by 0.27g, p = 0.0471, appendix figure D2) and growing feathers with thicker barbs (by 1.87

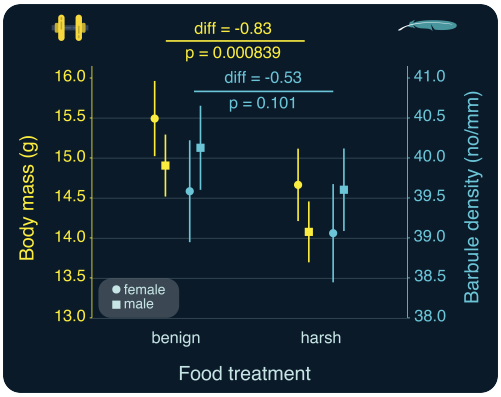


FIGURE 5.3 Comparison of the changes in morphometric indices of condition, showing the effects of the food treatment on body mass (left - yellow; a single measurement per individual in a given year) and barbule density (right - blue; a single measurement per individual averaged across 9 replicate measures in a given year), separated by sex. Circles (for females) and squares (for males) denote the predicted means, while error bars show 95% confidence intervals. Estimates are calculated for 2023, at the mean age (3.2 years).

x 10^{-3} mm, p= 6.4 x 10^{-8}) but with a lower density of barbules (- 0.46 no/mm, p = 0.0133, appendix figure D2). There were also significant differences between the sexes (figure 5.2b), with males weighing less (on average by - 0.58g, p = 0.0234, figure 5.3) and exhibiting feathers with wider barbs (by 1.033 x 10^{-3} mm, p = 0.0092) and higher barbule densities (by 0.61 no/mm, p = 0.0704, figure 5.3). The effect of age was the smallest relative to the other fixed effects (figure 5.2b), only significantly associated with a decline in barbule density (by - 0.23 barbules/mm with every year increase in age, p = 0.0177). Finally, the food treatment had a significant effect on body mass, with birds that experienced increased foraging costs weighing -0.83g less (p = 8.39 x 10^{-4}, figures 5.2b and 5.3). Moreover, we found that this decrease in body mass likely represented a loss of body fat, as birds under the two treatment conditions had similar skeletal sizes in terms of their wing length (harsh - benign difference = 0.21mm, p = 0.449, appendix figure D3), but individuals facing increased foraging costs had lower standardised mass indices (harsh - benign difference in SMI = -0.89, p = 3.39 x 10^{-5}, appendix figure D3). On the other hand, there were no significant effects on any of the variables related to feathers (figure 5.2), except that individuals from harsh foraging conditions grew feathers with a lower density of barbules, nearing significance (- 0.53 barbules/mm, p = 0.101, 5.3).

Variation in metabolic rate and insulation efficiency was not significantly associated with an individual's sex or age, although metabolic rate showed a slight decline with age (slope = - 0.93 kJ/day reduction each year, p = 0.1838), mass-corrected metabolic rates varied less in females (appendix figure D4), and males tended to have slightly better insulatory capacity (losing on average - 0.38 °C less heat through their plumage, p = 0.1288). However, both proxies of thermoregulation were affected by the food treatment, with individuals under harsh foraging conditions

(figure 5.4) expending less energy in the respirometry chambers (by -5.13 kJ/day, p = 0.0026), and exhibiting a weaker ability to retain their body heat (on average losing 0.46 °C more heat through their feathers compared to benign treatment birds, p = 0.0566). To test whether variation in insulation efficiency indeed reflected differences in heat loss through the plumage, rather than caused by shifts in core heat production, we compared surface body (T_{body}) and eye (T_{eye}) temperatures under the two treatment conditions. We found that while temperatures measured on the body were 0.71°C higher in the harsh treatment (p = 0.0379), measurements taken from the eye region did not differ significantly (harsh - benign difference = 0.19°C, p = 0.445, appendix figure D5), thereby suggesting that our proxy for insulation efficiency captures differences in the ability to retain heat through the plumage.

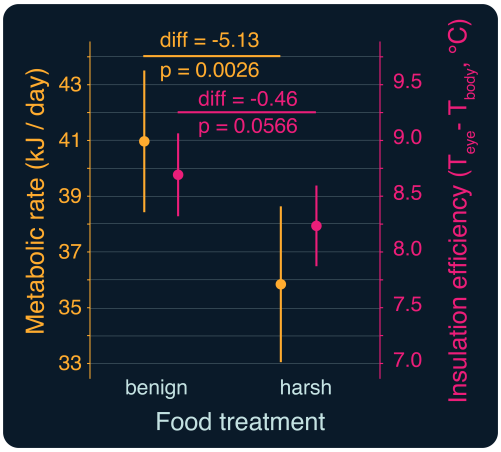


FIGURE 5.4 Comparison of the changes in physiological indices of condition, showing the effects of the food treatment on metabolic rate (left - orange; a single measurement per individual, averaged across c. 6 replicate measures) and insulation efficiency (right - pink; a single measurement per individual averaged across c. 6 replicate measures). Points denote the predicted means, while error bars show 95% confidence intervals. Estimates are calculated for females at the mean age (MR: 3.5 years, insulation: 3.0 years).

3.0.2 | Foraging costs alter individual morphology and thermoregulation

Overall, manipulating the costs that birds face while foraging for food had a much greater impact on body condition (in terms of body mass) than on plumage quality (in terms of barbule density), illustrated by an almost doubling of the standardised difference in body mass between benign and harsh treatment groups (- 0.59 SD, p = 8.39 x 10^{-4}) as opposed to barbule density (- 0.30 SD, p = 0.101). Differences in resource availability also affected the birds' ability to thermoregulate. The strongest observed responses to the food treatment were in terms of

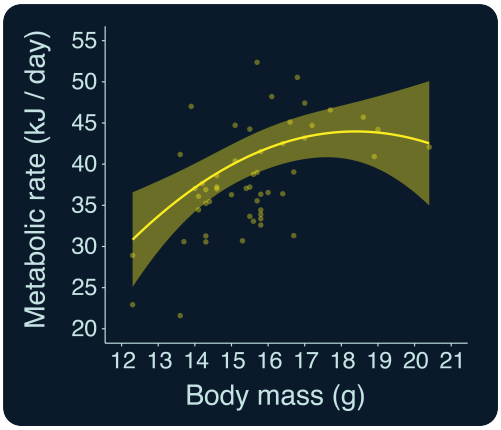


FIGURE 5.5 Relationship between body mass and metabolic rate. Points represent individual-level averages of c. 6 samples of metabolic rate and a single measure of body mass. Solid line shows mean predictions for benign treatment females at the mean age (3.5 years), and shaded areas indicate 95% confidence intervals. Log_{10}-transformed relationship with body mass is shown in appendix D6 to allow comparison against the wider literature.

changes to metabolic rates (- 0.81 SD lower for birds from harsh conditions, p = 0.0026), but the impact on insulation efficiency was also notable (- 0.50 SD lower in birds experiencing increased foraging costs, p = 0.0566).

3.0.3 | Differences in metabolic rate are associated with body condition, not feathers

To investigate whether changes in morphology might be linked with thermoregulatory dynamics, we tested various combinations of body and plumage quality indices (body mass, SMI, wing length, and the four feather structural elements) as explanatory variables in models partitioning the variance in thermoinsulative traits. We found that differences in metabolic rates among birds could be explained by body condition primarily, as models incorporating feather metrics did not improve AIC estimates. In particular, we observed a positive but asymptotic relationship between body mass and metabolic rate (figure 5.5), where initially, increases in body mass corresponded with elevated metabolic rates, however, beyond a certain threshold, further increases in body mass did not lead to higher metabolic rates. This suggests that metabolic rates do not scale linearly with body mass, but instead plateau at larger body sizes. To test whether differences in metabolic rates were due to differences in body condition (i.e. amount of body fat) after controlling for overall skeletal size, we also tested models with SMI instead of simply body mass, and found a very similar asymptotic relationship with a slightly better fit to the data (AIC_{SMI} = 327.74 instead of $AIC_{body\ mass}$ = 330.30, appendix figure D7).

3.0.4 | Optimal insulation efficiency is achieved at intermediate barbule densities

On the other hand, variation in insulation efficiency could be best explained by differences in plumage quality primarily, as the inclusion of body mass or SMI in models did not improve their fit (in terms of AIC). In particular, we found a quadratic relationship between barbule density and insulation (figure 5.6), where the greatest differences between eye and body temperatures (i.e. amount of heat retained) were associated with intermediate values of barbule density. This suggests that there might be an optimum value for barbule density in relation to the amount of heat that can be retained by feathers. Interestingly, birds from the harsh condition reached somewhat lower levels of insulation at the optimal barbule density value compared to their benign treatment counterparts.

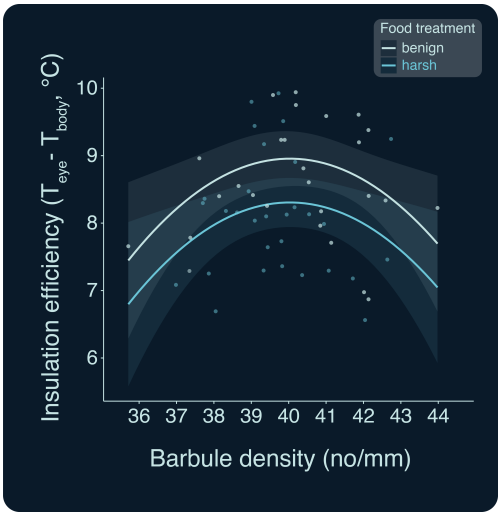


FIGURE 5.6 Relationship between barbule density (number of barbules / mm of barb) and insulation efficiency (temperature difference between eye and body region). Points represent individual-level averages of c. 6 samples of insulation efficiency and 9 measures of barbule density. Solid line shows mean predictions separated by food treatment (benign or harsh foraging costs), estimated for females at the mean age (3 years), and shaded areas indicate 95% confidence intervals.

3.0.5 | Shifts in morphology and insulation do not significantly impact survival probability

When testing whether foraging treatment-induced changes in individual morphology and thermoregulation were linked to differences in survival rates, we found that none of our measures of individual condition could predict whether a bird survived to the following year. This was further illustrated by the fact that survivors and non-survivors across both food treatment groups did not differ significantly in any of the traits tested

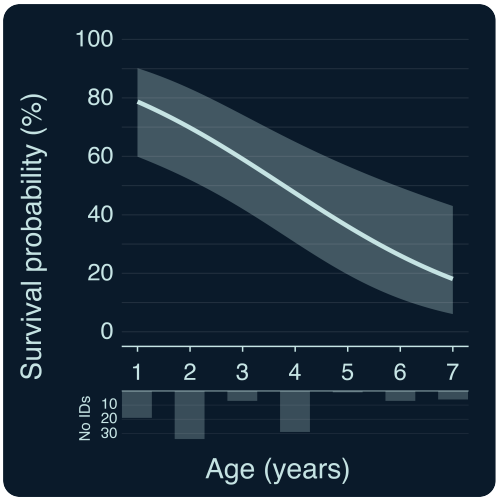


FIGURE 5.7 Relationship between age and survival probability (proportion of individuals of a given age surviving between year 1 and 2). Solid line shows mean predictions estimated for benign treatment females, and shaded areas indicate 95% confidence intervals.

(as shown in appendix figure D8). Instead, we found a strong association with age (figure 5.7), whereby older individuals had significantly lower odds of survival across both sexes and food treatment groups (log-odds of survival drop by -0.470 with each year increase in age, $p = 6.06 \times 10^{-4}$), translating to a 60.6% drop from 78.7% survival probability at age 1 (the youngest) to 18.1% at age 7 (the eldest), representing a 3.85-fold increase in mortality rates (from 21.3% mortality at age 1 to 81.9% at age 7).

4 | DISCUSSION

Overall, we found that foraging conditions significantly altered individual morphology and thermoregulation in our captive zebra finch population, but that these changes did not lead to significant differences in survival rates. These findings highlight the complex nature of responding to environmental stressors, with individuals constantly juggling the energetic trade-offs of adjusting various aspects of their morphology, physiology and behaviour.

4.1 | Prioritising feather quality over body condition

Individuals facing increased foraging costs had lower body mass, but similar feather structures, with the exception of a slight decrease in barbule density. The effect size of the manipulation was also stronger on aspects of body condition (body mass, and wing length corrected body mass: standardised mass index or SMI) compared to any element of feather structural condition. This suggests that bird may prioritise maintaining the integrity of their feathers, which they retain for a year

on average in this population (van der Heide, unpublished), compared to the more ephemeral nature of body mass and fat reserves, which can fluctuate more in response to day-to-day changes in environmental factors and individual behaviour.

The importance of maintaining structurally sound feathers may be even more pronounced for birds in the harsh treatment group that spend a larger proportion of their day flying due to the experimental feeder setup, as shown in earlier observations of this study system (Koetsier and Verhulst (2011)). As birds in both habitat types had unlimited food supply, the generally lower body mass observed in the harsh treatment group might not be the result of resource limitation, but rather it could be indicative of the energetic benefits of carrying less body mass for efficient flight (Witter and Cuthill (1993), Senar et al. (2002)).

4.2 | Insulation efficiency is maximised at intermediate barbule densities

Interestingly, our findings indicate that increased barbule density may not always offer better insulation efficiency, as previously suggested. Instead, we found a quadratic relationship, whereby individuals of intermediate barbule densities had the highest insulation capacity (i.e. lost the least amount of heat through their plumage). Perhaps this illustrates a trade-off between the different mechanisms by which feathers provide insulation. Specifically, a higher barbule density may be more effective for insulation via convection, as denser barbules create a more continuous, homogeneous barrier that limits the movement of air between the feathers, thereby minimising the displacement of warm air trapped near the body by the cooler air of the bird's surroundings (Dawson et al. (1999), Wolf and Walsberg (2000)). On the other hand, lower barbule densities might be more effective for insulation via conduction, by allowing more space for larger air pockets to be trapped, which form a poorly conductive barrier to prevent heat loss (Dawson et al. (1999), Wolf and Walsberg (2000)). Thus, intermediate barbule densities might strike a balance between the competing structural requirements of conductive versus convective insulation, thereby providing the most efficient overall insulation.

The quadratic relationship between barbule density and insulation was present in both treatment groups, however, birds experiencing increased foraging costs did not reach the same level of insulation even at the suggested optimal barbule densities. As neither body mass nor SMI was significantly associated with insulation efficiency, we rejected the idea that this insulation discrepancy could be explained by differences in body condition between the groups. Instead, perhaps the structural elements we measured might not provide a complete picture of the overall quality of feathers and there may be other aspects of feather quality in which harsh treatment individuals differed from the benign group. By spending a larger proportion of time foraging (Koetsier and Verhulst (2011)), their feathers may be subjected to increased wear-and-tear. Feather damage is more likely to occur as breakages and kinks near the wing tip, rather than in the form of individual barbules being lost in the middle of the feather where they are tightly interlocked and protected. Similarly, the food treatment could have caused differences in the amount of melanin deposited in the feathers (D'Alba et al. (2014)), which contributes to both its structural integrity and insulative power through infrared absorption (Dove et al. (2007)).

4.3 | The energy dynamics of thermoregulatory mechanims

Our results show that birds subjected to higher foraging costs were not only less well insulated but also had lower metabolic rates. This may reflect an energetic trade-off between dealing with the relative risks of starvation versus cold stress. The lower metabolic rate of harsh treatment birds points to a potential energy conservation mechanism at play, often seen across taxa when faced with resource limitation (McKechnie and Lovegrove (2002), Hohtola (2012)). Though a lower insulation efficiency could be detrimental, for small birds the risk of immediate starvation is often higher (Hohtola (2012)). Maintaining a higher metabolic rate to compensate for poor insulation would increase their metabolic burden in an environment where they do not have the means to adequately increase their food intake. Moreover, the outdoor aviaries still provide a more sheltered environment where the selective pressures for insulation may be weaker. Therefore, in this system, the costs of having a higher metabolic rate may outweigh the costs of having a decreased insulation efficiency. Additionally, birds may use alternate mechanisms to keep warm, for instance huddling together (Gilbert et al. (2010)) or reducing activity to conserve energy (Ben-Hamo et al. (2016)). These behaviors allow birds to conserve heat without the need to increase metabolic expenditure.

On the other hand, the lower metabolic rates of birds in the increased foraging costs group may not reflect adaptive responses. Instead, this observation might simply be explained by the lower body mass of harsh treatment birds and the positive relationship between metabolic rate and body mass (seen across species; McKechnie and Lovegrove (2002)). Additionally, a lower metabolic rate may also signal an overall weakened physiology of individuals facing higher foraging costs, as previous research on zebra finches has found a decrease in metabolic rates upon immune system activation (Sköld-Chiriac et al. (2014)).

4.4 | No apparent effects on survival

Despite the manipulated foraging costs significantly altering the morphology and thermoregulation of birds, these changes did not lead to significant differences in mortality in our study, and instead, survival rates could be predicted by age. This suggests that while morphology and thermoregulation respond to environmental conditions, they may not be the primary drivers of survival in this population. Equally, it might indicate that individuals under harsh foraging conditions were able to mitigate any adverse environmental effects adequately. However, due to the short timescale and small sample size of our survival

data, we likely lack the statistical power to distinguish small differences in survival effects. Additionally, the more sheltered conditions provided by the aviaries may have buffered the birds from environmental stressors that would otherwise impact their survival in the wild, such as the potential impacts of the morphological shifts on flight efficiency and predator avoidance dynamics (Gosler et al. (1995), Møller and Nielsen (2018)). Despite no apparent effects on mortality, the morphological and physiological changes induced by the foraging cost treatment could have longer-term consequences on other aspects of fitness, such as reproductive success, as previous work on this population has revealed that females experiencing elevated foraging costs tend to lay smaller clutches (Briga (2016) chapter 4).

4.5 | Concluding remarks

In this study, we tested for any evidence of plastic responses in feather structure under manipulated foraging conditions, to address the missing links between plumage structural quality and thermoregulation. Although our research provided novel insights into the relationship between feather structure and insulation efficiency, namely that heat retention is maximised at intermediate barbule densities, these findings rely on the assumption that structural elements of the wing feathers provide a good proxy for the overall plumage quality of an individual. However, future studies should explore the relationship between feather structure and insulation across a broader range of feather types to better understand the functional significance of feather variation in thermoregulation. Moreover, our data showed clear shifts towards decreased insulation efficiency and a lowering of metabolic rates in response to resource limitation. While we suggest that an indirect comparison between these trends can provide valuable inferences at the experimental treatment level, future work aimed at improving our understanding of the trade-offs between these thermoregulatory mechanisms as responses to environmental stressors should collect individual-based data on both metrics simultaneously.

AUTHOR CONTRIBUTIONS

A.K. conceived and planned the project with the help and supervision of C.R., E.C. and B.S. M.T. and S.V. carried out the food manipulation experiments. A.K. collected the data, with help from M.T., under the guidance of S.V. A.K. analysed the data. A.K., C.R., E.C., S.V. and B.S. contributed to the interpretation of the results. A.K. produced the figures and wrote the chapter, with feedback from B.S.

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ETHICAL NOTE

All methods and experiments were performed under approval of the Central Committee for Animal Experiments (Centrale Commissie Dierproeven) of the Netherlands, under license AVD1050020174344.

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APPENDIX D

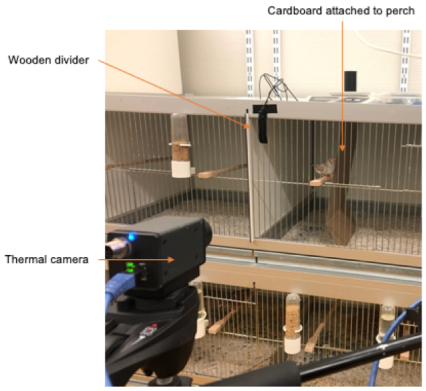


FIGURE 1 Infrared thermography data collection set-up, showing the thermal camera placed 0.5m from the cage, and a bird encouraged to perch close to the grid by the placement of a cardboard panel.

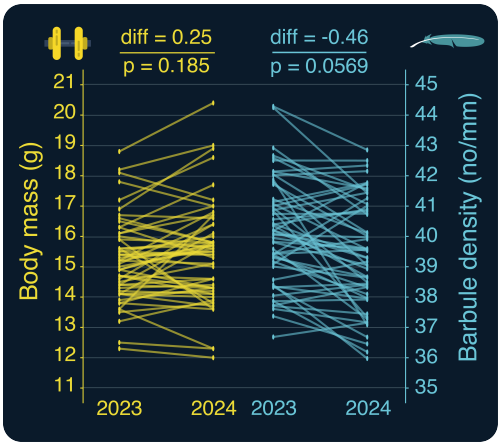


FIGURE 2 Comparison of the changes in body mass (left - yellow) and barbule density (number of barbules / mm of barb; right - blue) between the two years of data collection. Lines connect morphometrics taken on the same individual in two separate years. Points for body mass represent a single value for a bird in a given year, whereas barbue density is given as an average of 9 estimates for a bird in a given year.

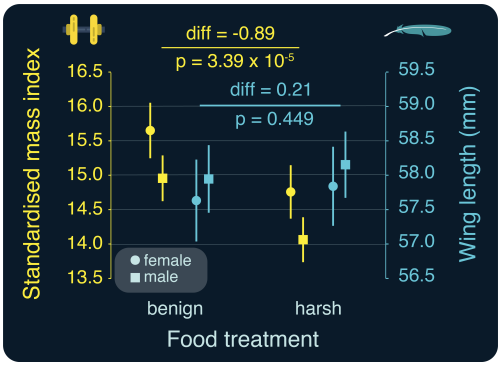


FIGURE 3 Comparison of the changes in standardised mass index (SMI; body mass scaled by wing length, shown left - yellow) and wing length (right - blue) in response to the food treatment (benign or harsh foraging costs). Both SMI and wing length use a single value for each bird in a given year. Circles (for females) and squares (for males) denote the predicted means, while error bars show 95% confidence intervals. Estimates are calculated for 2023, at the mean age (3.2 years).

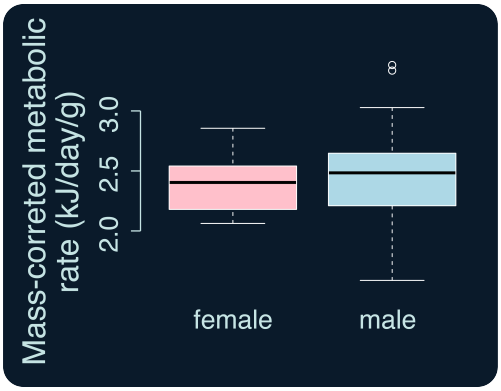


FIGURE 4 Boxplots comparing the distribution in metabolic rates corrected by body mass between the two sexes. Solid lines show the median, boxes represent the interquartile range (IQR), whiskers are drawn at 1.5 x IQR beyond the box, and points represent outliers.

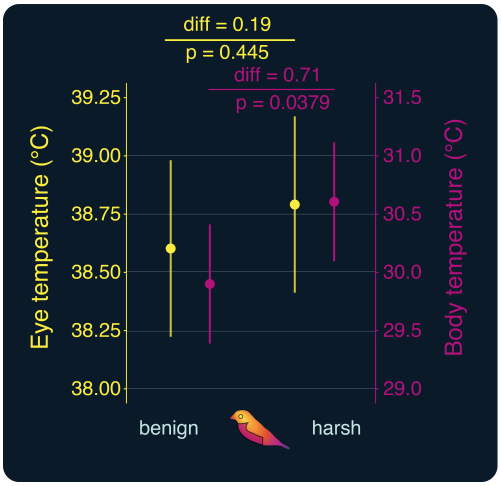


FIGURE 5 Comparison of the changes in eye (left - yellow) and body region (right - purple) temperatures as a result of the food treatment (benign or harsh foraging conditions). Both eye and body temperatures were extracted from c. 6 thermal images and averaged for an individual. Points denote the predicted means, while error bars show 95% confidence intervals. Estimates are calculated for females at the mean age (3 years).

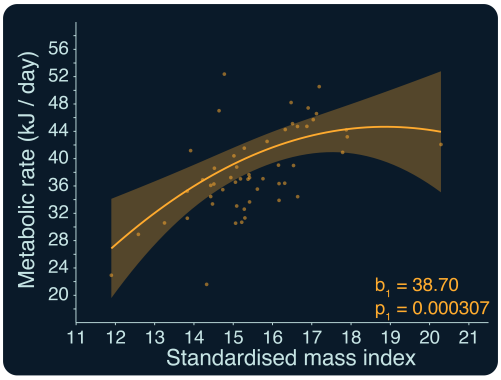


FIGURE 7 Relationship between standardised mass index (SMI = body mass scaled by wing length) and metabolic rate. Points represent individual-level averages of c. 6 samples of metabolic rate and a single value for SMI. Solid line shows mean predictions for benign treatment females at the mean age (3.5 years), and shaded areas indicate 95% confidence intervals.

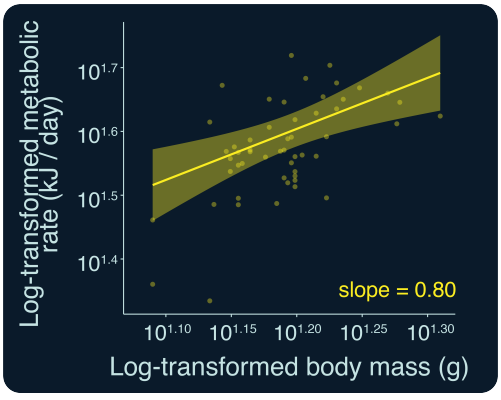


FIGURE 6 Relationship between $\log_{10}$-transformed body mass and metabolic rate. Results are estimated from a linear model with log-transformed metabolic rate as response, with food treatment, sex, age, barbule density and log-transformed body mass as predictors. Points represent individual-level averages of c. 6 samples of metabolic rate and a single value for body mass. Solid line shows mean predictions (slope = 0.80) for benign treatment females at the mean age (3.5 years), shaded areas indicate 95% confidence intervals.

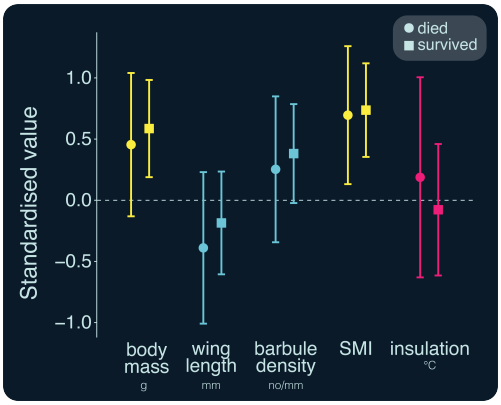


FIGURE 8 Comparison of the morphometric and insulative traits of birds measured in the first year of data collection, divided into groups based on individuals that survived to year 2 (circles) and those that died (squares). Points show predicted group means, z-scored for comparability, with error bars indicating 95% confidence intervals. Estimates plotted are for benign treatment females at the mean age (3 years).

6 | GENERAL DISCUSSION

Over the course of this thesis, I explored the complexity of life-history traits and the extrinsic and intrinsic factors that underlie their variation in birds, using a combination of theoretical, experimental and observational approaches.

6.1 | Questions addressed in this thesis

6.1.1 | How are life-history traits linked in seasonal environments?

In **Chapter 2**, I developed an agent-based model simulating the breeding behaviours of great tits (*Parus major*) and showed that different environmental conditions (temperature and resource availability) favour different optimal combinations of seasonal reproductive timing (lay date) and reproductive effort (clutch size) in terms of maximising the number and quality of offspring raised.

Specifically, I found that variation in lay dates of successful breeders (that fledged at least one young) was driven by both annual mean and seasonality in temperatures, such that increasing annual means resulted in population average lay dates to advance, but to a lesser degree under high temperature seasonality. On the other hand, variation in clutch sizes was only driven by differences in temperature seasonality, whereby successful breeders as a whole laid larger clutch sizes under high seasonality.

However, importantly, the optimal strategy that maximised fitness in each environment was not simply a combination of the average lay date and clutch size of successful breeders. Instead, the different optimal strategies that emerged under each environmental scenario likely reflected a shift in the trade-offs between the number and quality of offspring; that there is a difference between the clutch size that is possible under each environment, versus the one that actually results in high quality young. Similarly, birds may be able to fledge young within a range of dates that coincide with the presence of caterpillars, but the lay date that balances both the number and quality of young needs to also consider the time and energetic requirements of different sized clutches. For instance, it takes longer to lay a larger clutch (as tits lay one egg each day), which shifts the relative start date of chick provisioning and therefore its synchrony with the food peak as well as with the peak in competition with other provisioning parents. Indeed, across all environments, the earlier layers achieved the highest fitness, whereas the length of the caterpillar availability determined the optimal clutch size. In particular, high values of both annual mean and seasonality in temperature severely shortened the window of resource availability, constraining the range of optimal strategies that could

emerge. Overall, these patterns illustrate how different aspects of the environment may independently drive individual life-history traits towards different values, but that ultimately, fitness is maximised under an optimal compromise between multiple traits.

It was reassuring to find that optimal lay dates of the simulated populations also advanced under increasing annual mean temperatures, as we see this pattern in many species across taxa as a response to global warming (Thackeray et al. (2016)). However, the patterns in simulated optimal clutch sizes did not align with my initial predictions. Given the simplified dynamics of a significantly reduced number of factors interacting in my models compared to the complexity of the real world, I expected that the theoretically best strategy emerging from the simulations would consistently present larger clutch sizes than what birds in nature can realistically raise. As discussed in **Chapter 2**, the significant discrepancy between simulated and observed clutch sizes may be explained biologically (e.g. real birds are short-lived and invest more early in life, whereas simulated birds only consider decisions within a single year), but the effects of particular model specifications are the more likely culprit (e.g. a uniform clutch size distribution leading to a higher proportion of the breeding population struggling to provide for their offspring). Therefore, future work on these models should be informed by appropriate sensitivity analyses; testing how deviations in each model parameter (e.g. action costs) affect the outcome.

6.1.2 | How can we collect and analyse large amounts of feather quality data fast?

In **Chapter 3**, I introduced a novel data collection and analysis framework for extracting feather structural information from images taken on live birds of different species, that provided the tools for exploring how variation in plumage quality might mediate life-history trade-offs over different timescales.

Traditional methods to describe feather structural quality rely on invasive and time-consuming techniques of plucking feathers for manual counting and measurement of different structural elements under a microscope (unless looking at fault bars). Though plucking body feathers is arguably not heavily invasive, removing feathers of the tail and wing critical for flight need serious ethical considerations. Moreover, it could limit research into questions that deal with the consequences of individual differences in plumage quality, as the energetic costs of feather replacement can have considerable effects on survival (Jenni and Winkler (2020) chapter 3.4), and the degree of which can vary significantly between individuals.

Instead, the data collection protocol I developed for this thesis makes use of an inexpensive digital microscope (compared to traditional ones) that is also lightweight and portable (powered by USB connection to laptop), allowing data collection in the field directly on live birds, whether small or large (e.g. swans). However, this microscope is only capable of magnification up to

x200, therefore any structures smaller than barbules are unlikely to be captured clearly, especially with the increased likelihood of motion blur. Taking photographs of live birds is quick and easy on wing and tail feathers, however, imaging feathers of the body are unlikely to be achievable in a standardised manner (though I have managed to photograph the body feathers of museum specimens).

After improving the efficiency of data collection, I also needed to address the question of how to analyse a large backlog of feather images. To my knowledge, there are no tools as of yet that can identify and measure different feather structural features from images. Without the knowledge, or indeed a large enough dataset to use machine learning, I drew inspiration from simpler image analysis techniques commonly used in the biomedical sciences fields, to build a tool that could bridge this gap. Though there is still some work to be done, mainly in terms of flagging and filtering erroneous measurements due to low picture quality or irregularities in the feather, I hope that once all the functionalities of `featheRscan` are fine-tuned and released as an R package, it will provide a useful tool for the significantly faster analysis of vast amounts of feather images compared to manual counting.

6.1.3 | Can plumage quality mediate life-history trade-offs over different timescales?

In **Chapter 4**. I tested the role of feather quality in the short-term trade-off between the number and quality of offspring by manipulating the brood sizes of blue tits (*Cyanistes caeruleus*) and found significant impacts on both the feather and body condition of chicks. And in **Chapter 5**, I investigated how feather structure relates to insulation efficiency in zebra finches (*Taeniopygia guttata*) to begin to understand how plumage quality might link life-history traits over longer time frames through survival. I found that manipulated foraging costs altered feather condition to some extent, with intermediate barbule densities providing the best insulation, however differences in plumage quality did not result in differential survival in this system.

In order for plumage quality to act as a link between life-history traits, we need to show evidence that it is influenced by energy or time limitations, and that it impacts functions essential for survival. Firstly, manipulated differences in resource availability (via nestling competition in blue tits, and altered foraging costs in zebra finches) affected individuals' feather structures across both species, although in somewhat opposite directions. Brood enlargement in blue tit nestlings, which is intended to increase competition and therefore decrease resource allocation to each chick, resulted in the growth of higher barbule density feathers. On the other hand, increased foraging costs in zebra finches, which are intended to reduce resource intake, caused birds to grow lower barbule density feathers (though it was not statistically significant). However, across both species, the manipulated resource availability caused a greater impact on body mass, with an increase in brood size and in foraging costs significantly decreasing the body mass of both blue tits and zebra finches, respectively. This suggests that there

may be some inherent trade-offs between allocating resources to body and feather growth. Given that body mass in small birds is highly variable (Blem (1976)), birds may prioritise investment into more permanent structures, like feathers, when faced with resource or time constraints. It could also simply reflect that feather structure as a trait is less influenced by environmental factors compared to body mass. Indeed, variance estimates from the cross-fostering of blue tit chicks showed that the relative contributions of nest of rearing (ẽnvironmental effects) and nest of origin (g̃enetic effects) to the variation in feather structure are less pronounced compared to those in body mass (with environment:genetic proportions of 11:6% in feathers and 36:12% in body mass).

In terms of the consequences of variation in feather structure on survival, I could not discern any clear pattern of direct links across either species. In blue tits, brood size enlargement consistently resulted in decreased nestling survival, along with lower body mass and, surprisingly, higher barbule density feathers. The main function of feathers while still in the nest is their contribution to thermoregulation. Therefore, given that larger broods are less prone to experiencing hypothermia (Andreasson et al. (2016)), the structure of feathers are unlikely to directly impact survival before fledging, and instead, the increased mortality of enlarged broods likely resulted from their drop in body mass. Similarly, variation in the feather structure of zebra finches did not result in significant survival differences. Nor did variation in any other morphological trait tested, even though harsh foraging treatment birds suffered significant loss of body mass. Interestingly, variation in feather structure was associated with insulation capacity, whereby heat loss through the plumage was minimised at intermediate barbule densities, perhaps illustrating an optimal balance between the different primary mechanisms (convection vs conduction) by which feather structure contributes to insulation. However, differences in insulation capacity were not associated with significant differences in survival probability, perhaps due to birds being housed in (although outdoor) aviaries that might shelter them against selective pressures they would otherwise experience in the wild. Therefore, the direct links between how feather structure affects the survival of birds in the wild through its potential consequences on insulation, remains to be further researched.

6.2 | Additional questions raised

6.2.1 | Why is feather structure highly variable within individuals?

One of the unexpected findings of my thesis was the fact that measures of feather structure, mainly barbule density, were highly variable between feathers of an individual across all species. Firstly, it highlights the need to image multiple feathers when characterising the plumage quality of an individual. Secondly, it begs the question whether the structure estimated from a few feathers can reliably act as a proxy for the overall plumage quality of a bird. It is obvious that different feather types

(such as flight vs body feathers) will have overall different structural architectures (e.g. rigidity, proportion of plumulaceous vs pennaceous sections) as they fulfill different primary functions (e.g. flight vs insulation). But it is interesting to consider that even between feathers associated with the same overall function, in this case primaries adapted for flight, there may be consistent differences perhaps related to specialised sub-functional roles, such as their positioning within the wing shape. Such a pattern was highlighted by Dawson and colleagues (Dawson (2005)), who found that across several species, the outer primaries close to the wing tip exhibited higher mass relative to their length, perhaps providing greater rigidity to the leading edge of the wing, allowing it to cut through the air more efficiently, or have higher resilience against physical damage by being first to come in contact with the vegetation. Therefore, a systematic approach to characterise the quality of feathers across several feather tracts within an individual (and repeated across individuals) would provide useful information about whether the structural quality estimated from a few feathers provides a reliable proxy for the overall individual condition. If this proves to be the case, it might be an additional fast and easy way to estimate population health, whether from moulted feathers recovered from a study site, or measured directly on a subset of individuals.

6.2.2 | Why do decreased broods behave opposite to our expectations?

Another curious result arose from the brood size manipulation experiments on blue tit nestlings (**Chapter 4**), where decreased broods showed patterns inconsistent with expectations, and instead behaved more similarly to increased nests, especially at higher starting clutch sizes. Rather than producing chicks with higher body mass and increased fledging success, parents who raised reduced brood sizes fledged fewer and smaller young. Unexpected responses to brood reductions have been noted elsewhere (Nord and Nilsson (2012)), yet it remains unclear why these patterns arise. One possible explanation might be that parents perceive the removal of nestlings as a predation event and are therefore more wary to return to provision at the nest. Even if not perceived as predation, parents might decide to invest less into a brood that has already shown signs of failure. However, both of these explanations rely on parents being able to accurately count the number of nestlings before and after manipulation – evidence for which is contradictory (e.g. great tits can and do alter feeding rates in response to manipulated brood sizes Pettifor et al. (2001), but do not seem to adjust defence tactics Regelmann and Curio (1983)).

6.2.3 | How might plumage characteristics vary under continued environmental change?

Given that population-level estimates of plumage quality seem to be highly variable between years in zebra finches (**Chapter 5**), and genetic factors contribute relatively little to differences between individuals in blue tits (**Chapter 4**), it is unlikely that

we would observe any strong directional shifts in feather structure over a short timescale as a direct response to warming temperatures. Alternatively, climate change may have an indirect effect on feather quality through its influence on decisions about key life-history traits within the annual cycle and their interactions with moult. For instance, an advance in lay dates (often observed in response to warming spring temperatures, Parmesan and Yohe (2003), Thackeray et al. (2016)) could result in a more frequent occurrence of second broods (e.g. as observed in some great and blue tit populations, Glądalski et al. (2016)), thereby extending both the length and investment into the breeding season. A delay in the conclusion of breeding activities could, on the one hand, delay the onset of moult, leading to time constraints, or, on the other hand, increase the likelihood of moult-breeding overlaps – with both scenarios potentially impacting feather quality (e.g. producing shorter, lighter feathers Nilsson and Svensson (1996), Vagasi et al. (2012), Echeverry-Galvis and Hau (2013)).

Climate change has also increased the frequency of extreme climatic events (ECEs), including both unusually hot summer days as well as unexpected cold spells (Met Office (2018)). It is therefore unclear whether the main selective pressures shaping feather quality in the future will be related to heat retention or heat dissipation strategies (though the limiting effects of heat dissipation appear to be stronger Grémillet et al. (2012)), both of which present a significant energetic challenge, especially for small birds (Brodin et al. (2017)). The increased unpredictability of seasonal patterns in temperatures, however, might make it more difficult to predict and match plumage structure to upcoming ecological pressures. An overall increase in annual mean temperatures might encourage birds to produce feathers that dissipate heat better (e.g. shorter, with lower density of barbs and barbules), which is likely to carry fitness benefits, such as increased reproductive success and better survival (e.g. feather-clipped parents are able to rear larger offspring and maintain their own body mass better in blue tits, Nord and Nilsson (2019)). However, the same structure might not provide the most efficient insulation in the face of a sudden drop in temperatures, possibly affecting over-winter survival in temperate regions. Moreover, the change in the thermal microclimate might differ between habitat types, especially in urban environments (e.g. 'urban heat islands'; Oke (1982)). For instance, urban-raised nestlings tend to produce contour feathers with a lower density of barbs (Sándor et al. (2022)).

Besides a shift in the thermal environment potentially affecting the optimal feather structure, a shift in key resources necessary for feather production may also impact plumage quality in the future. In the case of organisms specialised on a seasonally occurring food source to complete different stages of their life cycle (e.g. reproduction, moult), differential shifts in life-history activities in response to climate change could disrupt some of the temporal overlaps in predator-prey dynamics. For instance, many birds adjust their diet during breeding and/or moult to increase protein intake required for feather production (e.g. great and blue tits rely on an abundance of winter moth caterpillars to provision their young (Perrins (1965)), many seabirds depend on the seasonal availability of sand eel to successfully raise offspring Rindorf et al. (2000)), and ordinally granivorous zebra finches switch to insect consumption during moult Pap et al. (2008)). With many species shifting the timing of key life-history

events in response to global warming (Parmesan and Yohe (2003), Thackeray et al. (2016)), many instances of mismatches between predator and prey have also been reported (Durant et al. (2007), Visser et al. (2012), Burgess et al. (2018)). It is unclear whether or how this would lead to constraints on plumage development, both within the nest and during moult, but given some observed negative effects of a protein-resitricted diet on feather quality (e.g. shorter, lighter feathers in house sparrows Pap et al. (2008)), future disruptions to food chains as a result of climate change may bring about similar consequences on plumage structure.

6.3 | Future directions

Though the ideas explored in this thesis have contributed to our understanding of how individuals may optimise multiple life-history traits under environmental change, some aspects of the techniques presented here could be further improved or combined with additional approaches to answer some of the follow-up questions that emerged from the results.

Firstly, although the trade-offs between lay dates and clutch sizes have been well-established (Rowe et al. (1994)), we have less of an understanding of how their combined optimal expressions may shift under different environmental contexts. Therefore, by building the agent-based model presented in **Chapter 2**, I tried to create some of the building blocks that might help us further investigate how multiple life-history traits may be co-optimised in response to environmental variation. Particularly hoping that it may be able to give us more accurate insights into how future climate change is expected to alter the links and balances between competing life history optima. To do this, however, the ABM presented here should be adjusted to consider other mechanisms by which organisms respond to environmental change.

For instance, we know that recent climate change has caused shifts in the phenology of many taxa, and that there is variation in the degree and direction of these shifts between species (Thackeray et al. (2016)). Therefore, it is not enough to simply simulate how species interact with the environment and each other given their current temperature sensitivities, as these might shift in future, and potentially to varying degrees. Instead, we could gain a more comprehensive picture by adjusting the current ABM's parameter values by setting different threshold temperatures that cue caterpillar and bird activities. This would allow us to test how potential changes to temperature sensitivity through microevolution might alter the range of likely shifts in life history optima under different environmental scenarios.

Additionally, the study of moult within the annual cycle and the role of plumage quality mediating life-history trade-offs appears to be a promising avenue of research needing further investigation. The agent-based model of **Chapter 2** could also be expanded to include decisions about moult, such as its onset (seasonal timing of the first feather replaced), intensity (number of

concurrently grown feathers), and its total duration. Taking inspiration from stochastic dynamic models investigating the optimal sequence of primary moult in migratory (Barta et al. (2008)) and non-migratory birds (Barta et al. (2006)), feather quality could be coded to deteriorate with time, while also influencing foraging efficiency, thereby directly linking feather quality to survival. Though previous models considered breeding as a key event in the annual cycle, they only included behaviours such as 'start breeding', 'care for young' or 'abandon brood'. Including decisions about the timing of laying, timing of incubation, and about clutch size, besides actions related to moult timing, intensity and duration, would help further test the role of feather quality linking life-history trade-offs over both short and long timescales.

For instance, such a model would allow us to explore how the trade-offs between breeding and moult might be shifted under different levels of environmental change. As suggested in **section 6.2.3** above, warming spring temperatures might encourage a higher frequency of multi-brooded individuals that may delay the end of breeding and therefore the onset of moult, with potential downstream effects on plumage quality. It would further allow us to explore whether moult constraints and/or moult-breed overlaps are more likely to occur under different environmental contexts, and how this may alter the relationships between investing into current reproduction (via clutch size) versus parental survival (via feather quality after moult). Similarly, a model including both breeding decisions combined with feather growth dynamics would enable investigation into how parental investment (clutch size, foraging rate) and timing of reproduction (laying date, incubation start, including incubation delays, hatch date) might influence offspring feather structure grown in the nest. In turn, it would allow us to test whether offspring feather structure could better explain patterns of first year juvenile mortality rates, as opposed to relying on transient predictors of success, such as body mass at fledging (Perrins (1965)).

Other patterns in life-history trade-offs may also be explained by their effect on plumage quality. For instance, a seasonal decline in reproductive success in birds has been well-documented (Hochachka (1990), Rowe et al. (1994), Verhulst et al. (1995)), but the mechanisms behind this pattern are not fully understood. Though previous research has highlighted negative effects of moult duration on plumage quality, we know very little about how the length of the nestling phase might influence the quality of the first feathers produced. Extrapolating from the above argument, one might hypothesise that late-hatched chicks (whether late within the season or relative to their siblings within the nest) might shorten their nestling phase to fledge at an earlier date and avoid the negative consequences of being late relative to conspecifics (e.g. experimentally induced late broods of great tits suffered significantly lower recruitment rates, Verhulst and Tinbergen (1991)). This may be achieved through an increase in feather growth speed, and/or by leaving the nest at a younger age with an underdeveloped plumage. The blue tit nestling plumage quality data presented in **Chapter 4** revealed a significant effect of hatch date on barbule density across both years, suggesting that seasonal patterns in offspring quality (e.g. as seen with body mass Verhulst and Tinbergen (1991)) extend to the plumage, as well. However, accurate information about individual fledging times, combined with feather quality

information prior to fledging are necessary to explore the mechanisms behind this pattern. Future projects investigating this relationship could employ radio-frequency identification (RFID) tags (such as those often attached to a bird's leg (McMahon et al. (2024)) and appropriate reading devices integrated into the nest boxes (Santema et al. (2024)) to gather real-time fledging data, alongside collecting feather quality metrics derived in the field using the digital microscope and image processing protocol described in **Chapter 3**.

Furthermore, we still do not have a clear understanding of what (if any) particular aspects of plumage structural quality could best predict survival once birds leave the nest and become independent. Neither blue tit nestling feather structure in **Chapter 4**, nor zebra finch plumage quality in **Chapter 5** could be linked to any significant differences in fledging success and survival to the following year, respectively. However, a significant relationship between barbule density and insulation capacity in zebra finches suggests that feather structure may at least be involved in functions related to survival. Future work should further investigate how feather structure relates to not only heat retention, but heat dissipation as well, as it is likely to be a greater energetically limiting factor influencing fitness under continued climate warming (Grémillet et al. (2012)). An experimental approach to address this could involve birds profiled for their feather structure (using techniques described in **Chapter 3**), and exposed to a range of temperatures in climate controlled rooms, where the heat transfer through the plumage could be measured using infrared thermography (as described in **Chapter 5**). Besides experimental studies on live birds, a systematic and non-invasive approach could make use of model wings with custom built synthetic feathers of different structural architectures (e.g. varying the density of barbs and barbules), combined with infrared thermography, to establish the direct causal relationships between feather structure and heat transfer.

To predict how plumage characteristics may shift under future global warming, we need to better understand how key elements of environmental change are associated with variation in feather structure. For instance, it is unclear whether temperatures experienced during feather growth have a direct effect on realised plumage properties. Studies have suggested that hotter nest temperatures may be responsible for the difference in feather structure between urban and forest birds (Sándor et al. (2022)). Observational approaches to test this relationship could include using small thermologgers placed inside nest boxes (e.g. iButton thermometers Simmonds et al. (2017)) to correlate average temperatures experienced in the nest with the quality of the juvenile plumage produced (using techniques as described in **Chapter 3**). Alternatively, experimental approaches could manipulate nest temperatures, either in the wild using heat packs (e.g. Andreasson et al. (2018)) or in captivity using climate controlled rooms.

Besides changes in temperatures, birds under future environmental change may experience shifts in resource availability, for instance due to mismatches with the temporal occurrence of their food source. It is not fully understood what dietary elements are required for feather production, except an obvious need for higher protein turn-over (Jenni and Winkler (2020)).

Therefore, future research should investigate whether shifts in the temporal overlap between feather growth and the availability of protein-rich food items are likely to impact plumage structural quality (as measured in **Chapter 3**). For instance, various sampling techniques (Zandt (1994)) exist to estimate caterpillar abundance over time in the classic great tit - winter moth system, which could be correlated with intra-seasonal patterns of nest-grown feather quality. Additionally, camera traps combined with automated image analysis techniques may be a flexible approach in many species to identify the temporal distribution of specific protein-rich food items brought to the nest, thereby improving our understanding of how climate change induced shifts in the temporal distribution of food resources might affect plumage quality in a natural setting.

6.4 | Concluding remarks

With this thesis, I aimed to contribute to our understanding of life history optimisation from a wide range of perspectives; by building models to understand the higher-level phenomena of how life-history traits are intricately linked under environmental variation, as well as testing the role of plumage quality in mediating life-history trade-offs over both short and long timescales, alongside developing tools that will allow more efficient data collection and analysis of feather structural variation for future research. In particular, the role of plumage quality in mediating life-history trade-offs is a promising but so far overlooked field requiring further investigation.

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