# The Properties and Potential Signal Value of Atlantic Puffin Bill Colouration

by

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# ABSTRACT

Conspicuous features in species with mutual ornamentation may be overlooked, especially concerning their potential adaptive function. Recent work has demonstrated that selection may act to produce and maintain features that are present in both sexes, but careful investigation is required to elucidate which selective force(s) are at play. The life history characteristics of the species, as well as the properties of the trait itself, are fundamental to generating informed hypotheses on what the adaptive function may be. In this thesis I explore the function of the colourful bill and rosette of Atlantic puffins (*Fratercula arctica*), a long-lived seabird with obligate bi-parental care. In Chapter 2, I assess whether bill colour aligns more closely with a signal of identity or quality based on three key properties: lability, condition-dependence, and degree of sexual dichromatism. My analysis had mixed results; bill colour was highly dynamic within individuals throughout the late breeding season, but no aspect of colour was condition-dependent and the sexes were monochromatic based on a model of avian visual perception. Although bill colour does not correspond to a traditional quality signal (i.e., correlation with body condition), it may signal a different aspect of individual quality, which I explore in Chapter 3. Specifically, I ask whether bill colour is related to parental quality, as reflected in offspring hatch date and growth patterns. Several aspects of maternal bill and rosette colouration predicted chick growth but not hatch date. Instead, timing of hatch itself was an important factor in determining patterns of offspring mass and wing growth. While these results provide a more nuanced picture of the colourful bill's role in Atlantic puffins, there is ample room for further investigation, as discussed in Chapter 4.

# CO-AUTHORSHIP STATEMENT

**Chapter 2** has been submitted for publication in *Ethology* and is available as a pre-print on bioRxiv (DOI: 10.1101/2023.04.11.536353). The following is a list of co-authors and their contributions to the manuscript:

Katja H. Kochvar – Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft/Review & Editing, Visualization

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## 1.1 Signal theory

Animals produce a dazzling variety of visual displays, from brightly coloured integuments to iridescent plumage. These colourful displays are often shaped by two common forms of sexual selection; male-male competition (i.e., intrasexual selection), whereby individuals with the most colourful or complex version of a display are best able to acquire and defend resources, or female choice (i.e., intersexual selection), in which these individuals receive the most mating opportunities (i.e., Andersson, 1994; Hill & McGraw, 2006b). Either of these processes may lead to individuals with the highest quality displays obtaining the highest fitness and thus, most genetic representation in the next generation. For displays to be sexually selected, they must stimulate the sensory system of another organism such that a change of behaviour occurs, a process known as communication or signaling (*sensu* Ruxton & Schaefer, 2011). In visual communication, as with all types of communication, an *informer* or *sender* stimulates a behavioural change in a *perceiver* or *receiver* by way of a *signal* (Owren et al., 2010; Ruxton & Schaefer, 2011). Not all conspicuous features are signals; only those that have evolved explicitly to alter the behaviour of a perceiver can be considered signals (Laidre & Johnstone, 2013; Ruxton & Schaefer, 2011; Searcy & Nowicki, 2005). A classic example that illustrates this distinction is the form of a bird's tail. Initially, tails evolved to aid in flight, but in some cases have been elongated beyond their optimum to influence females in mate choice contexts. In this case, the bird's tail itself is not a signal, but a property of the tail, namely its length, would be considered a signal (Searcy & Nowicki, 2005).

For a perceiver to notice and respond to a signal, the signal must be 1) perceptible, such that the perceiver can reliably detect the signal and 2) on average, honest, such that it benefits the

perceiver to respond to the signal (Johnstone & Grafen, 1993; Kokko, 1997; Searcy & Nowicki, 2005). Thus, when investigating a particular signaling system, the perceptual *umwelt* (i.e., environment, or self-centered world) of the perceiver must be considered (Caves et al., 2019).

The mechanism maintaining the honesty of a signal should also be taken into account. The handicap principle is one proposed mechanism for the maintenance of signal honesty, and it rests on the assumptions that 1) there is a cost to producing and/or maintaining a signal, 2) the cost differs based on the quality of the individual, and 3) only high quality individuals can successfully bear this cost (Johnstone & Grafen, 1993; Zahavi, 1975; Zahavi & Zahavi, 1999). Colourful traits may have production costs, such as acquiring and allocating sufficient pigment to a structure (i.e., carotenoid-based colouration), and/or maintenance costs, such as regular preening of feathers (i.e., iridescent plumage; Hill & McGraw, 2006b). The associated display of colourful features may also have costs, including energetic loss, material or temporal allocation from other vital tasks or processes, and exposure to predation events (Hill & McGraw, 2006b). Whatever the cost may be, it must be outweighed by the benefit to the informer in communicating information to the perceiver. This benefit is considered in terms of fitness, which is defined here as a contribution to survival and/or reproductive success (Endler, 1986). In avian species, a more colourful display might result in a higher quality territory, enhanced ability to defend a territory or group of receptive mates, more extra-pair mating opportunities, or higher investment by the female in egg yolks or broods, among others (Hill & McGraw, 2006b). Signals may also stay honest if their display is limited by a physical or physiological constraint of the informer. Such is the case with index signals, where the degree of trait expression is directly linked to an individual's quality (Bradbury & Vehrencamp, 2011). Pigmented-based colourful features can be limited by the physiological constraints of pigment absorption and deposition or the toxic effects of high concentrations in the body (i.e., carotenoid-based colouration; Simons et al., 2014; Svensson & Wong, 2011). Structural colours,

on the other hand, may be constrained by the physiological capacity, developmental environment, and/or genetic quality required to obtain precise feather arrangement and organization (Ghiradella & Butler, 2009; Shawkey et al., 2003; White, 2020). As with handicap signals, perceivers that notice and respond appropriately to index signals should receive fitness benefits.

## 1.2 Carotenoid colouration

### 1.2.1 Mechanisms of carotenoid colouration production

One form of pigmented colouration that is responsible for many of the yellow, orange, and red displays seen across avian species is based on a class of molecules termed *carotenoids*. Carotenoids not only act as a pigment in plumage and integumentary structures, but also play important roles in cellular processes, where they function primarily as antioxidants and immune-enhancers (Simons et al., 2014; Svensson & Wong, 2011). They are also a critical aspect of avian vision due to their presence in retinal oil droplets, where they serve to narrow the spectral absorption profiles of cone photoreceptors, permitting stronger discrimination between different wavelengths of light (Lim & Pike, 2016).

Carotenoids cannot be synthesized *de novo* in vertebrates and therefore must be acquired through the animal's diet (Goodwin, 1980; Hill & McGraw, 2006a). However, they can be modified by internal metabolic processes into related forms (McGraw, 2006). Most dietary carotenoids give rise to yellow pigmented structures, while metabolically converted ketocarotenoids are generally responsible for orange and red structures (Hill & McGraw, 2006a; Johnson & Hill, 2013)

### 1.2.2 Signal value of carotenoid colouration

Three non-mutually exclusive hypotheses have been proposed linking carotenoid pigmentation and individual quality: the foraging hypothesis (Endler, 1980; Hill, 1992), the trade-off hypothesis (Lozano, 1994; Moller et al., 2000), and the shared pathway hypothesis (Hill, 2011). The foraging hypothesis posits that the colour of a trait is directly linked to an individual's ability to forage; specifically, those who can catch more carotenoid-rich prey are able to produce more pigmented traits. Indeed, indicators of foraging ability have been linked to carotenoid-based colouration. For example, nestling provisioning rate was predicted by the yellow breast plumage of blue tits (*Cyanistes caeruleus*; García-Navas et al., 2012), and the propensity to travel offshore for prey was correlated with the gular skin ornament of brown boobies (*Sula leucogaster brewsteri*; Michael et al., 2018). Additionally, experimental manipulation of dietary carotenoids produces changes in colouration, indicating that carotenoid availability can limit trait expression in some cases (Koch et al., 2016), although carotenoids are not considered a limiting factor of colouration in most bird populations (Simons et al., 2012; Svensson & Wong, 2011b). It is important to note that strategies for absorption and utilization of carotenoids vary drastically among species (Svensson & Wong, 2011b), and high concentrations of circulating carotenoids do not necessarily lead to the production of colourful features. Indeed, most mammals lack the mechanisms to transfer carotenoids to hair (Tobin et al., 2005), such that many primates like the sooty mangabey (*Cerocebus torquatus atys*), orangutans (*Pongo pygmaeus*), and even humans (*Homo sapiens*) have high serum carotenoid concentrations but do not produce carotenoid-based displays, sometimes despite the presence of red-orange colouration (i.e., orange hair of orangutans has no detectable levels of carotenoids; Slifka et al., 1999; for an exception, see Galván et al., 2016).

The trade-off hypothesis instead focuses on the immune properties of carotenoids, arguing that there is a trade-off between immune function and degree of pigmentation such that only individuals with a strong enough immune system can afford the costs of using carotenoids to

produce a highly pigmented trait (Hamilton & Zuk, 1982; Lozano, 1994; von Schantz et al., 1999). This hypothesis is supported by work demonstrating that carotenoid supplementation results in not only more elaborate traits, but also stronger cell-mediated immune responses following phytohemagglutinin (PHA) injections (i.e., in zebra finches *Taeniopygia guttata*; Blount, 2003). However, it has been argued that tests of immune response without the use of live pathogens or parasites are biologically irrelevant and thus, insufficient to establish that carotenoids are essential to immune function (Koch & Hill, 2018). There is strong support for carotenoids functioning as antioxidants (Krinsky, 1989), but the overall relationship between antioxidation capacity and carotenoid-based colouration tends to be small and non-significant (Costantini & Møller, 2008; Simons et al., 2012). Yet, a link between carotenoid-based colouration and immune function can nevertheless be established in some species. For instance, in male blackbirds (*Turdus merula*), the carotenoid-based yellow-orange bill became significantly duller in the weeks following an immune challenge irrespective of changes in body mass (Faivre et al., 2003).

The shared pathway hypothesis similarly relates carotenoid pigmentation to health, but instead emphasizes the metabolic challenge of converting carotenoids from food into other usable forms, thus relating the overall condition and health of the individual to the trait colour. Ketocarotenoids, which are responsible for most red carotenoid-pigmented colouration, must be metabolically converted from dietary carotenoids (Johnson & Hill, 2013), imposing a potential energetic cost to the production of red features. General support for this hypothesis comes from a recent meta-analysis demonstrating that metabolically converted carotenoids rather than dietary carotenoids drive relationships between bird feather colouration and measures of individual quality (Weaver et al., 2018). One measure of individual quality directly related to metabolism is mitochondrial function efficiency, and in red crossbills (*Loxia curvirostra*), red ketocarotenoid-based ornaments seem to reflect this aspect of quality. Only individuals that initially had redder

plumage (and thus, were assumed to be initially high quality) exhibited higher feather ketocarotenoid concentrations and increased redness after being treated with a superoxide dismutase mimetic (mitoTEMPO) designed to increase circulating dietary carotenoid levels (Cantarero et al., 2020). As such, only high quality individuals were able to successfully exploit increased levels of dietary carotenoids for ornamentation (Cantarero et al., 2020).

## 1.3 Study species information

### 1.3.1 Life history

Atlantic puffins (*Fratercula arctica*; hereafter puffin) are a long-lived seabird in the family *Alcidae* that breeds in the North Atlantic. Puffins return to breeding colonies to reproduce beginning at the ages of 4-5 years, and continue to reproduce every summer for the next 20 or more years (oldest North American puffin aged 33 years; USGS, 2022). They spend most of the year at sea, returning to their natal colony only in the summers to breed. Puffins mate monogamously, with low levels of divorce (range: 9-13% annually; Harris & Wanless, 2011) and no evidence of extra-pair paternity (Anker-Nilssen et al., 2008). It may be wise to reconsider some of these life history characteristics, however, considering that the study on extra-pair paternity only sampled 38 chicks in a single Norwegian colony (Anker-Nilssen et al., 2008), and that males frequently attempt extra-pair copulations (26% of which are successful; Creelman & Storey, 1991). Once reunited on the colony, mated pairs partake in pair bonding behaviours such as "billing," which involves two individuals knocking their beaks together, often before a crowd of spectators (Harris & Wanless, 2011). During the breeding season, puffins copulate primarily at sea but move to burrows on land to lay and incubate their egg and raise their chick (Harris & Wanless, 2011). They exhibit high levels of interannual site fidelity, nearly always returning to the same general area to breed,

although this varies substantially between colonies (Kersten et al., 2021). In some cases, adults return to the same exact burrow despite fierce competition at the beginning of the season for nesting sites (Harris & Wanless, 2011).

Each puffin pair only has one egg per year, and both male and female parents contribute equally to parental care duties, with slight differences in sex roles (Creelman & Storey, 1991). In Newfoundland colonies, eggs are laid in middle to late May, and most chicks hatch approximately 42 days thereafter in late June to early July. The duration of the chick rearing period is more flexible; on average, chicks take 38-44 days to fledge, but under suboptimal environmental conditions (i.e., delayed or reduced prey availability), this period can be extended upwards of 80 days (Nettleship, 1972). Puffin chicks in Newfoundland colonies feed primarily on the small pelagic fish, capelin (*Mallotus villosus*), and capelin spawning and availability in inshore coastal waters during the chick rearing season is critical to successful fledging in these colonies (Harris & Wanless, 2011). For puffins living in colonies throughout Witless Bay Ecological Reserve, Newfoundland, capelin spawning on nearby coastal beaches directly coincides with the onset of the puffin chick-rearing period, generally occurring in June-July. Although capelin is their preferred prey, puffins also feed on sandlance (*Ammodytes sp.*), larval fish, crustaceans, and squid (Barrett et al., 1987; Rodway & Montevecchi, 1996). This diversity in diet allows Canadian colonies to withstand yearly fluctuations in capelin availability, as demonstrated by similar estimates of breeding success and chick fledging mass between colonies in Labrador (low capelin abundance) and Newfoundland (high capelin abundance; Baillie & Jones, 2003).

### 1.3.2 The colourful bill, cere, and rosette

Atlantic puffins are well-known for their conspicuous, brightly coloured red-orange bill. Bill colouration in puffins is at least partially carotenoid-based, as the presence of ketocarotenoids in the bill structure has been chemically confirmed (Doutrelant et al., 2013). In Newfoundland colonies, carotenoids are likely acquired via their main food source, capelin, which contain 37.8 mg of carotenoids per 1 kg of fish oil (Bjerkeng et al., 1999), and/or via consumption of carotenoid-rich zooplankton, such as krill (euphausiids) and other crustaceans (i.e., copepods, amphipods; O'Driscoll et al., 2001; Rautio et al., 2009). Carotenoids are deposited in integumentary structures via circulation through the bloodstream, so most vascularized structures are theoretically capable of incorporating carotenoid pigments (Hill and McGraw, 2006a). Because the cere, rosette, and epidermis connecting avian bills is vascularized (Lucas & Stettenheim, 1972), colouration of these features may change after initial production. Bare part features are also prone to colour fading, potentially due to light exposure (Blount & Pike, 2012), accumulation of dirt particles, or tissue degradation (Hill and McGraw, 2006a).

While the red tip of the beak is colourful throughout the year, the orange-red hard dermal plates on the inner part of the beak and the eye ornaments are only produced as part of their breeding plumage in the summer (Figure 1.1; Harris & Wanless, 2011). In Newfoundland, Atlantic puffins arrive back to the colony in late April or early May, and by this time their breeding plumage has already been fully established – only rarely do puffins at the colony still have remnants of winter plumage (Harris & Wanless, 2011). The breeding plumage remains until the end of the reproductive cycle or just after the birds leave the colony, which occurs in middle to late August in Newfoundland. At this time, the dermal plates, eye ornaments, nasal shield, sub-nasal plates, and lamellae overlapping the edge of the first bill ridge slough off to reveal much darker features below (Figure 1.1; Harris, 2014). Additionally, the bright orange rosette at the gape of the beak becomes

smaller and paler for the upcoming winter season, and previously white facial plumage darkens significantly (Figure 1.1; Harris, 2014).

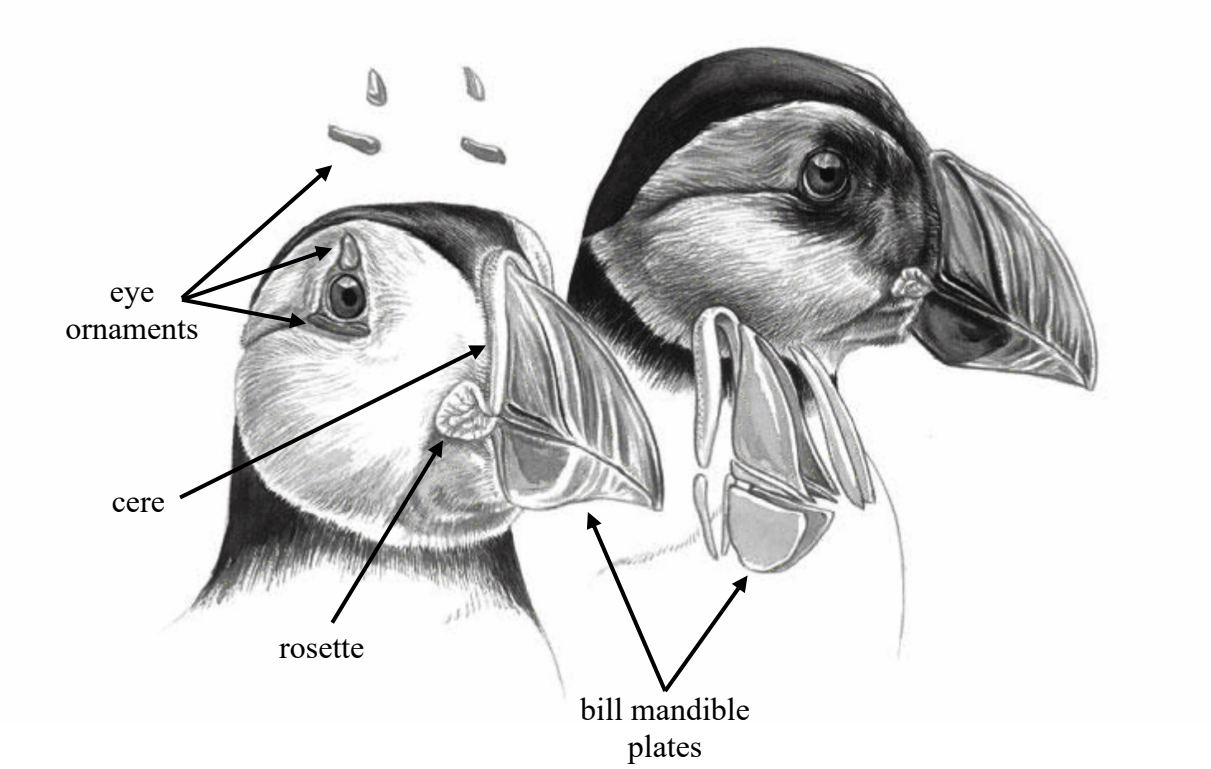


Figure 1.1 The transition from summer plumage (left) to winter plumage (right) is marked by the loss of the eye ornaments and bill plates, shriveling of the rosette, and darkening of the facial plumage. Diagram originally drawn by Keith Brockie and adapted from Figure 3.2 in *The Puffin* (Harris & Wanless, 2011).

### 1.3.3 Mutual ornamentation

Atlantic puffins, like many seabirds, exhibit mutual ornamentation. Mutual ornamentation in this context is defined as both males and females of a species displaying an elaborated trait. While some have defined ornamentation as imposing costs on the informer (Kraaijeveld et al., 2007), or specifically functioning to attract mates or intimidate rivals (Hare & Simmons, 2019), I employ a broader, more inclusive definition, as the associated costs and function of such traits are not always well described. In puffins, both sexes have monochromatic plumage (i.e., white ventrally and black dorsally), with several colourful bare-part displays during the breeding season (i.e., bright orange legs, a fleshy orange gape rosette, and an orange-red bill). Both male and female

adult puffins produce the colourful bill, and there is no evidence of sexual dichromatism, even when accounting for the bird's visual system (Doutrelant et al., 2013). However, it is important to note that males and females are slightly sexually dimorphic and can be distinguished with 88% accuracy using traditional morphometrics, owing to males' larger bills and overall size (Harris, 1979; Bond et al., 2016). While true that genetic linkage between the sexes can play a role in mutual ornamented trait evolution, it is unlikely to offer a complete explanation as to why both sexes continue to produce a highly conspicuous trait like the colourful bill (Kraaijeveld et al., 2007). The genetic correlation hypothesis posits that elaborate features like the colourful bill have been selected for and are functional in males, but arise in females simply because they have not yet evolved a less ornamented version of the trait (Lande, 1980). Indeed, in zebra finches, bill colour is genetically correlated between the sexes despite contrasting selection differentials (i.e., redder bills preferred in males but associated with negative reproductive outcomes in females), thereby preventing the evolution of sexual dimorphism and maintaining mutual ornamentation in this species (Kraaijeveld et al., 2007; Price, 1996). However, a genetic correlation between the sexes does not preclude selection from acting additionally to maintain an elaborated feature. In fact, a genetic correlation is usually not strong enough to prevent the evolution of sexual dimorphism, especially if the trait is costly to produce or maintain (Kraaijeveld et al., 2007). Therefore, selective forces such as social selection or mutual sexual selection likely play a role in this species. Social selection results from competition among conspecifics over non-sexual resources, which in puffins would most likely act during burrow site competition (Harris & Wanless, 2011; Kraaijeveld et al., 2007). In contrast, mutual sexual selection results from competition over sexual resources or between sexual partners, and can occur pre-copulation, such as during initial mate selection, or post-copulation, when parents may adjust investment levels in egg incubation and chick rearing (Kraaijeveld et al., 2007). Because puffins exhibit high interannual mate fidelity and rarely engage

in extra-pair mating, pre-copulatory mechanisms of sexual selection cannot fully explain why the colourful bill is continuously produced over the course of their long life. Put simply, initial mate choice at age 4-5 does not sufficiently explain why puffins still produce a colourful bill at age 25! Instead, post-copulatory mechanisms are likely to play a larger role, especially considering that hatching and fledging success can vary substantially among pairs (i.e., Nettleship, 1972).

## 1.4 Parental investment

Many seabirds, including puffins, exhibit obligate bi-parental care (Alcock, 2016). Parental removal experiments confirm the obligative nature of parental care in this species, as chicks raised by a singular adult have significantly slower growth rates and increased mortality (Harris, 1978). Indeed, both male and female puffins are present throughout the breeding season and contribute nearly equally to parental duties. There is a slight division of parental roles, with females involved more in the direct care of the offspring (i.e., incubating, feeding) and males involved more in indirect care (i.e., nest defense and maintenance; Creelman & Storey, 1991).

Parents are attuned to their chicks' resource requirements and can flexibly adjust their investment depending on offspring demands. An investment manipulation experiment successfully rejected the alternative hypothesis of fixed level investment (i.e., that parental investment remains constant regardless of changes in offspring demand; Johnsen et al., 1994). Because puffin parents do not recognize their own young and will continue to feed regardless of the chick's identity (Harris & Wanless, 2011), cross-fostering experiments can be successfully conducted. When 6-day-old chicks were replaced with 20-day-old chicks, the new 20-day-old chick grew at a similar rate to equally old control chicks (Johnsen et al., 1994). Offspring food requirements increase steadily with age until 7-10 days before fledging (Ashcroft, 1979; Harris & Wanless, 2011), so parents had

to respond to changing demands by dramatically increasing provisioning rate when the 20-day-old chick was introduced to maintain a similar growth rate to controls (Johnsen et al., 1994). The lack of difference in growth rate between the experimental and control groups provided clear evidence that parents can manipulate feeding effort in relation to offspring status. However, when 20-year-old chicks were replaced by 6-year old chicks, the growth rate of the foster chicks declined after day 30 of age compared to those in the control group, and several of the foster chicks were deserted by parents toward the end of the nestling period, demonstrating a temporal limit to the flexibility of parental investment in this species (Johnsen et al., 1994). Parents in the prolonged nestling period group were also lighter and in poorer condition at the end of the breeding season compared to controls, providing evidence for a cost to above-threshold investment levels that likely extends to survival and future reproductive success (Johnsen et al., 1994). Additional evidence for flexible adjustment comes from supplementary feeding experiments, where parents of pufflings given extra food markedly decreased feeding rate compared to controls (Cook & Hamer, 1997; Dahl et al., 2005; Fitzsimmons, 2018).

Flexible adjustment in puffins occurs with the aid of a parent-offspring feedback loop, whereby chicks relay information about their current status and adults respond in an appropriate fashion. Pufflings communicate to parents through two distinct types of begging calls; short peeps, which relate to aspects of quality such as body condition, and long screeches, which seem to indicate both short-term hunger and longer-term need (Rector et al., 2014). Indeed, puffin parents can be experimentally manipulated to increase their feeding rate by playing recordings of peep calls in the burrow (Harris, 1983).

## 1.5 Breeding phenology

Phenology refers to the timing of seasonal activities; in the case of avian breeding, this refers to activities such as arrival to the breeding site, egg laying, chick hatching, and chick fledging. In general, those that breed earlier or more synchronously with peak food availability tend to have higher breeding success (i.e., match-mismatch hypothesis; Stenseth & Mysterud, 2002; Verhulst & Nilsson, 2008). In many species, phenology has shifted as a result of climate-associated changes in the marine environment, consisting mainly of 1) warming oceans and 2) increasingly variable sea-surface temperatures. Specifically, multiple Arctic seabird species have shifted to breeding earlier due to advancement of spring temperatures (although not all; Descamps et al., 2019; Keogan et al., 2018). Additionally, unpredictable changes in sea-surface temperature have resulted in increased variation in local food availability prior to egg laying, thus reducing the ability of seabirds to appropriately "match" phenology to their environment (e.g., common murres *Uria aalge* and black-legged kittiwakes *Rissa tridactyla*, Shultz et al., 2009). Individuals that can better adapt to these climactic shifts are predicted to fare better and produce more surviving fledglings.

In puffins, there is evidence that early breeding is advantageous; on Great Island in Newfoundland, fledging success was higher for chicks that hatched early in the season, regardless of nest habitat type (59.3% vs. 42.5%; Nettleship, 1972). A similar relationship was observed on St. Kilda, United Kingdom, although this only held for burrows in dense areas (compared to sparse areas; Harris, 1980). Yet, puffins have responded to climate change in recent years by *delaying* hatch date by approximately one week (Major et al., 2021), which may in part be a response to the persistent delay in capelin spawning after the Newfoundland and Labrador stock collapse in 1991 (Buren et al., 2014; Murphy et al., 2021). Synchrony of hatch date with capelin spawning may also be an important factor in determining puffin breeding success. However, in a study that compared puffin breeding parameters of years with normal, expected capelin spawning timing (1968-1969; Nettleship, 1972) to two years with an anomalous one month delay (1992-1993), puffin chicks

hatched 5-9 days later than normal and 6-8 days after capelin spawning, but did not suffer from reduced fledging or breeding success overall (Regehr & Rodway, 1999). Thus, the relationship between timing of breeding and reproductive success remains contentious, especially in the face of climate change.

## 1.6 Chick growth

Growth analysis is an important and well-studied component of avian biology, and various aspects of growth can be related to overall health and well-being of an individual (Karkach, 2006). Patterns of chick growth differ between species, as well as within a species, as a function of geographic location, phenology, nutrition, and genetics (Ricklefs, 1968). However, seabirds as a whole tend to have low growth rates compared to other avian species and are some of the only species in which offspring mass can surpass adult mass, although this rarely occurs in puffins (Ricklefs, 1968). Several aspects of seabird biology explain these patterns, including the relatively long chick-rearing period, low clutch size, high degree of independence at fledging, and heavy reliance on flight for foraging (Ricklefs, 1968).

Like other seabirds, puffin chicks exhibit a typical growth pattern, although there is a notable amount of inter-individual variation in the shape and features of their growth curves. After hatch, most chicks regularly increase in mass until reaching a peak of 70% of the adult body weight (~270 g), which is achieved 24-28 days post-hatch (see Chapter 3 for more details; Harris & Wanless, 2011). After this point, they begin to decline in mass for the next 7-10 days until fledging. In a minority of cases, pufflings do not achieve their peak mass until fledging. Daily mass increments fluctuate considerably, with chicks gaining and/or losing weight day to day, even when provided a constant amount of food (in captivity; Hudson, 1983). However, among pufflings, daily

patterns of growth are quite synchronized within the colony, with all individuals gaining or losing more weight on the same days (Ashcroft, 1976; Hudson, 1983). The relationship between food and growth is therefore less straightforward than one might predict. In fact, one study on Skomer Island, United Kingdom, suggests that the most important factors predicting offspring growth between days 5-25 are the individual's age, initial weight, and growth the previous day, rather than the number of feeds received that day (Davidson, 1994). Nonetheless, access to food clearly affects growth, as those given supplementary food at the burrow tend to reach peak weights earlier and weigh more at fledging (Harris, 1978; Harris & Wanless, 2011).

In contrast to mass, skeletal measurements such as wing length increase regularly with age, peaking predictably at the time of fledging (see Chapter 3 for more details). Wing length and bill length are thus considered better indicators of age, although growth curves for these traits are colony-specific (Harris & Wanless, 2011). Interestingly, there seems to be a trade-off between mass and skeletal growth, with pufflings prioritizing growth of the head and wings over body mass when food intake was experimentally reduced (Øyan & Anker-Nilssen, 1996).

## 1.7 Objectives

The overarching goal of this thesis is to gain a better understanding of the potential signal value of the Atlantic puffin's colourful bill. In Chapter 2, I will explore the signaling properties of the bill, cere and rosette. This includes an examination of colour change across the breeding season, both cross-sectionally and longitudinally, as well as the condition-dependence and degree of sexual dichromatism of bill colour. In Chapter 3, I will assess the relationship between the colouration of adults in mated pairs and various chick outcomes, including hatch date and offspring growth metrics. The results of these two chapters will allow me to make informed conclusions about the

potential role(s) of Atlantic puffin bill colouration during the breeding season, which I will address in Chapter 4.

## 1.8 References


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# CHAPTER 2 Signaling properties of Atlantic puffin bill colouration

## 2.1 Abstract

Sexually monomorphic species have been historically overlooked in the sexual/social selection literature, but there is growing evidence that mutual ornamentation can be driven by selective forces such as mutual sexual selection or selection for individual recognition. Examining the properties of a trait may elucidate which forces most likely play a role, especially when comparing the characteristics of quality and identity traits. Atlantic puffins *(Fratercula arctica)* are an example of a mutually ornamented monomorphic species, where both males and females display a bright orange-red bill and orange gape rosette during the breeding season and are ornamented to similar degrees. In this study, I investigate whether the properties of the colourful bill and rosette, specifically lability across the breeding season and condition-dependence, more closely align with signals of quality or identity. I first confirmed that the puffin bill is statistically and perceptibly sexually monochromatic, supporting prior findings in this species. I then determined that the bill changes in a discriminable way within individuals and is especially dynamic in the fleshy cere and rosette. However, no metric of colour on any region of the bill or rosette was significantly related to current body condition. Yet, because bill colour is carotenoid-pigmented and highly labile, I argue that this trait more likely functions as an alternative quality signal, although further study is needed to determine which aspect of quality colouration signals, if not condition. These results provide a basis for experimentally testing the signal value of the colourful bill in Atlantic puffins, and more broadly, a framework for investigating the properties of mutual ornamentation in avian species.

## 2.2 Introduction

The selective forces that shape colourful displays have piqued scientific attention in recent decades. Most of this research has focused on sexually dimorphic species, in which the degree of sexual selection is presumed to be high (Andersson, 1994). Yet, in many species, males and females are conspicuously ornamented to similar degrees. In some cases, a nonadaptive genetic link between the sexes may play a role in explaining monomorphic features, as proposed by the genetic correlation hypothesis. This hypothesis posits that elaborate features are only functional in males, and are simply expressed as a genetic by-product in females (Lande, 1980, 1987). However, this ignores the fact that such traits are usually costly to develop and maintain and ultimately fails to fully explain why they are expressed in both sexes (Amundsen, 2000). Indeed, there is growing evidence that selective forces also shape ornamentation in monomorphic species (Amundsen & Pärn, 2006; Dale et al., 2015; Kraaijeveld et al., 2007).

Mutual sexual selection, social selection, and selection for recognition are the primary selective mechanisms that may explain the continued presence of elaborate displays in a monochromatic species (Kraaijeveld et al., 2007). In mutual sexual selection, bidirectional mate choice or mate competition selects for an ornamented trait, whereas in social selection, competition over non-sexual resources such as territories or food resources is responsible (Kraaijeveld et al., 2007). Selection for recognition occurs when distinctive variants of a trait facilitate recognition, whether that be between species, kin, mates, or neighbors (Sherman et al., 1997; Tibbetts & Dale, 2007). In mutually or socially selected traits, there is often a link between the display and some aspect of individual quality. Quality may represent a feature inherent to the signaler, such as physical condition, good genes, or age, and/or may reflect abilities acquired through experience, such as the capacity to provide parental care or secure a good territory (Dale et al., 2001). These

traits are thought to be reliably maintained because low-quality individuals cannot afford the costs of elaborate trait expression, thus providing "honest" information to receivers (Andersson, 1994; Kirkpatrick & Ryan, 1991; Kodric-Brown & Brown, 1984; Zahavi, 1975). In contrast, individual recognition traits are not necessarily linked to quality, but rather are unique such that perceivers can consistently identify the informer (Dale et al., 2001; Tibbetts & Dale, 2007). Individually recognizable traits can be useful in relocating mates or offspring, as well as reducing costs of territorial defense or competition in group dominance hierarchies (Tibbetts & Dale, 2007).

A trait's features can lend insight into which selective forces play a larger role. The properties of signals of quality and signals of identity share some similarities; both types have high degrees of phenotypic variability within a population (Dale et al., 2001). However, there are also four important differences between quality and identity signals: 1) signals of quality fluctuate between and/or within years for a given individual, whereas signals of identity remain highly stable over time, 2) frequency distributions are generally unimodal for signals of quality and complex for signals of identity, 3) only signals of quality are expected to be condition-dependent and correlate with aspects of fitness, and 4) signals of quality are more heavily influenced by environmental factors, whereas signals of identity are more heavily influenced by genetic factors (Dale et al., 2001). Trade-offs between the two types of signals make it theoretically challenging for a single trait to provide both types of information, although it is still possible if different features of the trait vary independently of each other (i.e., segregation of information; Marler, 1960).

Carotenoid-pigmented features have received significant attention for their role as signals of quality (Hill & McGraw, 2006a,b). Carotenoids serve as key pigments for red, orange, and yellow features, must be acquired from the animal's diet, and play important roles as antioxidants and immune-enhancers (Hill & McGraw, 2006a). For these reasons, many hypotheses have proposed a relationship between carotenoid-pigmented features and an individual's foraging ability

(Endler, 1980; Hill, 1992), immune function (Lozano, 1994; Moller et al., 2000), and overall health (Hill, 2011). There is evidence supporting these hypotheses in some species, but there are many caveats and contradictions that make it challenging to deem carotenoid features as broadly reliable signals of quality (Svensson & Wong, 2011a).

Seabirds often have carotenoid-pigmented bare part colouration, standing in stark contrast to their generally achromatic plumage. Atlantic puffins (*Fratercula arctica;* hereafter: puffin) are no exception, displaying bright red-orange mandibles, an orange rosette, and orange legs and feet throughout the breeding season. The bill, which is referred to throughout this chapter as the region including the upper and lower mandibles, cere, and rosette, is very conspicuously ornamented in puffins; the difference between the colourful bill during the summer and the mostly black mandibles and pale rosette during the winter is stark, even to the human eye. This transition in ornamentation has been well documented in puffins (Harris & Wanless, 2011), but little is known about the functional role of the colourful bill and rosette during the breeding season (see Doutrelant et al. 2013 for some discussion).

When considering the potential adaptive function of bill colouration, several aspects of puffin life history must be taken into account. Puffins are long-lived (25+ years), socially and genetically monogamous seabirds with high interannual adult survival and low divorce rates (Anker-Nilssen et al., 2008; Harris & Wanless, 2011). Therefore, after initial pairing at age 4-5 years, most puffins stay with their mate each breeding season; only in the unlikely event of partner death or divorce (9-13% of pairs annually) do they need to select a new mate (Harris & Wanless, 2011). Additionally, puffins exhibit obligate bi-parental care of a single chick, with equal levels of care provided by males and females (although roles differ slightly; Creelman & Storey, 1991). It is thus unsurprising that puffins are sexually monochromatic (accounting for avian vision;

Doutrelant et al., 2013), which classically would indicate that puffins experience low levels of intersexual selection.

However, the potential for mutual sexual selection, social selection, or selection for recognition in shaping the colourful bill should not be ruled out. Mutual sexual selection may operate on dynamic assessment traits used to make decisions concerning parental investment (Kraaijeveld et al., 2007), especially in species like puffins where there is a strong trade-off between current reproductive effort and future survival/breeding attempts (Erikstad et al., 2009; Johnsen et al., 1994; Trivers, 1972). Support for this trade-off in puffins comes from a study in which parents were experimentally manipulated to prolong the provisioning period; those with lower body masses had marked decrease in survival to the next year, potentially indicating that they could not sufficiently bear the cost of increased investment (Erikstad et al., 2009). Social selection may operate when individuals engage in fierce, at times physically aggressive competition for burrows at the beginning of the breeding season (Harris & Wanless, 2011). The most preferable burrows are likely those closer to the cliff edge with a steeper slope, as they experience lower levels of predation/kleptoparasitism and, consequently, allow individuals to obtain higher degrees of breeding success (Nettleship, 1972). Additionally, puffins have high interannual mate and site fidelity, so it is plausible that social networks remain consistent from year to year. Individual recognition could be especially useful in reuniting with a long-term mate or limiting costly territorial defense between familiar neighbors.

While the function of bill colour and the selective forces that shape it remain unknown, knowledge of its properties can be used to generate more informed hypotheses. In Atlantic puffins, bill and rosette colour may 1) communicate some aspect of individual quality (to conspecifics, including mates), and/or 2) provide information on individual identity. A central aim of this chapter is to determine whether puffin bill colouration more closely corresponds to the properties of a

quality signal or an identity signal, or alternatively, whether some aspects of the bill resemble quality signals while other aspects resemble identity signals. Specifically, I will focus on the lability and condition-dependence of bill colouration. The degree to which Atlantic puffin bill colouration changes is unknown, but could theoretically fluctuate within a few days, as in other species with carotenoid-pigmented bills (Ardia et al., 2010; Rosenthal et al., 2012). The condition-dependence of bill colouration has been previously investigated in puffins, but with conflicting results (support from Doutrelant et al., 2013; lack of support from Kelly, 2015).

This chapter lays the foundation for exploring the adaptive value of colour in the puffin's bill, cere, and rosette. First, I seek to evaluate the validity of Doutrelant et al.'s (2013) key finding that puffins are sexually monochromatic on the bill. Doutrelant et al. (2013) assessed the dichromatism of two regions on the bill and the rosette with a non-parametric multivariate model to compare the colour space occupied by the two sexes, as well as a generalized linear model with the four photoreceptor responses of each region as predictors of sex. My analysis will build on this work by 1) employing both statistical and perceptual approaches to evaluate dichromatism, 2) incorporating a robust sample size of hundreds of individuals (compared to 36 in Doutrelant et al., 2013), and 3) sampling the cere in addition to the bill and rosette. I then characterize key properties of puffin bill colouration to assess the bill's potential role as a quality signal and/or identity signal, and whether mutual sexual selection, social selection, or selection for individual recognition are more likely to be acting. Specifically, I examine cross-sectional and longitudinal change in bill colouration, as well as the relationship between current body condition and bill colouration. Quality signals are predicted to be highly labile across the breeding season, change in a discriminable way within individuals, and exhibit condition-dependence, whereas identity signals are predicted to be stable overall and lack condition-dependence.

## 2.3 Methods

### 2.3.1 Study site

This study was conducted on Gull Island in the Witless Bay Ecological Reserve of Newfoundland and Labrador, Canada (47.26, -52.77; Figure 2.1). The Atlantic puffin colony on Gull Island is one of the largest in the Northwestern Atlantic, with ~120,000 breeding pairs according to a 2012 population survey (Wilhem, 2017).

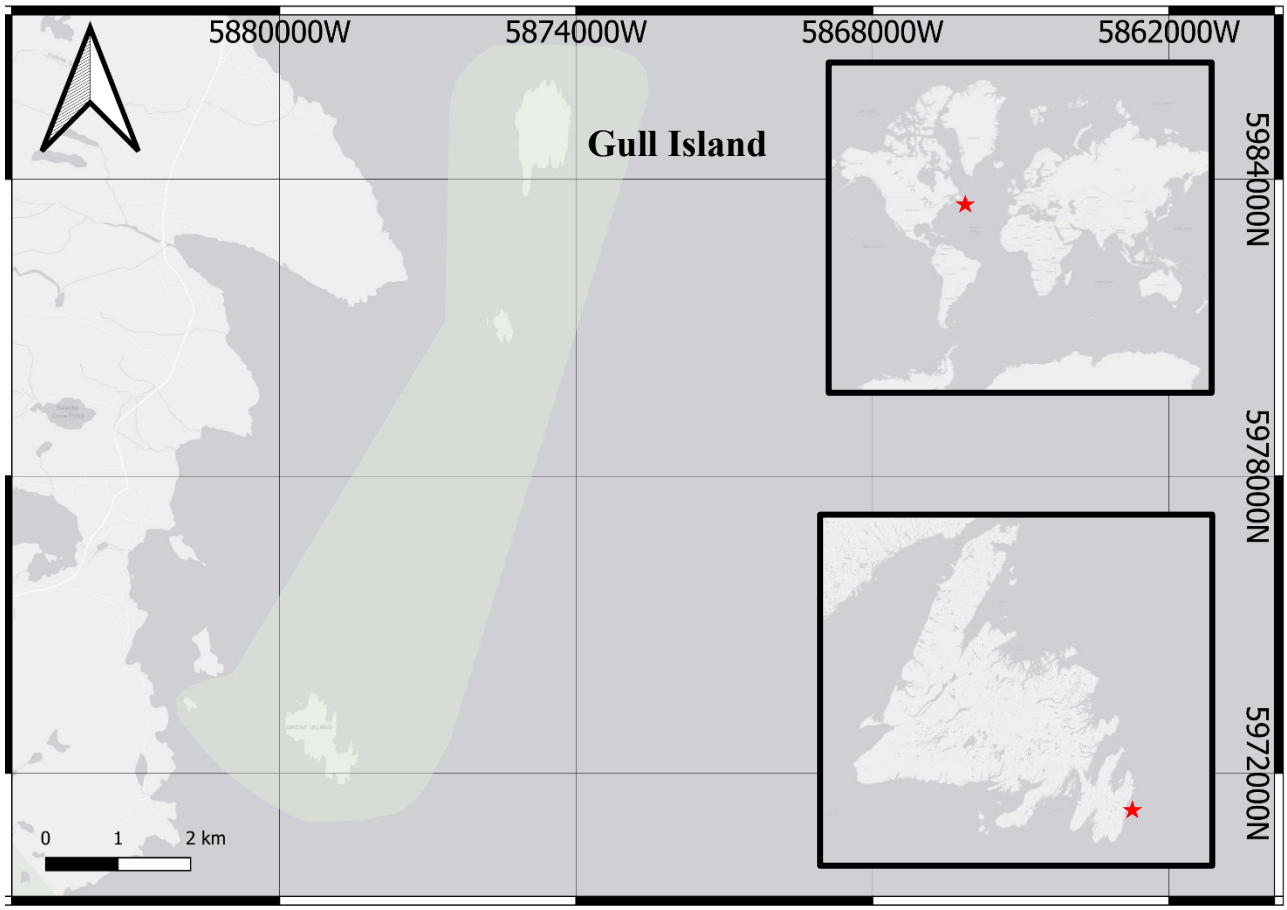


Figure 2.1 Map of the Witless Bay Ecological Reserve of Newfoundland, Canada (in green), with my study site, Gull Island, identified.

### 2.3.2 Field methods

To assess whether Atlantic puffin bill colouration changes within a breeding season, I employed a cross-sectional (across individuals) and longitudinal (within-individual) approach. For

the cross-sectional analysis, I used morphometric and colour data from 229 individuals, sampled during late June to mid-August of 2019, 2020, and 2021. Some individuals were sampled multiple times within or between years, which I account for in my statistical analyses (Section 2.3.6). I focused on the brood rearing phase of the breeding season because puffins are particularly sensitive to human disturbance during the incubation phase, and are much less likely to abandon after chicks have hatched (Rodway et al., 1996). The mean hatch date in this population tends to be in the last week of June or first week of July, so I began sampling after most adults were no longer incubating. In addition, when extracting puffins from their burrows, I assessed whether there was an egg or chick present and did not further handle the adult if an egg was identified. To assess colour changes longitudinally, I opportunistically sampled 41 individuals twice within the same year during late June to mid-August of the years 2019, 2020, and 2021. The two sampling dates took place 1-28 days apart, with a mean of 12.02 ± 5.22 days.

Individuals were captured by hand in their burrows between the hours of 22:00 and 3:00. At first capture, individuals were given a Canadian Wildlife Service stainless steel band for subsequent identification, mass was measured with a 600 g Pesola spring scale to the nearest 5 grams, flattened wing chord was measured with a ruler to the nearest 1 mm, and a blood sample was taken with a 26.5-gauge needle from the brachial vein on #2 filter paper (to collect the sample and stop the bleeding) for sex determination. Individuals were then taken into a blind, where auto-exposure bracketed ultraviolet (UV, 320-380 nm) and visual spectrum (400-680 nm) RAW 20 megapixel images were taken of the left side of the beak and rosette with a full spectrum converted Samsung NX1000 (following instructions from Troscianko, 2018) using two 2-inch Baader lens filters (Figure 2.2). UV and visual spectrum images were obtained because unlike humans with three photoreceptors, puffins have a fourth photoreceptor sensitive to UV wavelengths (Endler &

Miekle, 2005), and my goal was to measure bill colours as they would be perceived by a puffin. The photos were illuminated with a full-spectrum Metal Halide 150W ballast that passed through a light diffuser, and all photos included optic grade white (99% reflectance) and dark (10% reflectance) standards (Lake Photonics, Uhldingen-Mühlhofe, Germany), a ruler, and a small white board to record the date and band number of the individual puffin. The bill was cleaned of debris with a toothbrush prior to photo capture and was held in place with a wooden bill stabilizer. Handling time for these procedures was typically no more than 15 minutes, and in no case did a puffin exhibit typical signs of distress (i.e., panting). This process was repeated for the second capture, except that redundancies were avoided by excluding the need to band the bird, measure the wing chord (moult occurs well after the breeding season ends, so it should remain stable; Harris & Wanless, 2011) and collect blood (only one sample needed for sexing).

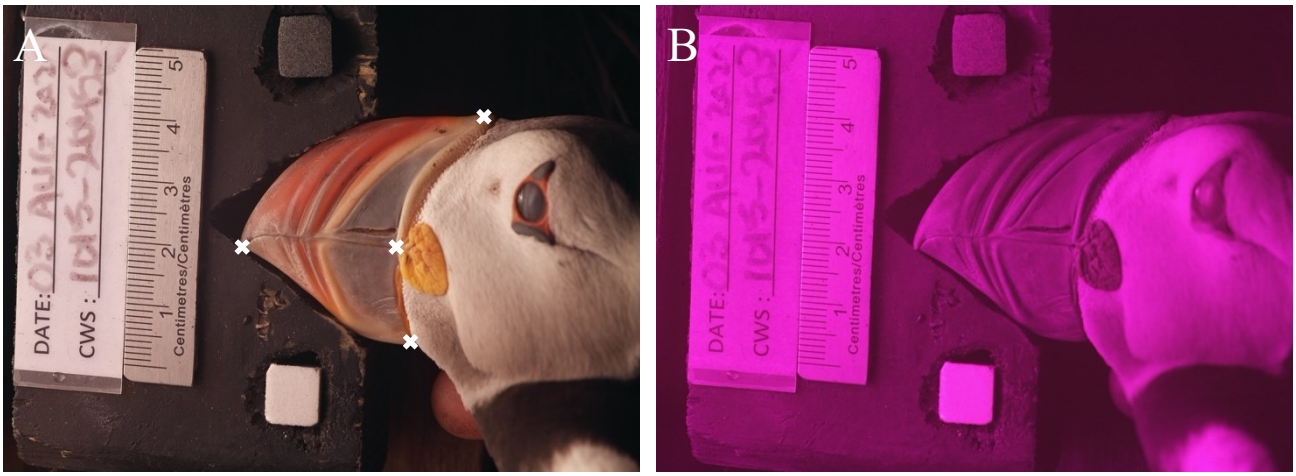


Figure 2.1 Example photos of an adult puffin from 2020 following the methodology outlined in 2.3.2. Each puffin was photographed in the A) visual spectrum and B) ultraviolet spectrum. The white x symbols on the visual spectrum photo indicate the locations of the points that were used to generate multispectral images with the "Affine align" tool.

### 2.3.3 Molecular methods

Sex of each individual was determined molecularly from blood samples collected in the field. Blood was stored at room temperature until molecular procedures could begin. At this time,

a ~1 cm section of paper saturated with blood was extracted with sterilized scissors and placed in a 1.5 ml collection tube. DNA was extracted using a DNeasy® Blood & Tissue Kit (Qiagen Inc., Toronto, ON, CA) following protocols outlined in the DNeasy® Blood & Tissue Handbook (2020) and stored at -20 °C.

Polymerase chain reaction (PCR) was run with extracted DNA to amplify the chromo-helicase DNA 1 (CHD1) gene on the avian W and Z chromosomes. Following standard procedures for sexing seabirds (Fridolfsson & Ellegren, 1999), 12.5 µl Thermo Scientific™ PCR Master Mix, 2 µl of both primers 2550F and 2718R, 6.5 µl of nuclease-free water, and 2 µl of extracted DNA were added to each PCR tube. All batches were run with a no template control (NTC) tube, which contained an additional 2 µl of nuclease-free water instead of extracted DNA. The PCR was performed with an Eppendorf Mastercycler® ep gradient S on a program of 95 °C for 5 minutes, 35 cycles of denaturing, annealing, and extension at 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 60 seconds, extension at 72 °C for seven minutes and a cooling period of 4 °C for 10 minutes. PCR samples were stored at -20 °C unless proceeding directly to gel electrophoresis.

PCR samples were run on a RedSafe™ agarose gel with 100 base pair reference ladders on a Thermo Scientific™ EC 300 XL for 50 minutes at 130 amps. The gels were then imaged using Image Lab software and stored digitally. All procedures were carried out at Memorial University of Newfoundland following standard lab safety protocols.

### 2.3.4 Calculation of body condition index

Body condition was determined from the residuals of a best fit linear regression with mass as the response variable. Typically, body condition is either given as the residuals of a linear regression of mass on wing chord length or simply mass (Doutrelant et al., 2013; Fitzsimmons,

2018; Labocha & Hayes, 2012). However, mass in puffins is known to change across the breeding season, vary due to fluctuations in prey availability and environmental conditions, and differ between the sexes, so these variables (Ordinal date of capture, year of capture, and sex) were included in the regression model. To account for potential sex-biased differences in mass change within and between years, I also included the two-way interactions between sex and Ordinal date and sex and year, as well as the three-way interaction between sex, Ordinal date, and year in the full model. Prior to fitting the model, mass and wing length were centered by subtracting the mean value to reduce instances of structural multicollinearity between main-effect and interaction terms (Cohen et al., 2014). The full model was reduced by stepwise removal of non-significant terms based on results from ANOVA tables. The final linear regression model met the assumptions of homogeneity of variance, normal distribution of the residuals, and low multicollinearity among predictors.

### 2.3.5 Assessment of colour

Multispectral images of the left side of the beak were generated using micaToolbox 2.2v in ImageJ for each individual at a given capture date (van den Berg et al., 2020). One visual spectrum RAW photo and one UV RAW photo were chosen from the two sets of bracketed photos for alignment using the Photoscreening tool, permitting selection of the most illuminated sample without evidence of RGB camera pixel oversaturation. Visual spectrum and UV images of the puffin bill were aligned and merged with either the 'Affine align' tool by selecting four consecutive points on the bill of each image (uppermost intersection of upper mandible and cere, bill tip where mandibles meet, bottommost intersection of lower mandible and cere, intersection of cere and rosette; Fig. 2.2), or the 'Manual align' tool by manually positioning the photos such that the bills

exactly match up. The quality of the alignment was evaluated by creating a false colour image with the 'Make Presentation Image' tool, using yellow to represent the visual R normalized channel and blue to represent the uvB normalized channel. False colour images without evidence of poor alignment on the bill were considered aligned and could be used in subsequent analysis. From these multispectral images, cone catch images were generated using the sensitivity of a violet-sensitive (VS) avian visual system (peak cone sensitivities: $\lambda$ = 410, 450, 505, 565 nm; Ödeen et al., 2010; Table A.1). The spectral sensitivity of puffin vision is currently unknown, but other closely related species in the family *Alcidae* (i.e., common murres *Uria aalge* and razorbills *Alca torda*, Ödeen et al. 2010) are VS based on sequencing of the SWS1 opsin gene. Additional parameters in the cone catch model included double cones representing perception of brightness, which was modelled as the combined sensitivities of the medium- and long-wavelength photoreceptors, and standard illuminant D65 (CIE) as a typical spectrum under ambient light conditions. Quantum catch values for five regions of interest (ROIs, or simply regions) on the bill were extracted from the cone catch images: two on the tip of the upper and lower mandible, where puffins generally have the most saturated carotenoid-pigmented colouration, one on the base of the upper mandible, which is black and presumably melanin-pigmented, one on the semi-fleshy cere, and one on the fleshy rosette, both of which are more vascular than the bill and may be more likely to respond to fluctuations in carotenoid availability (Lucas & Stettenheim, 1972; Figure 2.3; Table A.1). Each ROI was the same shape and size (30x30 pixel circle) and positioned to obtain the clearest measure of colour possible (i.e., avoiding obvious scratches, pieces of dirt, or contour/shadow lines). Average quantum catch values from the ROIs were modelled in tetrahedral colour space using the 'colspace' function, with space set to tetrachromatic ("tcs") and qcatch set to quantum catch ("Qi"; Maia et al., 2013; Table A.1). A tetrahedral colour space defines a 3-dimensional space of perceivable colours, where the central point is the achromatic center, representing equal stimulation of the

cones, and each vertex is the absolute stimulation of one of the four cones. Colours from ROIs plotted in this space have both Cartesian coordinates (x,y,z) and spherical coordinates ($\theta, \phi, r$), the latter of which was used in both the cross-sectional and longitudinal analyses.

From the spherical coordinates, a colour vector can be defined between the achromatic center and the position of the measured colour in colour space. The colour vector can be subsequently used to identify two measures of hue and one measure of chroma for each ROI (Table A.1). Hue is the direction of the colour vector (drawn from the achromatic center to the locus) and is defined by the angular position of the locus in tetrahedral colour space, both in terms of the azimuth (horizontal axis, $\theta$) and elevation (vertical axis, $\phi$; Stoddard & Prum, 2008). Hue $\theta$ (hereafter "hue VIS") represents the contribution of the visible spectrum (red-blue) to perceived colour. Hue VIS ranges from -π to +π, such that perceived reds and purples have negative values, perceived yellows and oranges are close to zero, and perceived greens and blues have positive values (Dakin & Montgomerie, 2013). Across the ROIs in my study sample, hue VIS ranged from red-orange (-0.354) to orange-yellow (0.507). Hue $\phi$, hereafter "hue UV," represents the contribution of violet and UV to perceived colour. Hue UV ranges from -π/2 to +π/2, with more UV rich colours having more positive values (Dakin & Montgomerie, 2013). UV reflection is low across the measurements in my study sample, with the maximum value on the upper mandible being -0.375, the minimum value on the cere being -1.170, and a -0.704 average across the regions. Chroma is the saturation of a colour and is defined as the magnitude of the colour vector ($r$) from the achromatic center (Stoddard & Prum, 2008). Because the colour space is a tetrahedron, different hues have different potential maximum chroma ($r_{max}$), so achieved chroma ($r/r_{max}$) was calculated for each ROI as a more informative estimate (Stoddard & Prum, 2008). These measures were determined for all four chromatic regions (upper and lower mandible, cere, and rosette).

Brightness was calculated from the relative stimulation of the double cone. Brightness is the sole achromatic measure and represents the quantity of perceived light reflected from the surface. Brightness was measured for all five regions, including the achromatic mandible base. All colour variables were calculated with the R package *pavo* (Maia et al., 2013, 2019).

Because these colour variables do not give us information on the perceptual aspect of colour (i.e., discriminability), a modeling approach was employed to statistically quantify the degree of sexual dichromatism in the population, as well as to evaluate longitudinal differences in colour within a breeding season. Rather than working with Cartesian or spherical coordinates, I directly transformed the quantum catch values using the 'coldist' function in the R package *pavo* (Maia et al., 2013, 2019). The noise-weighted Euclidean distance between the two sets of quantum catch values for each individual was calculated using the receptor noise-limited (RNL) model, which models colour and brightness discrimination thresholds based on receptor noise (Vorobyev & Osorio, 1998). Chromatic differences (dS) were calculated from the quantum catch values of the four single cone channels, whereas achromatic differences (dL) were calculated from the quantum catch value of the double cone channel (Table A.1). Receptor noise was estimated from the relative densities of photoreceptor types, which I approximated as 1:1:3:3.55 (UV-wavelength sensitive, short-wavelength sensitive, medium-wavelength sensitive, and long-wavelength sensitive photoreceptors, respectively) for Atlantic puffins based on the proportion of oil droplet types in the retina (Émond, 2016). The Weber fraction, which is used to determine the signal-to-noise ratio, was set at 0.10 for the chromatic channel and 0.15 for the achromatic channel based on average results from behavioural tests of other avian species (Olsson et al., 2018). Noise-weighted Euclidean distances generated from this model correspond directly to perceptual differences (termed just noticeable differences, JNDs; Vorobyev et al., 1998; Vorobyev & Osorio, 1998). The general rule is that a chromatic or achromatic difference is likely perceptible to conspecifics if the

JND > 1, whereas two colours should be indistinguishable if the JND < 1. However, this is often only the case under optimal viewing conditions (i.e., controlled laboratory studies), so JND values of two and three may be more relevant to animal communication in a natural setting, where illumination changes, environmental distractions, and temporal constraints may detract from the ability to reliably discriminate colours (Fleishman et al., 2016).


Figure 2.2 Regions of interest (ROIs) for each puffin were selected on the 1) upper mandible, 2) lower mandible, 3) base of the mandible, 4) cere, and 5) rosette. Selection areas are enlarged on the illustration to show detail.

### 2.3.6 Statistical analyses

#### 2.3.6.1 Sexual dichromatism

To evaluate whether puffins are sexually dichromatic on the bill, cere, and rosette, I calculated whether each bill region was statistically separate in colour space between males and females, as well as perceptibly discriminable. Following the recommended methodology outlined by Maia and White (2018), statistical separation was evaluated using noise-corrected colour and brightness distances (generated with the 'coldist' function) to run a distance-based PERMANOVA (hereafter: distance PERMANOVA) with the 'adonis' function in the R package *vegan* (Oksanen et al., 2007). Distance PERMANOVA is a nonparametric test that simulates a null distribution by randomizing distances between observations, resulting in the generation of a pseudo-F statistic

(Anderson, 2017; Maia & White, 2018). I ran this on each ROI with 999 permutations to determine whether the region was significantly different in colour and/or brightness space between the sexes ($\alpha = 0.05$) and estimate the effect size of the analysis ($R^2$). *P* values were adjusted with the false discovery rate across all distance PERMANOVA tests (n = 9; one brightness test for each ROI, one colour test for all ROIs except base of mandible) to correct for type 1 error (Benjamini & Hochberg, 1995).

Perceptible discrimination was evaluated using colour and brightness distances generated from a bootstrapping procedure with the 'bootcoldist' function in *pavo* (see 2.3.5 for 'coldist' settings). This function samples with replacement 1000 times ("boot.n" = 1000) to generate a distribution of mean distances between the two groups, in this case males and females. From this, 95% confidence intervals around the geometric mean distance could be estimated. Distances with 95% confidence intervals above 1 JND were considered theoretically discriminable, whereas those with intervals above 3 JND were considered reliably discriminable across sensory environments. Both thresholds are useful in assessing the degree to which males and females are dichromatic from a puffin's perspective. For both the statistical and perceptible dichromatism analyses, 162 of 229 samples were retained (77 male, 85 female), as they represented unique observations of genetically sexed individuals.

#### 2.3.6.2 Trends in colour change over the breeding season

The cross-sectional dataset was used to assess broad temporal trends in colouration across the late breeding season. Linear models were generated for each colour variable, where hue, chroma, or brightness was the response variable and Ordinal date was the sole predictor variable. Diagnostic plots were generated to ensure the residuals met the assumptions of normality, linearity,

and homoscedasticity. For the 26 individuals that were sampled more than once within and/or between years, only one of the sampling dates was retained in the dataset to avoid pseudoreplication. This was randomly chosen using an R sampling procedure, such that some regions from the same individual retained values from different sampling dates. There were 53 instances where individuals were sampled multiple times within or between years, so only 176 of 229 observations were included in each model.

#### 2.3.6.3 Discriminability

The longitudinal dataset was used to assess whether individuals perceptibly change colour within the breeding season. Colour and brightness change was evaluated by calculating the distance in JNDs between within-individual measurements on each region of the bill, cere, and rosette. To assess the scope of discriminable changes, the percentage of regions that changed colour or brightness in a discriminable vs. non-discriminable way within individuals was calculated for each ROI separately at JND values of one, two and three. Achromatic differences were calculated for all five ROIs, whereas chromatic differences were calculated for four of the five ROIs, excluding the black, achromatic base of the upper mandible. These percentages give a general assessment of whether puffin bill colouration perceptibly fluctuates during the breeding season.

I also investigated the effect of time between sampling on the discriminability of colour or brightness using unpaired t-tests, with discriminability at a JND value of one as the dependent variable (categorical, 2 levels) and the number of days between sampling as the independent variable (continuous). To determine if the data met the assumptions of normality and equal variance, a Shapiro-Wilk's test of normality and a Levene's test of homogeneity of variance was run on the original discriminability group distributions. If these assumptions were not met ($\alpha = 0.05$), a Mann-

Whitney U test was performed. Outliers were identified with boxplots, and for parametric t-tests only, one outlier was removed when analyzing each region separately because it fell well outside of the whiskers of the boxplot (i.e., more than 1.5x the inter-quartile range). The magnitude of the difference in days between sampling for discriminable vs. non-discriminable colours was calculated based on the group medians and reported alongside interquartile ranges (Q1, Q3). Effect sizes for t-tests or Mann-Whitney U tests were also calculated and interpreted using Cohen's cut-offs (small: 0.1 –< 0.3, moderate: 0.3 –< 0.5, large: >= 0.5). Finally, a linear regression was used to predictively assess the temporal threshold for colour and brightness discriminability of each region, with change in Euclidean distance (measured in JNDs) as the response variable (y) and time difference (in days) as the predictor variable (x). The regression equation was reverse solved to determine the threshold for discriminability (i.e., when the regression equation equals one JND).

$$y = b_0 + b_1 x, where\ y = 1$$

$$x = \frac{1 - b_0}{b_1}$$

( 1 )

This equation was solved for all individuals, as well as males and females separately, to evaluate sex-biased discriminability differences. The mean and standard error of the threshold was calculated by sampling the variables $b_0$ and $b_1$ from their respective ranges (estimate ± standard error) with replacement 1000 times. Only the thresholds of significant linear relationships after correction for type I error with a false discovery rate are reported (Benjamini & Hochberg, 1995).

#### 2.3.6.4 Colour and time interval between sampling

The longitudinal data was also used to determine if changes in colour over the breeding season are due to individual changes over time. Specifically, the relationship between change in

colouration/brightness and the number of days elapsed between sampling dates was assessed with linear models, controlling for date of first capture. The interaction between time difference and date of first capture was also included in the initial model and retained if the interaction was significant. This was analyzed for each ROI and each colour variable separately. Change in colouration and brightness was assessed as the raw difference between these values on successive sampling dates ($C_2 - C_1$, where $C_2$ is the value at second capture and $C_1$ is the value at first capture). Positive values indicated that the metric increased, whereas negative values indicated that the metric decreased. Diagnostic plots were produced for each model to determine if the residuals met the assumptions of normality, linearity, and homoscedasticity. Cook's distance was used to identify influential outliers by removing values that had a Cook's distance value four times greater than the mean. All models met the assumptions of linear regression after removal of outliers.

#### 2.3.6.5 Colour and condition

The relationship between colour and condition was evaluated with the cross-sectional dataset using linear models, where hue, chroma, or brightness were the response variables and body condition was the predictor variable. Importantly, this approach differs from that of Doutrelant et al. (2013) and Kelly (2015), as this study examines the link between condition and colour variables obtained from a model of puffin vision, rather than principal components obtained from colour descriptors of raw spectra. Because Ordinal date of capture, year, and sex were included in the regression from which body condition was calculated, these variables were not included as separate predictor variables in the models. Diagnostic plots were produced for each model to determine if the residuals met the assumptions of normality and homoscedasticity. Cook's distance was used to identify influential outliers by removing values that had a Cook's distance value four times greater

than the mean. For individuals that were sampled multiple times within or across years, only one observation was randomly chosen to be retained in the dataset, yielding a final dataset of 162 individuals.

## 2.4 Results

### 2.4.1 Sexual dichromatism

After applying the false discovery rate correction, the distance PERMANOVA showed that male and female colouration was not statistically different in terms of colour or brightness on any region ($P > 0.05$, Table 2.1; Table 2.2).

All the chromatic bootstrapped estimates had 95% confidence intervals either completely below 1 JND (upper mandible, lower mandible, base of mandible, cere), or overlapping with 1 JND (rosette; Figure 2.4). For the achromatic bootstrapped estimates, the base of the mandible was the only region that overlapped with 1 JND (Figure 2.4). Neither the chromatic nor achromatic bootstrapped estimates approached 3 JND. Based on these estimates, none of the five bill regions are perceptibly different between males and females, even under ideal conditions. Importantly, our results are not influenced by differences in sampling between the sexes, as there was no statistical difference between the capture dates of males vs. females ($t = 0.94$, $P = 0.347$).

Table 2.1 Distance PERMANOVA table for chromatic distances between males and females

| Region | Source | Df | SS | MS | F | $R^2$ | P |
|---|---|---|---|---|---|---|---|
| *Upper mandible* | Sex | 1 | 14.330 | 14.330 | 3.892 | 0.023 | 0.162 |
| | Residuals | 167 | 614.941 | 3.682 | | 0.977 | |
| | Total | 168 | 629.271 | | | 1.000 | |
| *Lower mandible* | Sex | 1 | 1.795 | 1.795 | 0.478 | 0.003 | 0.757 |
| | Residuals | 167 | 627.476 | 3.757 | | 0.997 | |
| | Total | 168 | 629.271 | | | 1.000 | |
| *Cere* | Sex | 1 | 0.380 | 0.380 | 0.105 | 0.001 | 0.882 |
| | Residuals | 167 | 603.363 | 3.613 | | 0.999 | |
| | Total | 168 | 603.743 | | | 1.000 | |
| *Rosette* | Sex | 1 | 9.319 | 9.319 | 0.366 | 0.002 | 0.757 |
| | Residuals | 167 | 4248.858 | 25.442 | | 0.998 | |
| | Total | 168 | 4258.177 | | | 1.000 | |

Table 2.2 Distance PERMANOVA table for achromatic distances between males and females

| Region | Source | Df | SS | MS | F | $R^2$ | P |
|---|---|---|---|---|---|---|---|
| *Upper mandible* | Sex | 1 | 5.282 | 5.282 | 2.064 | 0.012 | 0.398 |
| | Residuals | 167 | 427.307 | 2.559 | | 0.988 | |
| | Total | 168 | 432.589 | | | 1.000 | |
| *Lower mandible* | Sex | 1 | 0.054 | 0.054 | 0.021 | 0.000 | 0.882 |
| | Residuals | 167 | 432.535 | 2.590 | | 1.000 | |
| | Total | 168 | 432.589 | | | 1.000 | |
| *Base of mandible* | Sex | 1 | 22.342 | 22.342 | 5.790 | 0.034 | 0.126 |
| | Residuals | 167 | 644.358 | 3.858 | | 0.966 | |
| | Total | 168 | 666.700 | | | 1.000 | |
| *Cere* | Sex | 1 | 3.663 | 3.663 | 1.169 | 0.007 | 0.495 |
| | Residuals | 167 | 523.446 | 3.134 | | 0.993 | |
| | Total | 168 | 527.109 | | | 1.000 | |
| *Rosette* | Sex | 1 | 7.029 | 7.029 | 2.002 | 0.012 | 0.398 |
| | Residuals | 167 | 586.397 | 3.511 | | 0.988 | |
| | Total | 168 | 593.426 | | | 1.000 | |

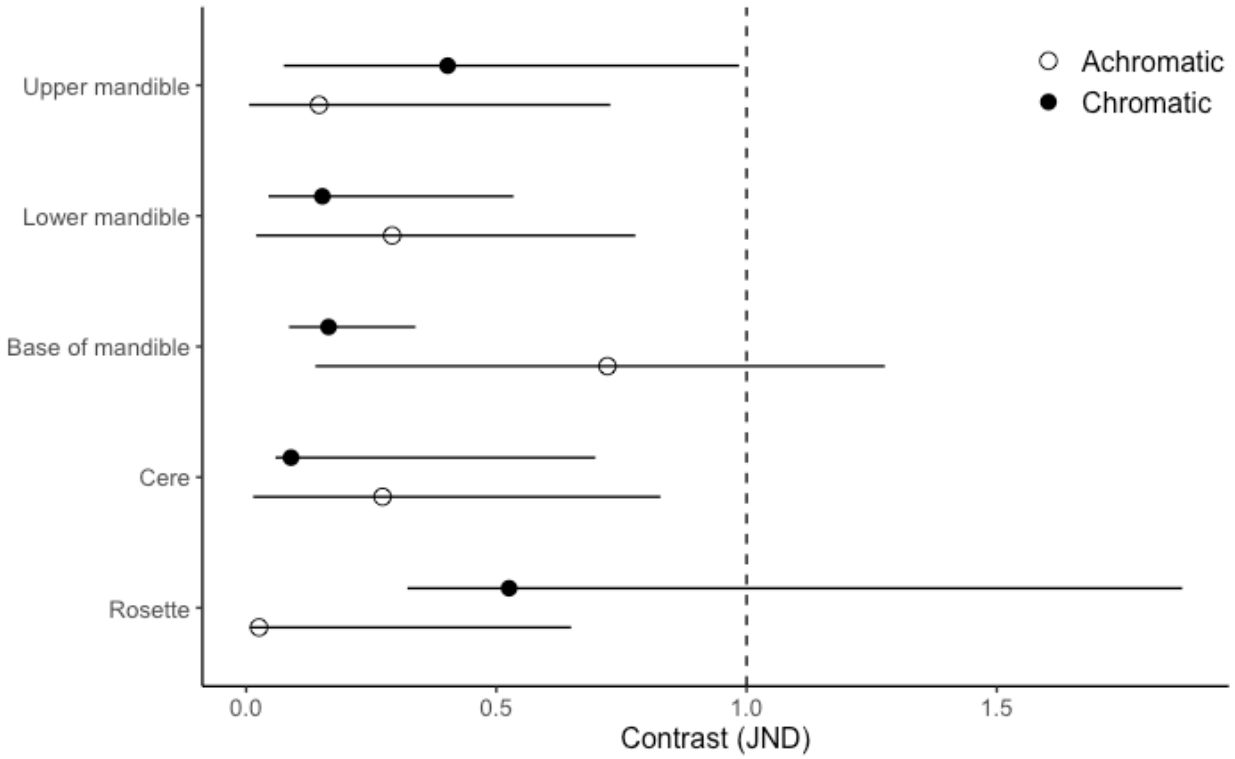


Figure 2.4 Bootstrapped 95% C.I.'s for mean chromatic and achromatic distances between males and females in colour space, grouped by region of the bill

### 2.4.2 Cross-sectional colour trends over the breeding season

The cross-sectional dataset was used to evaluate broad trends in colour change across the breeding season. I found that several colour variables in different regions of the bill, cere, and rosette changed within the breeding season (Table 2.3):

1. *Upper mandible*

   Both measures of hue and brightness significantly changed over the breeding season. Brightness decreased and the hue became more orange and less UV-reflective (Table 2.3; Figure 2.5A, B, D)

2. *Lower mandible*

   Achieved saturation significantly increased over time, whereas brightness significantly decreased over time (Table 2.3; Figure 2.5C, D). Neither measure of hue significantly changed over the breeding season (Table 2.3).

3. *Base of mandible*

   Brightness did not significantly change across the breeding season (Table 2.3). Measures of hue and chroma are not relevant to black features like the base of the mandible and thus were not tested.

4. *Cere*

   Achieved saturation and the UV component of hue significantly increased over the breeding season (Table 2.3; Figure 2.5B, C). Hue VIS and brightness did not change in a significant way (Table 2.3).

5. *Rosette*

   Both measures of hue significantly changed over the breeding season, such that the rosette became more yellow and less UV reflective later in the season (Table 2.3; Figure 2.5A, B).

Brightness significantly increased, whereas achieved saturation remained unchanged (Table 2.3; Figure 2.5D)

The effect of sex on these relationships was evaluated with linear models, where the main effects of capture date and sex, as well as the interaction between date and sex, were included as predictor variables. None of these relationships varied between males and females, as the interaction term was nonsignificant for all the colour variables after controlling for multiple testing with the false discovery rate ($P > 0.05$).

Table 2.3 Change in colour variables across the breeding season

| ROI | Response: colourimetric variable | Estimate | Std. Error | t | P |
|---|---|---|---|---|---|
| *Upper mandible* | **Hue vis** | 0.0007 | 0.0003 | 2.222 | 0.047* |
| | **Hue UV** | -0.0015 | 0.0006 | -2.474 | 0.030* |
| | Achieved saturation | 0.0013 | 0.0006 | 2.000 | 0.062 |
| | **Brightness** | -0.0013 | 0.0006 | -2.280 | 0.045* |
| *Lower mandible* | Hue vis | 0.0006 | 0.0004 | 1.728 | 0.104 |
| | Hue UV | 0.0000 | 0.0006 | 0.033 | 0.974 |
| | **Achieved saturation** | 0.0023 | 0.0006 | 3.928 | <0.001*** |
| | **Brightness** | -0.0024 | 0.0006 | -3.908 | <0.001*** |
| *Mandible base* | Brightness | 0.0002 | 0.0004 | 0.543 | 0.624 |
| *Cere* | Hue vis | -0.0008 | 0.0004 | -2.065 | 0.062 |
| | **Hue UV** | 0.0019 | 0.0005 | 3.891 | <0.001*** |
| | **Achieved saturation** | 0.0024 | 0.0007 | 3.356 | 0.002** |
| | Brightness | -0.0015 | 0.0008 | -2.015 | 0.062 |
| *Rosette* | **Hue vis** | 0.0024 | 0.0005 | 5.068 | <0.001*** |
| | **Hue UV** | -0.0018 | 0.0003 | -6.174 | <0.001*** |
| | Achieved saturation | -0.0004 | 0.0006 | -0.661 | 0.577 |
| | **Brightness** | 0.0058 | 0.0013 | 4.338 | <0.001*** |

*P* values corrected with false discovery rate method. Significant variables bolded at $p$ = 0.05*, 0.005**, 0.0005***

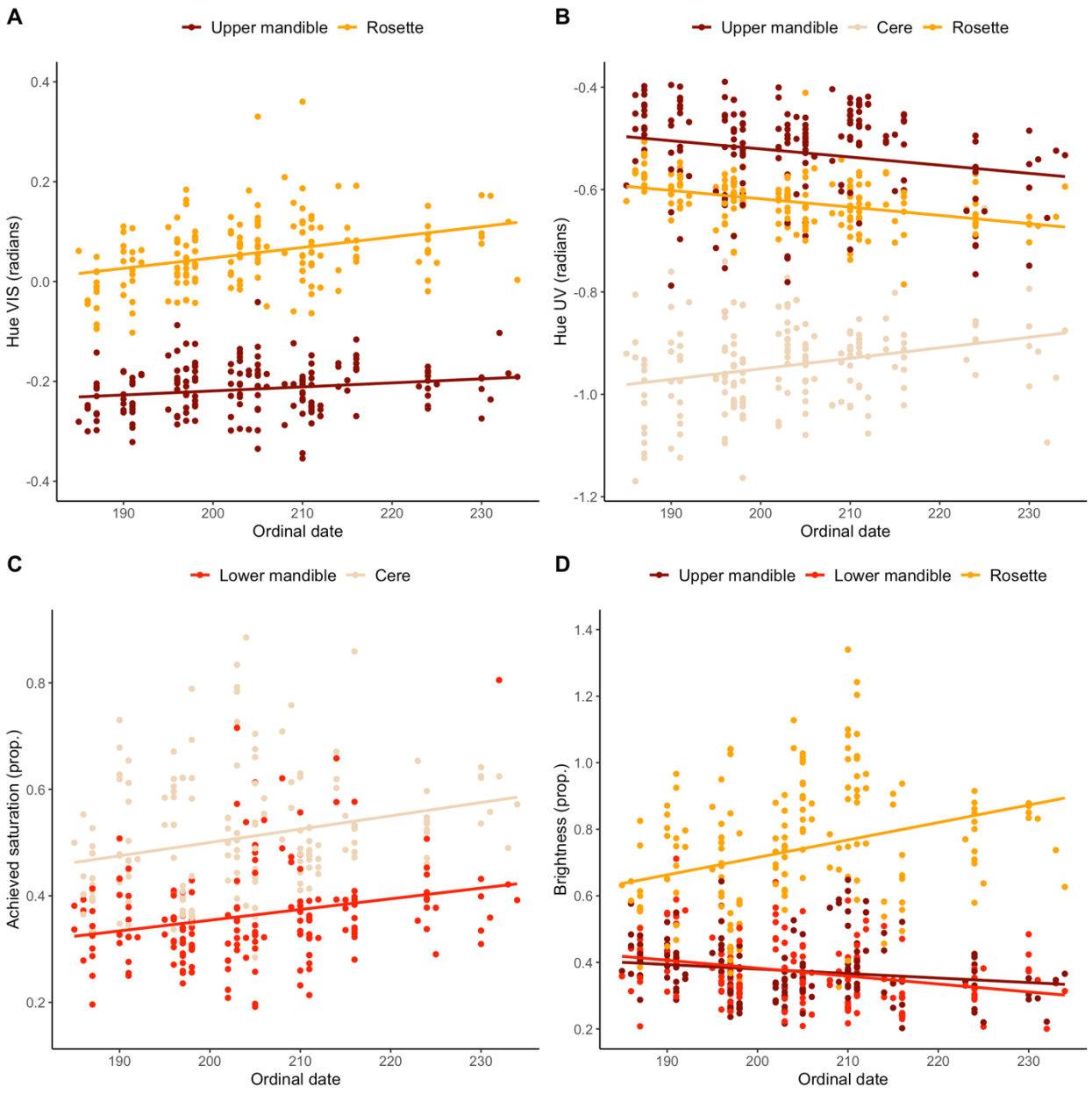


Figure 2.5 Across the late breeding season, significant changes were observed in A) hue VIS of the upper mandible and rosette, B) hue UV of the upper mandible, cere and rosette, C) achieved saturation of the lower mandible and cere, and D) brightness of the upper and lower mandible and rosette. Significance was obtained from linear regressions, and only significant trends after correction for the false discovery rate are displayed.

### 2.4.3 Longitudinal perceptible changes in colouration

The longitudinal data was used to evaluate whether colour changes in a perceptible way within individuals. Overall, slightly more than half of the changes in colouration on the bill, cere, and rosette were discriminable based on a model of puffin vision (56.7%, Table A.2; Figure 2.6; JND = 1). This was driven mostly by changes in the rosette, where 80% of colour changes were considered discriminable. This contrasts sharply with the lower mandible, where only 34% of changes were discriminable. As expected, fewer colour changes were discriminable at higher values of JNDs; at JND = 2, less than a quarter of changes were discriminable, and at JND = 3, less than 10% of all colour changes were discriminable (Table A.2).

The number of days between sampling dates only weakly predicted whether the observed colour change was discriminable. The assumption of homogeneity of variance was met for all regions, but several did not meet the assumption of normality, in which case Mann-Whitney U tests were performed. Across all regions, the changes that were discriminable at JND = 1 had significantly more days between capture dates than changes that were considered non-discriminable (W = 2707.5, $P$ = 0.046). However, the median number of days was the same for both discriminability groups (discriminable: *Mdn* = 13.0 days, IQR = 7.5-14.0 days; non-discriminable: *Mdn* = 13.0 days, IQR = 8.0-15.0 days), and the effect size was low (r = 0.156). The rosette exhibited a much stronger contrast (W = 59.5, r = 0.376, $P$ = 0.017), such that discriminable changes had five more days between sampling dates (discriminable: *Mdn* = 14.0 days, IQR = 12.0-15.0 days; non-discriminable: *Mdn* = 9.0 days, IQR = 4.25-13.0 days). For all other bill regions (i.e., upper and lower bill mandible, and cere), this relationship was nonsignificant.

The threshold of discriminability could only be calculated for the rosette, representing the only significant relationship between a region's colour difference and time difference (Figure 2.7).

The regression was significant for both males and females together ($t_{1,39}$= 2.73, $P$ = 0.009) and for females only ($t_{1,18}$ = 3.96, $P$ = 0.011). The threshold was calculated at 4.49 ± 0.98 days for males and females (at JND = 1) and 7.79 ± 0.07 days (at JND = 1) for females only (Figure 2.7). None of the linear relationships between colour difference and time difference were significant for males and females separately.

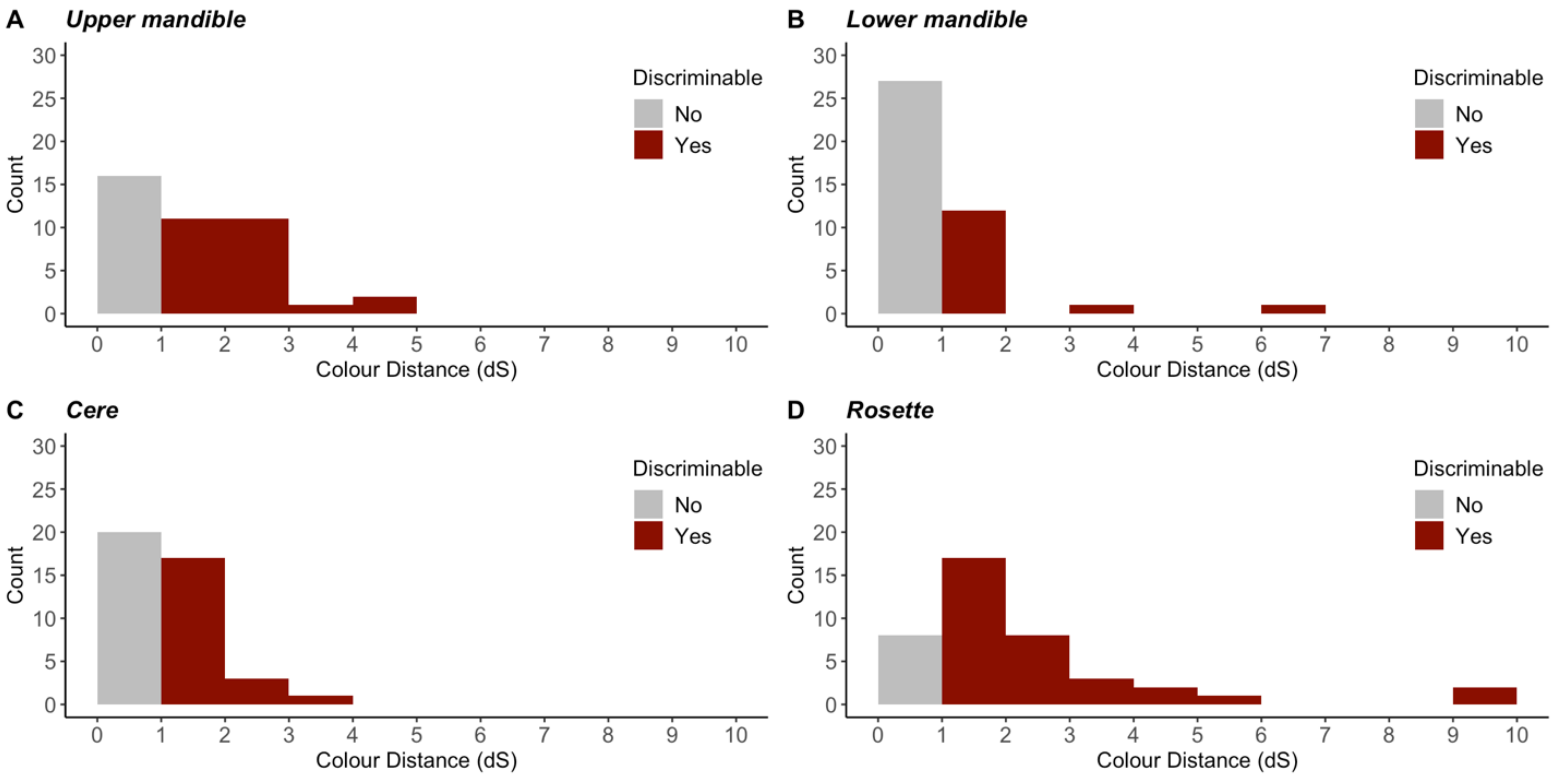


Figure 2.6 Distribution of colour distances between measurements sampled within individuals for the A) upper mandible, B) lower mandible, C) cere, and D) rosette.

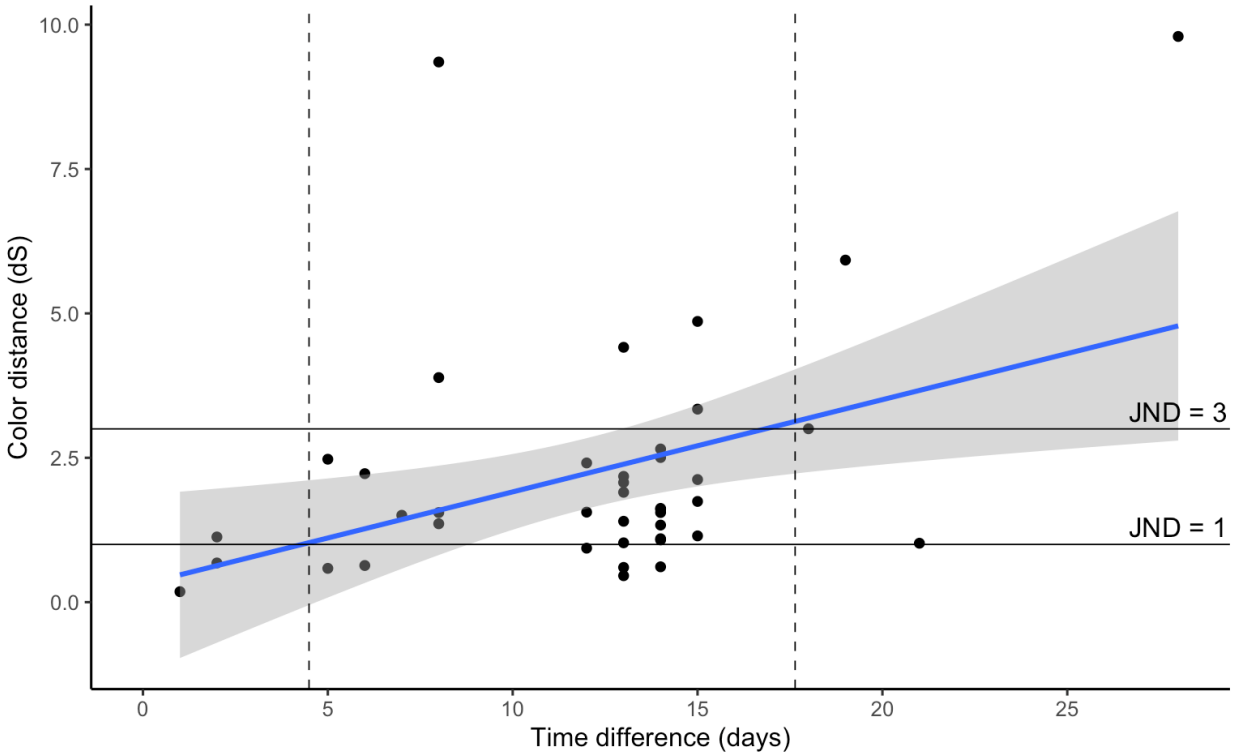


Figure 2.7 The relationship between rosette colour distance and time between sampling was significant, permitting calculation of thresholds of discriminability at JND = 1 (4.49 days) and JND = 3 (17.64 days).

### 2.4.4 Longitudinal perceptible changes in brightness

Over 70% of all changes in brightness were discriminable (71.7%, Table A.3, Figure 2.8), and the majority of changes in all five regions were discriminable. As with changes in colouration, the rosette had the highest proportion of discriminable brightness changes, with 88% of brightness changes considered discriminable (87.8%, Table A.3).

Mann-Whitney U tests were performed to assess the relationship between discriminability and time between sampling, since the assumption of normality was violated in all cases. There was a significant difference in the number of days between sampling dates for discriminable (*Mdn* = 13.0, IQR = 12.0 - 15.0) vs. non-discriminable changes (*Mdn* = 12.0, IQR = 7.0-14.0), although the difference was small and the effect size was low (W = 1971.5, $P = 0.004$, r = 0.223). The rosette was the only individual region with a significant difference and a moderate effect size, where discriminable changes had about six and a half days more between sampling dates (discriminable: *Mdn* = 7.0, IQR = 5.0-8.0; non-discriminable: *Mdn* = 13.5, IQR = 12.0-14.25; W = 28.5, $P = 0.014$, r = 0.387).

The threshold of discriminability (JND = 1) was not calculated for any of the five regions of the bill because no significant relationships were detected between brightness difference and time difference. When examined separately for each sex, two relationships were initially significant for males only (base of the mandible, $t_{1,18} = 2.54$, $P = 0.021$; rosette, $t_{1,18} = 2.76$, $P = 0.013$), but were nonsignificant after correcting for multiple testing with the false discovery rate (base of the mandible, $t_{1,18} = 2.54$, $P = 0.154$; rosette, $t_{1,18} = 2.76$, $P = 0.154$).

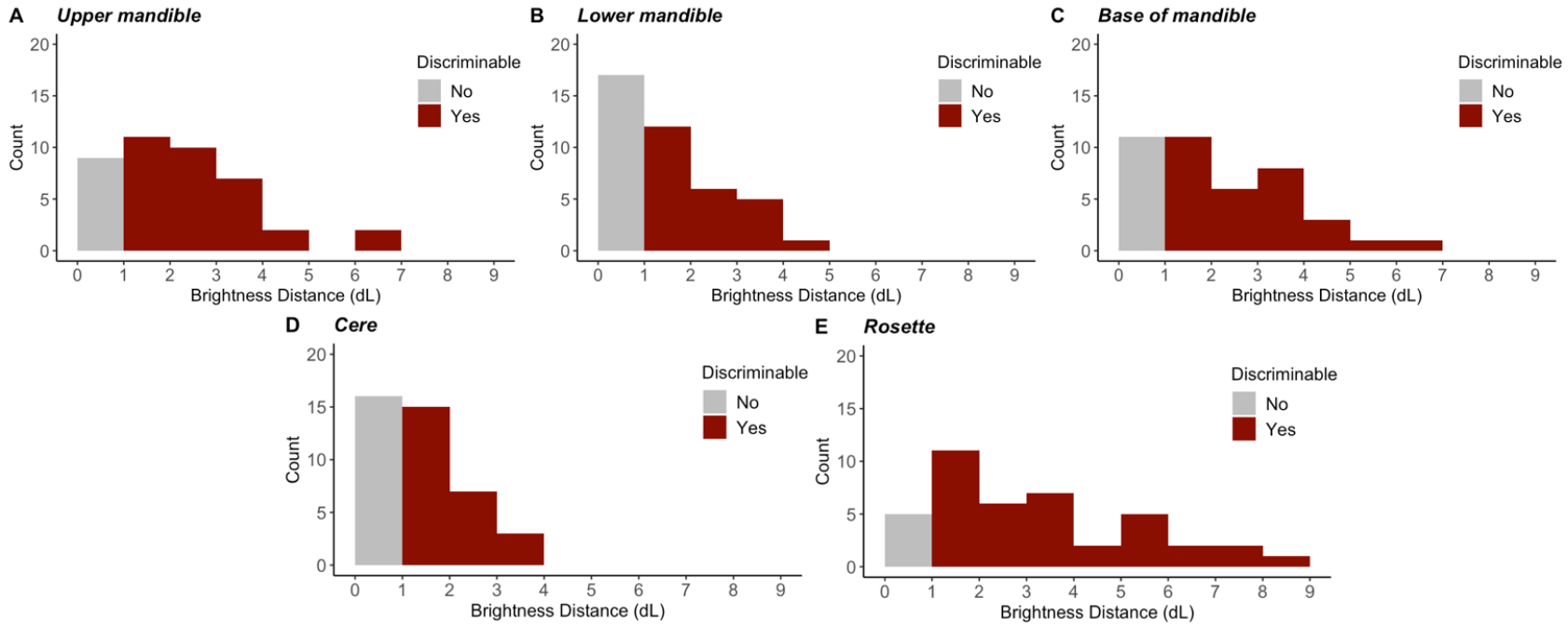


Figure 2.8 Distribution of brightness distances between measurements sampled within individuals for all five regions of interest: A) upper mandible, B) lower mandible, C) base of mandible, D) cere, and E) rosette

### 2.4.5 Longitudinal changes in colour variables

After applying the false discovery rate error correction, none of the colour variables were significantly influenced by the number of days between sampling (Table A.4). Sampling interval was initially significant for cere hue VIS, such that the cere became more orange with increasing sampling interval. Interestingly, the interaction between hue VIS and date of capture was also significant, such that the opposite trend (i.e., significant decrease with sampling interval) was observed for individuals captured later in the season (Figure 2.9). These results should be interpreted with caution, however, as all terms in the model were nonsignificant after application of the false discovery rate error correction. Sampling interval approached significance with the false discovery rate correction ($\alpha \leq 0.1$) for achieved saturation of the cere and brightness of the rosette only. As the time between capture dates increased, the cere's achieved saturation and the rosette's brightness decreased (Figure 2.10).

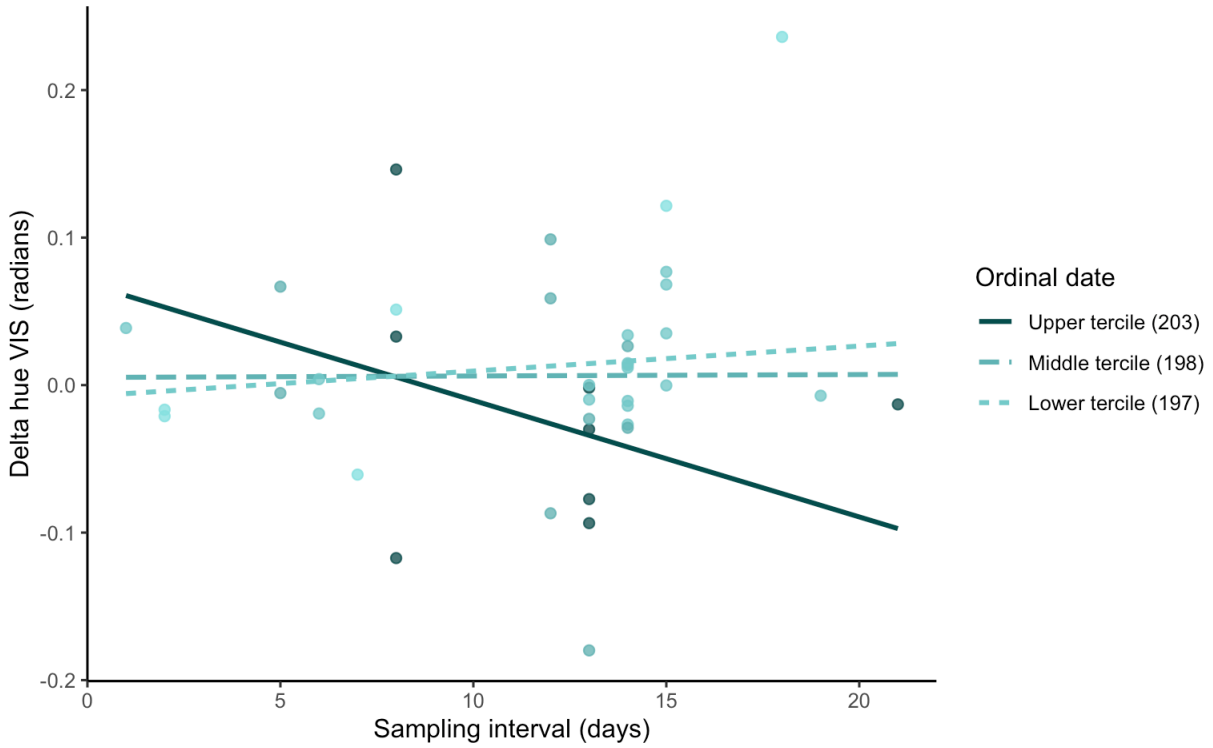


Figure 2.9 The two-way interaction between delta hue VIS and sampling interval was significant for the cere. Each line represents predictions for the mean Ordinal date of three equally sized terciles. For individuals captured later in the season (upper tercile), cere hue VIS negatively changed (i.e., becomes yellower) with increases in sampling interval. In contrast, cere hue VIS slightly positively changed (i.e., becomes more orange) with increases in sampling interval for individuals captured earlier in the season (lower tercile).

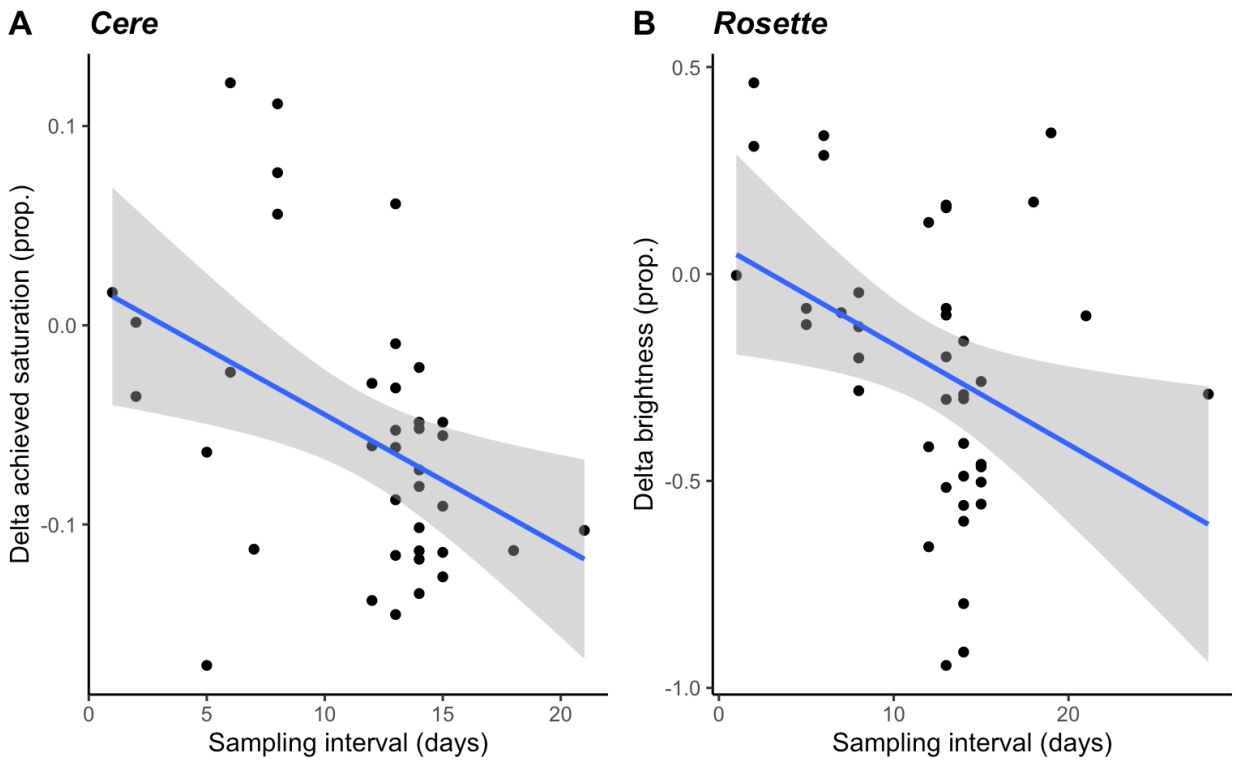


Figure 2.10 A) Larger decreases in the cere's achieved saturation, as well as B) larger decreases in the rosette's brightness were observed with increasing sampling interval.

### 2.4.6 Colour and condition

Body condition was calculated based on the residuals of a linear regression model with all four main-effect variables: wing length (scaled), Ordinal date of capture, year of capture, and sex. Mass was positively related to wing length ($2.02 \pm 0.43$, $F_{1,162} = 49.83$, $P < 0.001$; Figure A.1), but negatively related to Ordinal date ($-0.73 \pm 0.21$, $F_{1,162} = 11.39$, $P < 0.001$; Figure A.1). Mass was also higher in males compared to females ($34.21 \pm 3.63$, $F_{1,162} = 88.59$, $P < 0.001$; Figure A.1). Additionally, a post-hoc assessment of estimated marginal means found that mass was significantly lower in 2022 compared to 2019 ($14.59 \pm 5.14$, $t_{162} = 2.84$, $P = 0.026$; Figure A.1) or 2020 ($16.22 \pm 5.64$, $t_{162} = 2.88$, $P = 0.024$; Figure A.1). All the included variables were significant ($P < 0.05$; Table 2.4) and no evidence of multicollinearity was detected in the final model.

Hue VIS on the lower mandible was significantly positively related to condition before correcting for multiple testing ($t_{1,160} = 2.344$, $P = 0.020$). However, after application of the false discovery rate correction, no significant relationships were detected between any of the colour variables and condition (Table A.5). Assumption checks identified multiple outliers (three for upper mandible achieved saturation, two for lower mandible achieved saturation, one for base of mandible brightness, two for cere hue VIS, one for cere brightness and three for rosette hue VIS, and three for rosette hue UV), but the results were qualitatively the same with and without outliers, so only the models containing all data are reported.

Table 2.4 Analysis of variance table for body condition regression, 2019-2022

| Predictor | Df | GVIF | Sum Sq | Mean Sq | F | P |
|---|---|---|---|---|---|---|
| *Wing length* | 1 | 1.13 | 25422.82 | 25422.82 | 49.83 | <0.001 |
| *Ordinal date* | 1 | 1.64 | 5813.52 | 5813.52 | 11.39 | <0.001 |
| *Year* | 3 | 1.71 | 8375.07 | 2791.69 | 5.47 | 0.001 |
| *Sex* | 1 | 1.09 | 45203.18 | 45203.18 | 88.59 | <0.001 |
| *Residuals* | 162 | | 82657.40 | 510.23 | | |

## 2.5 Discussion

I was first able to replicate Doutrelant et. al.'s (2013) finding that puffins are sexually monochromatic from an avian visual perspective using a much larger sample size (n = 162 in this study vs. n = 36 in Doutrelant et al., 2013). This supports my underlying assumption that females and males are ornamented to similar degrees.

My investigation of the characteristics of the puffin's colourful bill, cere, and rosette yielded conflicting results. The cross-sectional dataset revealed population-level changes in the colouration of multiple regions across the breeding season. In line with this finding, the longitudinal dataset showed that colour and brightness perceptibly fluctuate within individuals over the course of the breeding season and are especially dynamic in fleshy structures like the cere and rosette. These analyses support the hypothesis that bill colour could act as a signal of quality, with dynamic fluctuations in colour potentially corresponding to changes in relative quality during the breeding season. However, I found no relationship between any of our colour metrics and a commonly employed proxy of quality, current body condition. A lack of condition-dependence suggests instead that the colourful bill does not align with a signal of quality.

These mixed results led me to further explore the stability of bill colour by investigating the time scale over which perceptible colour changes occur using my longitudinal data. I found that the rosette was the only region that consistently became more perceptible with time, although discriminable changes generally required a longer time interval than non-discriminable changes for all regions. Perhaps unsurprisingly, this fleshy innervated tissue changed on a very rapid time scale (Lucas & Stettenheim, 1972; Rosenthal et al., 2012); on average, the difference in colour observed after five days is theoretically discriminable based on an approximation of puffin visual perception. It is unclear what is driving these colour changes in the rosette, as none of the colour

variables I investigated (hue VIS, hue UV, achieved saturation) varied according to the time interval between sampling. However, two colour variables (hue VIS and achieved saturation) of the other fleshy structure I examined (cere) did exhibit some evidence of change with sampling interval, such that it became more yellow-orange and less saturated over time. Dynamic changes in the cere and rosette support prior work demonstrating rapid colour change in fleshy tissues, such as the 48-hour change in foot colour observed in food-deprived blue-footed boobies (*Sula nebouxii*, Velando et al., 2006). The relationship between time and colour was nonsignificant for the bill, so I could not calculate a threshold of discriminability, but perceptible changes in bill colouration still occurred within the 21-day time frame. Previous studies have shown that most changes in structures like the keratinized dermal plates of avian bills occur over the course of several weeks (e.g. European blackbirds *Turdus merula*, Baeta et al., 2008; zebra finches *Taeniopygia guttata,* Blount et al., 2003; spotless starlings *Sturnus unicolor*, Navarro et al., 2010), which may partially explain why I was unable to detect a clear relationship in my sample. Yet, avian bills can change rapidly in some species (three days in zebra finches, Ardia et al., 2010; one to three days in goldfinches, Rosenthal et al., 2012), and in my sample some instances of rapid colour change occurred (i.e., 4 individuals $\leq$ 6 days in the upper mandible, 2 individuals $\leq$ 6 days in the lower mandible). Alternatively, changes in bill or rosette colour may be responses to key events such as egg laying or peak food availability, resulting in a more sudden shift that does not vary linearly with time. Regardless of the trajectory of these colour changes, any feature capable of fluctuating on the order of days to weeks is too unstable to function as a signal of identity on its own.

The cross-sectional dataset permitted the evaluation of broad trends in colour over time across the population, which should not exist if bill colour functions as an identity signal. I found several significant changes in colour variables over time; across all regions, hue tended to become

more orange-yellow and less UV reflective, while achieved saturation increased and brightness decreased. These results can be juxtaposed with a previous study by Kelly (2015) that examined colour change in Atlantic puffin bare part colouration by comparing individuals sampled during the incubation phase (n = 17) and chick rearing phase (n = 17). Kelly (2015) found that saturation in the UV spectrum and brightness of the red and black regions of the mandible were significantly lower in individuals sampled during the chick rearing phase compared to the incubation phase, with no observed differences in hue or saturation in the visual spectrum. While Kelly (2015) investigated a slightly different portion of the breeding season, the observed trends in brightness and UV wavelength contribution are similar to the results presented in this study. Kelly (2015) posits that the observed difference may be partially due to the bill's function in signaling, such that the bill is more relevant to pair communication earlier in the breeding season. However, I believe this interpretation not only extends beyond the limits of Kelly's methodology (i.e., cross-sectional), but also fails to recognize more parsimonious explanations.

These cross-sectional trends may instead point to colouration shifts as the end of the breeding season nears and adults prepare to shed their keratinized bill sheaths and reduce the prominence of their rosettes (Harris & Wanless, 2011). The sampling period for this study was constrained to the chick rearing phase, so a considerable portion of individuals were captured in the last few weeks of the breeding season, when this shift is likely to begin. While an increase in saturation doesn't typically fit into this narrative, a concurrent shift in hue from red-orange to orange-yellow may explain why *achieved* saturation increases. Achieved saturation is dependent on the maximum possible saturation value ($r_{max}$), which is highest at the photoreceptor vertices (i.e., pure red, pure green, etc.) and lowest at the midpoint between the vertices in a tetrahedral colour space. Therefore, as the hue shifts from red to orange-yellow, the $r_{max}$ value decreases and

a vector of the same length (i.e., equivalent absolute saturation) would have a higher achieved saturation. Another possible explanation for the observed cross-sectional colour trends is that sampling bias occurred, such that only certain individuals remained on the colony and visited their burrows late in the breeding season. These individuals may be low quality if early breeding is advantageous (as in common terns *Sterna hirundo,* Arnold et al., 2004; roseate terns *Sterna dougallii,* Burger et al., 1996; chinstrap penguins *Pygoscelis antarctica,* Moreno et al., 1997; king penguins *Aptendodytes patagonicus,* van Heezik et al., 1994); in this case, their chicks would be less developed than those of high quality individuals and would require more frequent provisioning visits, increasing the likelihood of adult capture in the burrow. In contrast, individuals captured later in the season may be high quality if later breeding is advantageous (i.e., potentially higher synchrony with prey availability, as in thick-billed murres *Uria lomvia,* Gaston et al., 2009; Baird's sandpiper *Calidris bairdii,* McKinnon et al., 2012; rhinoceros auklets, *Cerorhinca monocerata,* Watanuki et al., 2009), or they are the only individuals with surviving chicks, such that lower quality individuals have already left the colony. Typically, carotenoid-pigmented colours that are more red-shifted, more saturated, brighter, and have less UV reflection are considered "high quality" (e.g., red grouse *Lagopus lagopus scoticus*; Mougeot et al., 2007) and preferred by potential partners or mates in a breeding attempt (e.g., red-legged partridge *Alectoris rufa*, Alonso-Alvarez et al., 2012; reviewed in Hill & McGraw, 2006b). This would support the first hypothesis that low quality individuals are more likely to be captured later in the breeding season. However, because I did not find a relationship between any of the colour variables and condition, I cannot draw definitive conclusions on what aspect of quality may be reflected in the puffin's bill colouration.

My study adds to the mixed evidence for the condition-dependence of puffin bill and rosette colour. Doutrelant et al. (2013) showed that bill colouration was redder (higher value of hue and chroma, lower brightness) for individuals in better body condition and the rosette was more orange (higher values of hue and chroma) for females in better condition. In contrast, Kelly (2015) found no relationship between bill colouration and condition or health. I sought to add clarity to this dispute by employing a dataset containing hundreds of individuals over multiple years. I also used a more repeatable and relevant methodology by 1) directly relating colour variables to body condition, rather than principal components derived from colour descriptors of spectra, and 2) calculating body condition from the residuals of a stepwise linear regression including multiple relevant predictors of mass (Ordinal date of capture, year, and sex). I ultimately found that none of the examined colour variables were significantly associated with body condition, supporting Kelly's (2015) conclusion that bill colouration is not condition-dependent. One potential explanation for the discrepancy with Doutrelant et al.'s (2013) findings is that the condition-dependence of colour differs between colonies. In Atlantic puffins, access to forage prey during the breeding season differs substantially between populations; colonies in Atlantic Canada mainly rely on capelin (*Mallotus villosus*), whereas colonies in the North Atlantic more heavily rely on sandlance (*Ammodytes sp.*; Harris & Wanless, 2011). Interestingly, sandlance oil has a slightly higher concentration of carotenoids compared to capelin oil (41.4 mg/kg vs. 37.8 mg/kg), such that North Atlantic colonies may have access to carotenoid-richer foods (Bjerkeng et al., 1999). Doutrelant et al. (2013) assessed the relationship between colour and condition in a colony in Norway, whereas this study and Kelly (2015) examined populations located in Atlantic Canada. If body condition is related to foraging success, then this difference in forage prey carotenoid content may partially explain why Doutrelant et al. (2013) found significant relationships between

colour and condition, while the present study and Kelly (2015) did not find such associations. An additional consideration is that while body condition indices are generally good estimates of body fat mass, the index I employed has not been empirically validated, so it is possible that a different measure of body condition is related to colouration (Labocha & Hayes, 2012). Another possibility is that bill colour is a stronger indicator of quality when selection pressures are high (i.e., years with poor breeding conditions), as seems to be the case in least auklets (*Aethia pusilla*; Jones & Montgomerie, 1992). Nevertheless, the most parsimonious explanation is that bill colouration is not condition-dependent in puffins, and that variability in colour is not a meaningful quality signal (as in red-tailed tropicbirds, *Phaethon rubricauda*; Veit & Jones, 2003). The trade-off hypothesis (i.e., one of the primary mechanisms linking carotenoid pigmented features to immune function and health) rests on the assumption that carotenoids are scarce, but recent work shows that carotenoids are generally not a limiting resource physiologically (Simons et al., 2014). There is also an especially weak link between dietary access to carotenoids and bare part colouration in birds (Olson & Owens, 2005), so it is unlikely that condition as it relates to foraging ability would be correlated with bill colour. Additionally, the mechanisms of carotenoid metabolism vary substantially between and within species, making it difficult to detect relationships within a population, let alone within a species (Svensson & Wong, 2011a).

Taken together, my results discount the hypothesis that bill colouration signals identity in puffins and provide only partial support for bill colouration as a putative signal of quality. The presence of discriminable differences in colour over the course of a few weeks demonstrates that bill colour fluctuates in a perceptible way, but due to a lack of condition-dependence, it remains unknown what these changes represent. Fleshy structures such as the cere and rosette may play an especially important role in signaling because of their ability to change colour on a rapid timescale.

Future work should focus on whether colour change in the cere and rosette correspond to alternative aspects of quality, such as the ability to provide parental care.

## 2.6 References


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# CHAPTER 3 Chick growth predicted by maternal bill colouration and timing of hatch in a seabird

## 3.1 Abstract

In species with obligate bi-parental care, investment by both parents in a current reproductive bout is critical to offspring growth and survival. The degree to which an individual can invest relies on their quality as a parent. Ornamental features are particularly interesting in this context, as they may honestly reflect some aspect of parental quality, either in terms of direct or indirect contribution. In this study, I investigated whether Atlantic puffin's (*Fratercula arctica*) red-orange bill colouration honestly reflects two proxies of parental quality: hatch date and offspring growth. No aspect of parental colouration predicted hatch date, but several metrics of maternal colouration predicted offspring mass gain, peak mass, and normalized wing growth. I also explored whether hatch date influenced patterns of chick growth and found that timing (early vs. late) but not synchrony with food availability significantly influenced mass and skeletal growth, albeit in opposing directions. Early hatching chicks achieved higher peak masses but exhibited reduced wing growth rates, potentially reflecting alternative strategies between investing primarily in weight gain or structural development. Taken together, these results highlight seabird chick growth as a complex metric of parental quality, associated with both phenology and parental phenotype.

## 3.2 Introduction

One of the primary functions of signals is to alter the behaviour of a perceiver based on the attributes of the informer (Owren et al., 2010; Searcy & Nowicki, 2005). Such attributes may include the informer's identity, current condition or breeding status, resource holding potential, or their intentions or future actions (Laidre & Johnstone, 2013). The content and reliability of a signal depend on the identity of the informer and the intended perceiver (Laidre & Johnstone, 2013). In species that form long-term monogamous pair bonds and exhibit bi-parental care, such as seabirds, some of the most critical communication occurs between two breeding adults of a mated pair.

Reproduction is a costly activity, with trade-offs between current reproductive effort and future survival/reproductive attempts (Trivers, 1972). Breeding success rests on the ability of a mated pair to communicate current abilities and make informed reproductive decisions. While females ultimately decide whether to reproduce and when to lay an egg, other decisions require communication between both partners, such as choosing and defending a nest site or timing incubation and feeding events (Griffith, 2019). Indeed, pairs that communicate effectively can better coordinate anti-predator defense, incubation, and offspring provisioning (e.g., Halkin, 1997; Mainwaring & Griffith, 2013; Mariette & Griffith, 2012; Spoon et al., 2006). Within-pair coordination also leads to higher reproductive success, as demonstrated by the 'mate familiarity effect,' where pairs that have been together longer enjoy higher breeding performance (Rowley, 1983). This phenomenon has been documented in several seabird species, including red-billed gulls (*Larus novaehollandiae scopulinus*; Mills, 1973), Manx shearwaters (*Puffinus puffinus*; Brooke, 1978), black-legged kittiwakes (*Rissa tridactyla*; Coulson & Thomas, 1983), short-tailed shearwaters (*Puffinus tenuirostris*; Bradley et al., 1990), and blue-footed boobies (*Sula nebouxii*; Sánchez-Macouzet et al., 2014).

Many types of signals can be used to coordinate pair behaviour throughout a breeding attempt. Courtship behaviours such as ritual greeting displays (e.g., penguins *Aptenodytes sp.*, Nelson & Baird, 2002), elaborate dances (e.g., North American western grebe *Aechmophorus occidentalis*, Nuechterlein & Storer, 1982), or allopreening (e.g., petrels *Procellariiformes*, Warham, 1996) can be used to ensure the pair is compatible and aid in partner recognition (Williams, 2021). Incubation duties may be negotiated between mated pairs via acoustic signals (e.g., structured duets in zebra finches *Taeniopygia guttata*; Boucaud et al., 2016), or a combination of acoustic and visual signals (e.g., vocalizations and flight patterns in Northern lapwings *Vanellus valennus*; Sládeček et al., 2019). To synchronize provisioning visits, mated pairs may assess partner vocalizations (e.g., *Wee* calls in black phoebes *Sayornis nigricans*; Ferree et al., 2021), partner displays (e.g., pre-flight postures in gannets *Sulidae* and Abbott's booby *Papasula abbotti*; Nelson, 1978), indirect offspring cues (e.g. begging calls in Cory's shearwaters *Calonectris diomedea*, Granadeiro et al., 2000; Quillfeldt & Masello, 2004), or colourful ornaments (e.g., plumage colour in tree swallows *Tachycineta bicolor*, Dakin et al., 2016; crown colour in blue tits *Parus caeruleus*, Limbourg et al., 2004).

Of the types of colourful ornaments, carotenoid-pigmented features can be especially useful in breeding contexts because they often convey something about an individual's current condition or ability (Lozano, 1994; McGraw, 2006). Since parental care is an energetically costly activity that can have a direct effect on body condition, only those that can offset this cost are predicted to invest in the current reproductive attempt (Houston et al., 2005; Kokko, 1998). Therefore, a signal that transmits information on condition or ability may serve as an indicator of an individual's parental quality. For perceivers, signals encoding parental quality information may be pivotal in deciding whether to continue investing in a reproductive attempt or abandon it.

Ultimately, parental quality of the individuals in a mated pair is a key determinant of reproductive success and thus fitness (i.e., common terns, *Sterna hirundo,* Arnold et al., 2004; black-legged kittiwakes, Coulson & Porter, 1985; Eurasian oystercatchers, *Haematopus ostralegus*, Ens et al., 1992).

How does one quantify "parental quality" in this context? Parental quality has been measured in a number of ways, but is always characterized by the degree of investment in the *current* offspring compared to *other potential* offspring (Trivers, 1972). In seabirds that lay a single chick during the breeding season, like puffins, *other potential* offspring refers to siblings that may be reared in future reproductive attempts. Parental investment occurs at all stages of breeding, and thus can be measured across the entire span of a reproductive attempt. Prior to hatching, investment may be quantified via egg size or content (i.e., levels of carotenoids or immunoglobins; Blount et al., 2002), as well as the frequency and duration of incubation visits. After hatching, investment can be related to parental provisioning effort, measured as the rate of feeding events or the sizes of meals brought to the nest or burrow (i.e., Gladbach et al., 2009). Parental provisioning rate can vary throughout the breeding season, such that paired individuals may flexibly adjust provisioning rate based on their own changes in condition and ability, signals conveying information on their partner's condition and ability, or signals from chicks indicating nutritional need (i.e., begging calls; Gillies et al., 2022; Rector et al., 2014). Indeed, in some avian species, provisioning rate differs according to a partner's plumage colouration. In tree swallows, structurally coloured dorsal plumage corresponds to reproductive performance and survival, with bluer-hued individuals faring better than greener-hued individuals (Bitton et al., 2008; Bitton & Dawson, 2008). Male and female tree swallows exhibit negative differential allocation/reproductive compensation by increasing their provisioning rate when paired to a

partner with greener, more saturated dorsal plumage, effectively compensating for the inability of their low quality partner to provide parental care (Dakin et al., 2016; Gowaty et al., 2007; Haaland et al., 2017). In blue tits, higher degrees of ultraviolet reflectance on the crown reflect attractiveness in both sexes (Hunt et al., 1999) and viability in males (Griffith et al., 2003; Sheldon et al., 1999). In contrast to tree swallows, female blue tits exhibit positive differential allocation by decreasing provisioning rate to offspring when paired to a male with an experimentally reduced ultraviolet crown, thereby reducing their investment when paired to a lower quality partner and limiting unnecessary tolls on survival and reproductive success (Burley, 1986; Haaland et al., 2017; Limbourg et al., 2004).

The relationship between parental investment and ornamental colouration has only been investigated in one species of seabird to my knowledge (e.g., blue-footed booby *Sula nebouxii*, Velando et al., 2006). In support of the positive differential allocation hypothesis, female blue-footed boobies paired to males with experimentally duller feet decreased the size of their second egg compared to control females (Velando et al., 2006). The Atlantic puffin is a well-suited candidate for expanding this exploration, as both males and females display a conspicuously red-orange bill and bright orange rosette during the breeding season and exhibit high degrees of parental care (Harris & Wanless, 2011). The adaptive significance of the bill and rosette is currently unknown, but as a dynamic carotenoid-pigmented feature (Chapter 2; Doutrelant et al., 2013), it has the potential to honestly signal individual quality. While there does not seem to be a relationship between puffin bill colouration and current body condition (Chapter 2), relationships to other aspects of quality, such as foraging prowess or overall health status, may nonetheless play a role. Alternatively, there may be direct links between carotenoid-pigmented features like the bill and maternal quality via carotenoids deposited in egg yolks, which reduce the likelihood of

dangerous free radical attacks on the growing embryo's fragile immune system (Blount et al., 2002; Haq et al., 1996; Surai & Speake, 1998). Regardless of the mechanism, if bill colouration can be related to some aspect of parental quality, then it may be useful as a signal of an individual's ability to successfully raise offspring.

Puffins are long-lived (25+ years), socially and genetically monogamous seabirds with high interannual survival and low divorce rates. Breeding in this species is characterized by obligate bi-parental care of a single chick, with equal levels of care provided by males and females (although roles differ slightly; Creelman & Storey, 1991). Pufflings (i.e., puffin chicks) are fully reliant on parental foraging trips to survive and properly develop, and parents can flexibly adjust their provisioning rate as a function of offspring need communicated via begging calls (Cook & Hamer, 1997; Dahl et al., 2005; Fitzsimmons, 2018; Harris, 1983; Johnsen et al., 1994; Rector et al., 2014). Because of these life history characteristics, signals that reflect a partner's capacity to provision offspring could potentially mediate decisions concerning parental investment.

However, my attempts to collect provisioning rate data on Atlantic puffins were largely unsuccessful for several reasons: 1) puffins do not have stereotyped visit patterns, making it challenging to track all burrow visits; 2) puffin burrow networks are complex and heterogenous, with varying shapes and sizes, multiple entrances, and interannual fluctuations, posing obstacles to deployment of a reliable radio-frequency identification (RFID) system; and 3) a high proportion of visits (~45%) do not include feeding events, such that provisioning rate cannot simply be deduced from visitation rate (Fitzsimmons, 2018). For these reasons, hatch date and chick growth are much more reliable proxies of parental quality.

In many avian species, timing of egg laying (and thus hatch date) is an important factor in determining breeding success, with those that lay earlier or more synchronously with food

availability being of higher quality and producing nestlings in better condition (e.g., roseate terns *Sterna dougallii,* Burger et al., 1996; chinstrap penguins *Pygoscelis antarctica,* Moreno et al., 1997; common terns and thick-billed murres *Uria lomvia,* Gaston et al., 2009). In puffins, fledging success is higher for early hatching chicks, providing support for early hatch date as an indicator of high parental quality (Harris, 1980; Nettleship, 1972). In years with low capelin spawning synchrony, puffin breeding success remained unchanged, which may indicate that synchronicity with food availability is a less salient factor in this species (Regehr & Rodway, 1999). However, this "mismatch" hypothesis warrants further attention, as there is strong evidence that synchrony with food availability is key to growth and survival in other seabirds (McKinnon et al., 2012; Stenseth & Mysterud, 2002; Watanuki et al., 2009).

Puffling growth may also be related to parental quality. Although growth does not directly measure parental effort, offspring development is molded by the direct (i.e., genetic) and indirect (i.e., behavioural) benefits parents can provide. In pufflings, mass gain is characterized by an approximately linear increase for the first ~24-28 days, after which it declines until fledging ~7-10 days thereafter. Prior studies generally quantified linear growth rate, peak mass, and fledging mass as metrics of puffling mass growth (Baillie & Jones, 2003; Diamond, 2021; Kress et al., 2017). In contrast, growth of skeletal structures such as wing chord and tarsus have been reported as linear throughout the rearing period (Harris & Wanless, 2011), so growth rate is an easily extractable metric commonly employed in the literature.

The adaptive function of conspicuous ornamental colouration in Atlantic puffins is currently unknown and has yet to be explored as it relates to parental quality. In Chapter 2, an in-depth examination of puffin ornamental colouration found that the bill, cere, and rosette dynamically fluctuate across the breeding season, such that conspecifics could theoretically notice

and respond to changes in colouration. This aligns with the hypothesis that bill colour functions as a quality signal, which I explore in this chapter by assessing whether colour can be linked to timing of hatch or metrics of offspring growth, both of which influence breeding success and thus reflect parental quality.

## 3.3 Methods

### 3.3.1 Study site

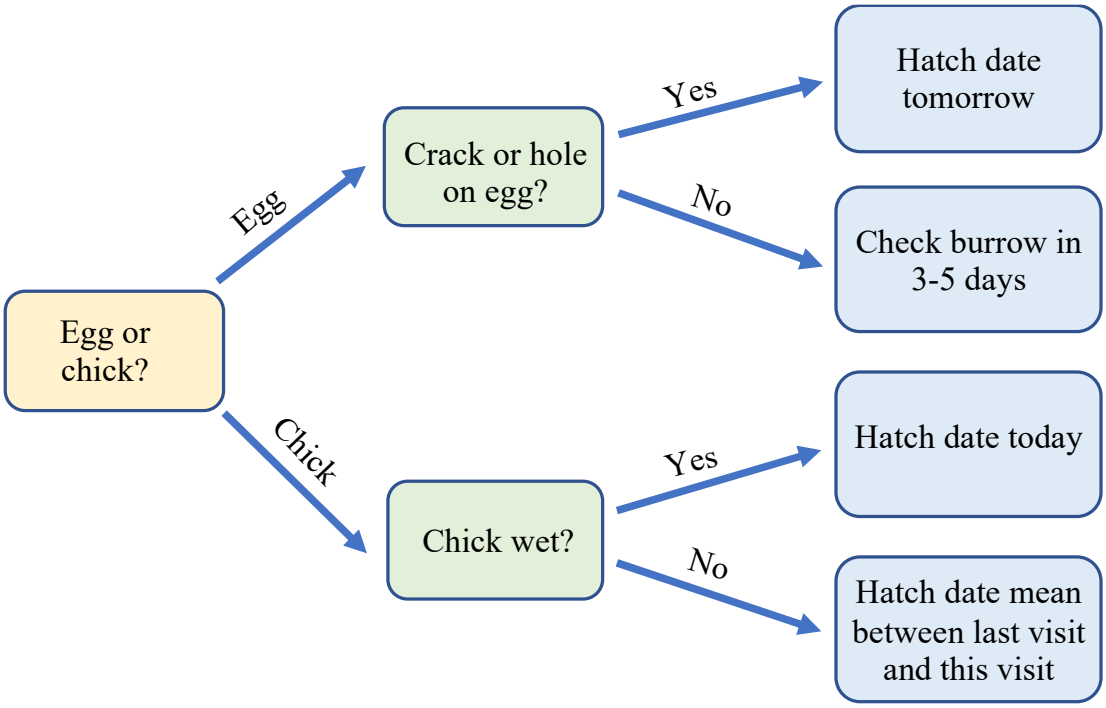
This study was conducted on Gull Island in the Witless Bay Ecological Reserve of Newfoundland and Labrador, Canada (47.26, -52.77). The Atlantic puffin colony on Gull Island is one of the largest in the Northwestern Atlantic, with approximately 120,000 breeding pairs according to a 2012 population survey (Wilhem, 2017).

### 3.3.2 Field methods: Hatch date

To assess the relationship between bill colouration and parental quality, 58 burrows were monitored and targeted for adult capture during the 2022 breeding season. On 13 and 24 June, occupied burrows were identified using an infrared burrowscope camera (EMS2021 Gopher Tortoise Camera System with infrared detection, Environmental Management Services, Canton, Georgia, USA) and marked if an egg was present. From 24 June until 20 July (i.e., egg hatching phase), burrows were checked with a burrowscope camera every 3-5 days for evidence of hatching. Nine of the originally 58 burrows were discovered empty before a chick hatched, so data is only available for 49 burrows. Hatch date was determined based on the contents of the burrow at each visit (Figure 3.1). If a burrow contained a chick during a given visit and contained an egg during the previous visit, the hatch date was assumed to be the midpoint between the visits (Baillie & Jones, 2003). If the chick still appeared to be wet, the hatch date was designated as the date of the current visit. If a burrow contained an egg, the burrow was re-checked after 3-5 days. However, if the chick appeared to be hatching (cracks or holes in the egg), the hatch date was designated as the date after the current visit, and the burrow was re-checked during the next visit to confirm there was a puffling present. Because exact hatch date could not be determined and was especially crude

for the earliest hatch dates (7 chicks hatched between 13 June and 24 June; logistical difficulties precluded more frequent visitation), nestlings were categorized based on timing and synchrony with local food availability. Each chick was classified as either an early hatcher (18 June – 29 June) or a late hatcher (30 June – 18 July), using 29/30 June as the cut-off date because 29 June was the median hatch date and 30 June was the mean hatch date among the monitored burrows. Coincidentally, the median hatch date (29 June) was the day when capelin was confirmed to be spawning in Witless Bay (eCapelin, 2017). Considering the median hatch date as the beginning of the period of peak food availability (Regular, 2014), chicks could also be classified as either synchronous (i.e., within $\pm$ 3 days from capelin spawning; 26 June – 2 July) or asynchronous (i.e., outside of this range; 18 June – 25 June , 3 July – 8 July). Of the 39 total chicks, 25 were classified as early, 14 as late, 16 as synchronous, and 23 as asynchronous.

Fig. 3.1 Decision flowchart for determining hatch date




### 3.3.3 Field methods: Chick data collection

Once a chick hatched, data on mass and wing length were collected at least five times for each individual. Each puffling was measured three times during the linear growth period (approximately 10-, 20-, and 30-days post-hatch), and every three to six days thereafter until the chick reached fledging size (wing length >= 130 mm). Offspring were captured by carefully extracting them from the burrow, occasionally with the aid of rubber-tipped tongs for particularly deep burrows. At each capture, mass was measured with a 600 g Pesola to the nearest 5 g, wing chord length was measured with a ruler to the nearest 1 mm, and notes on the stage of development were taken (i.e., downy, pin feathers, ready to fledge, etc.). Once the pufflings had pin feathers, the length of the tenth primary (hereafter p10) was measured with a ruler to the nearest 1 mm as

an exploratory metric of feather growth, and once their wing chord length was >125 mm, they were banded with a Canadian Wildlife Service (CWS) stainless steel band.

I was unable to measure ten chicks because of the depth or complexity of the burrow, and 14 additional burrows were empty or the puffling was discovered dead at the first check (10 days post-hatch). Two additional chicks were discounted because their burrows ultimately connected with the burrows of other monitored offspring later in the season and thus were assumed to be the same individuals. Full data could be collected for 15 individuals, with partial data collected on the remaining eight.

### 3.3.4 Field methods: Adult data collection

Once a chick was confirmed to have hatched, both parents were targeted for capture. Adult puffins were either extracted from their burrows after nightfall (22:00-3:00), or after several failed attempts, targeted for capture with a noose carpet. Noose carpets were fixed to the ground of the burrow entrance with gardening stakes, covered with soil, and placed so that the large loops of the nooses were protruding up from the carpet. A hunting blind was set up at the bottom of the slope with clear lines of sight to all target burrows, and at least one observer watched the slope while the traps were deployed for signs of capture (i.e., minor struggle). In all cases, birds were caught in the noose trap while exiting the burrow, so it was highly likely that the captured individual corresponded to the target burrow. Once a bird was caught, a field assistant or I quickly attended to the trap and carefully extracted the bird from the noose(s), a process that never lasted more than 2-3 minutes. If the bird had already been captured (identified via CWS number), the noose carpet was removed and the bird was immediately returned to the burrow. All other birds were secured in a cloth bag and transported to the blind to be measured and photographed.

Adult data collection proceeded following the methods outlined in Chapter 2.3.2. In short, individuals were first given a CWS band for subsequent identification, mass and wing chord were measured, and a blood sample was taken for sex determination. They were then taken into a blind, where ultraviolet (UV, 320-380 nm) and visual spectrum (400-680 nm) RAW 20 megapixel images were taken with a full spectrum converted Samsung NX1000 (following instructions from Troscianko, 2018) using two 2-inch Baader lens filters. The photos were illuminated with a full-spectrum ballast that passed through a light diffuser, and all photos included white (99% reflectance) and dark (10% reflectance) standards. The bill was cleaned of debris with a toothbrush prior to photo capture and was held in place with a wooden bill stabilizer. If noose carpets were deployed during these procedures, a research assistant regularly monitored the slope in case another bird was caught in a noose carpet. At no point did a bird get caught in a noose carpet while another bird was being processed. Noose carpets were always removed from the entrance prior to returning processed birds to their burrows.

Thirteen mated pairs (26 individuals) were successfully captured and measured, along with 13 additional individuals (n = 39). Full chick growth data was recorded for ten of the mated pairs and three individuals (n = 23), partial data was available for two mated pairs and five individuals (n = 9), and for five individuals, only hatch data was available.

### 3.3.5 Assessment of colour

Multispectral images, cone catch models, and colour coordinates from regions of interest were generated following the methodology outlined in Chapter 2.3.5 (van den Berg et al., 2020). In brief, one photo from the visual spectrum and one photo from the UV spectrum were aligned along the bill and merged in ImageJ. The multispectral image was converted to a cone catch model

using the spectral sensitivity of a violet-sensitive avian visual system with a standard illuminant D65 (CIE). Quantum catch values were extracted from four regions of interest (ROIs): one on the tip of the upper mandible, one on the base of the mandible, one on the cere, and one on the rosette. Quantum catch values were modelled in tetrachromatic colour space with the R package *pavo* (Maia et al., 2013; Table A.1)

Colour variables (hue VIS, hue UV, achieved saturation) were identified based on the features of a colour vector, which is defined by the position of the ROI colour coordinates in tetrahedral colour space with respect to the achromatic center (Table A.1). Hue represents the direction of the colour vector, in terms of azimuth (VIS) and elevation (UV). Hue VIS ranges from $-\pi$ to $+\pi$, such that perceived reds and purples are negative values, perceived yellows and oranges are close to zero, and perceived greens and blues are positive values (Dakin & Montgomerie, 2013). Hue UV ranges from $-\pi/2$ to $+\pi/2$, with more UV rich colours having more positive values (Dakin & Montgomerie, 2013). Chroma is the saturation of a colour and is defined as the magnitude of the colour vector ($r$) from the achromatic center (Stoddard & Prum, 2008). Achieved saturation is simply the magnitude controlling for the potential maximum chroma of the given hue ($r/r_{max}$; Stoddard & Prum, 2008). These measures were calculated for all four chromatic regions (upper and lower mandible, cere, and rosette). Brightness was calculated from the relative stimulation of the double cone. Brightness was measured for all five regions, including the achromatic mandible base. All colour variables were calculated with the R package *pavo* (Maia et al., 2013, 2019).

### 3.3.6 Molecular methods

Sex of the 39 adults was determined molecularly from blood samples collected in the field following the methods outlined in Chapter 2 (2.3.3). Briefly, DNA was extracted using a DNeasy®

Blood & Tissue Kit (Qiagen Inc., Toronto, ON, CA) following protocols outlined in the DNeasy® Blood & Tissue Handbook (2020) and stored at -20 °C. Polymerase chain reaction (PCR) was run on extracted DNA to amplify the chromo-helicase DNA 1 (CHD1) gene on the avian W and Z chromosomes. The PCR was run with an Eppendorf Mastercycler® ep gradient S on a program of 95 °C for 5 minutes, 35 cycles of denaturing, annealing, and extension at 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 60 seconds, extension at 72 °C for seven minutes and a cooling period of 4 °C for 10 minutes. PCR samples were run on a RedSafe™ agarose gel with 100 base pair reference ladders on a Thermo Scientific™ EC 300 XL for 50 minutes at 130 amps. The gels were then imaged using Image Lab software and stored digitally. Of the 39 sexed individuals, 19 were sexed as male and 20 were sexed as female. All procedures were carried out at Memorial University of Newfoundland following standard lab safety protocols.

### 3.3.7 Calculation of body condition index

Body condition was determined from the residuals of a best fit linear regression with mass as the response variable. This was calculated using the full dataset on hatch date (n = 39) for use in all models described in Section 3.3.9. Typically, body condition is either given as the residuals of the linear regression of mass on wing chord length, or simply mass (Doutrelant et al., 2013; Fitzsimmons, 2018; Labocha & Hayes, 2012). However, mass in puffins is known to change across the breeding season and differ between the sexes, so these variables (Ordinal date of capture and sex) were included in our regression model. To account for potential sex-biased differences in mass change within years, I also included the two-way interaction between sex and Ordinal date in the full model. The full model was reduced by stepwise removal of non-significant terms based

on results from ANOVA tables. The final linear regression model met the assumptions of homogeneity of variance, normal distribution of the residuals, and low multicollinearity.

### 3.3.8 Model selection for chick growth

To generate metrics of chick growth, six different growth models were evaluated for mass, wing length, and p10 length (as described below). Each model was assessed using averages of the response variable (mass, wing length, or p10 length) at each age point to avoid pseudoreplication. The linear and quadratic models (equations 1 and 2 below, respectively) represent classic systems of equations that may be relevant to growth. Equations and interpretations of models 3-6 are taken from the analysis by Tjørve & Tjørve (2010) on typical offspring growth curves. Variables that appear in multiple equations are only defined once, at its first appearance.

1. *Linear model*

The linear model is a simple relationship with two parameters, given as:

$$y = b_0 + b_1 x, \quad (2)$$

where $y$ = the response variable, $b_1$ = rate of change, $x$ = age (number of days since hatch), and $b_0$= mass at hatch. Linear models are often applied to specific parts of the growth curve (i.e., "linear growth rate"; Coulson & Porter, 1985; Nisbet, 1978), and may be especially useful for measures of structural growth such as wing or tarsus length.

*2. Quadratic model*

The quadratic model consists of three terms and can be defined as:

$$y = ax^2 + bx + c,$$

( 3 )

where $a$ is the quadratic coefficient, $b$ is the linear coefficient, and $c$ is the constant (i.e., free term). The quadratic model is not a typical growth curve model but was considered because the stereotypical pattern of puffling mass gain visually resembles a quadratic curve.

*3. Logistic model*

The logistic growth model contains three parameters, and is given as:

$$y = \frac{K}{1+e^{-r(x-x_i)}},$$

( 4 )

where $K$ = asymptotic body mass and $x_i$ = the age at the inflection point. This model has a fixed inflection point at 50% of the upper asymptote and is symmetrical on both sides of the inflection point (Ricklefs, 1968).

*4. Gompertz model*

The Gompertz model also consists of three parameters, and is usually given as:

$$y = Ke^{-e^{-r(x-x_i)}}.$$

( 5 )

This model, like the logistic growth model, has a fixed inflection point, but it is lower on the slope at 36.79% of the upper asymptote (Ricklefs, 1968).

*5. Extreme value function (EVF) model*

The EVF model also consists of three parameters, and can be given as:

$$y = K\left(1 - e^{-e^{r(x-x_i)}}\right).$$

( 6 )

This model has a fixed inflection point higher than that of the logistic growth model, at 63.21% of the slope. While the EVF model is not commonly used in modelling chick growth, it was found to best represent tarsus growth of African black oystercatchers (*Haematopus moquini*) in Tjørve & Tjørve (2010) and thus may be useful for some biometrics.

*6. von Bertalanffy model*

The von Bertalanffy model contains three parameters, and is given as:

$$y = K(1 - e^{-r(x+x_0)}),$$

( 7 )

where $x_0$ is a starting point on the x-axis for the curve, at $r = -x_0$. Because the curve has a starting point on the x-axis, it does not have a lower asymptote, unlike the previous three models. The inflection point on this model is at 29.63% of the upper asymptote, even lower than the inflection point of the Gompertz model. While this model is no longer commonly used to describe chick growth, it is still considered one of the classic models (Ricklefs, 1968).

The best model was chosen for each metric of chick growth (mass, wing length, p10 length) based on comparison of the corrected Akaike's information criteria ($AIC_c$), which is useful for comparing models generated from relatively small sample sizes. I ran the models both with and without the last data point of the oldest recorded individual in our dataset, who was estimated to be 58 days old when captured for the last time. This was 5 days older than the next oldest observation and demarcated a clear separation in the spread of the data, signs that it may unduly influence the results of our model selection. However, the $AIC_c$ values of the two sets of models

followed the same ranking order, so only results from the models including the outlier are presented here. In each case, the model with the lowest $AIC_c$ was selected as the preferred model. Following Tjørve & Tjørve's (2010) approach, the preferred model was compared to the model with the second lowest $AIC_c$ value to determine the probability that I selected the correct model of the two:

$$probability = e^{AIC_c(preferred\ model) - AIC_c(second\ model)/2}.$$

### 3.3.9 Chick growth parameters

Once the preferred models were selected for each growth metric, growth curves were separately fitted to each individual's data points. Because of differences in hatch date and an opportunistic sampling regime, pufflings were not measured at the same ages across the cohort. Generating separate growth curves for each individual allowed me to incorporate all the recorded measurements, regardless of age discrepancies. I excluded pufflings that were sampled fewer than three times for a given biometric; therefore, I was only able to generate mass and wing length growth curves for 18 of 25 measured individuals. An additional three chicks had fewer than three measurements of their tenth primary, so p10 growth curves were only produced for 15 individuals.

Growth curves were generated using the 'nlsList' function in the *nlme* package, grouping by individual ID and estimating variance separately for each growth curve (Pinheiro et al., 2022). Due to logistical challenges associated with field sampling, a few pufflings only had three or four measurements. In these cases, growth curves could sometimes not be produced due to a near perfect fit of the data or an incompatibility with the model type. In such cases, I used the 'nls' function in the *nlme* package to separately generate curves for each of these individuals (Pinheiro et al., 2022). The curves were then visually assessed to determine if the model properly fit the data.

In some cases, logical growth curves were created, (two individuals for mass, one individual for wing length), whereas in other cases, illogical or nonsensical curves were created (i.e., model clearly did not fit the data points) and could not be used (one individual for wing length, three individuals for p10).

For logistic and EVF models, four informative parameters could be extracted from the growth curves: growth rate (r), normalized growth rate (r/K), the y value at the inflection point ($y_i$), and the asymptotic value (K). For the quadratic model, a measure of growth rate ($y_{max} - y_{min}$)/ ($x_{ymax} - x_{ymin}$) and maximum value ($y_{max}$) could be calculated.

## 3.3.10 Statistical analyses

### 3.3.10.1 Predictors of hatch group

The relationship of parental colouration and condition to hatch group was evaluated with generalized linear models (link = "logit"), with hatch group as a binomial outcome variable. I tested whether adult colouration was associated with the timing of hatch (early vs. late; hereafter *timing*), as well as the synchrony of hatch with prey availability (synchronous vs. asynchronous; hereafter *synchrony*).

Timing and synchrony models were generated separately for male features versus female features. I chose to create separate models for each sex rather than include sex as a variable in the model because male and female parental colour and condition were differentially available, with some pufflings having data on both parents and others having data only from a single parent. This ensured that each puffling was only represented once in any given model.

The models were further split based on colour attributes (chromatic vs. achromatic variables) to avoid singularity errors associated with overfitting. Pearson's correlations were used

to select a maximum of six (males) or eight (females) colour variables for inclusion in the full models. Since models were generated for chromatic and achromatic colour variables separately, correlations were only calculated within chromatic or achromatic variables. Colour variables that were correlated ($p < 0.05$) *within* a region were first condensed by a) retaining the variable that was correlated to the most other variables (i.e., if achieved saturation is correlated to both hue VIS and hue UV, only achieved saturation was retained), and for chromatic variables, b) prioritizing hue VIS and achieved saturation over hue UV. For the remaining colour variables, those that were correlated *between* regions were further condensed based on the same criterion. If more than the allotted colour variables remained, variables from the same region were preferentially eliminated. Table 3.1 details which colour variables were retained for each full model.

Each model was reduced using backward stepwise selection from a model including all main effect colour variables presented in Table 3.1 and parental condition (Equations 1-8; Table 3.3). Interactions between predictor variables were not included in the full model to avoid overfitting. Models were reduced based on *P* values reported in the 'summary' function until only significant terms remained. *P* values of final models were adjusted for type 1 error with the false discovery rate correction (Benjamini & Hochberg, 1995).

#### 3.3.10.2 Predictors of chick growth

The relationship of parental bill colouration, parental body condition, and hatch group to chick growth outcomes was tested using linear regressions. To avoid overstating the conclusions, I first tested whether the response variables for each growth metric were correlated using a Pearson's correlation test (assumption of linearity met). For wing and p10 length growth, all four measures (growth rate, normalized growth rate, y value at the inflection point, asymptotic value)

were correlated (Table 3.2), so models were only generated for normalized growth rate (r/K) because it references both growth rate and asymptotic value. Since the normalized growth rate of wing length and p10 length were also highly correlated (0.731, $p =< 0.001$), I only report the results of wing growth models here. Rate of mass gain and maximum mass were also correlated (Table 3.2), but models were generated for both response variables because 1) normalized growth rate cannot be calculated for quadratic models, 2) linear growth and asymptotic mass are commonly assessed metrics in the literature and 3) each variable may indicate biologically different aspects of development.

Chick growth models were also split by sex and colour attributes (chromatic vs. achromatic), yielding four models for each biometric. The procedure outlined in Section 3.3.8.1 was used to select a maximum of four colour variables (male chromatic models) or six colour variables (female chromatic models) for inclusion in the full models. The same colour variables were chosen as for the hatch group generalized linear models (Table 3.1).

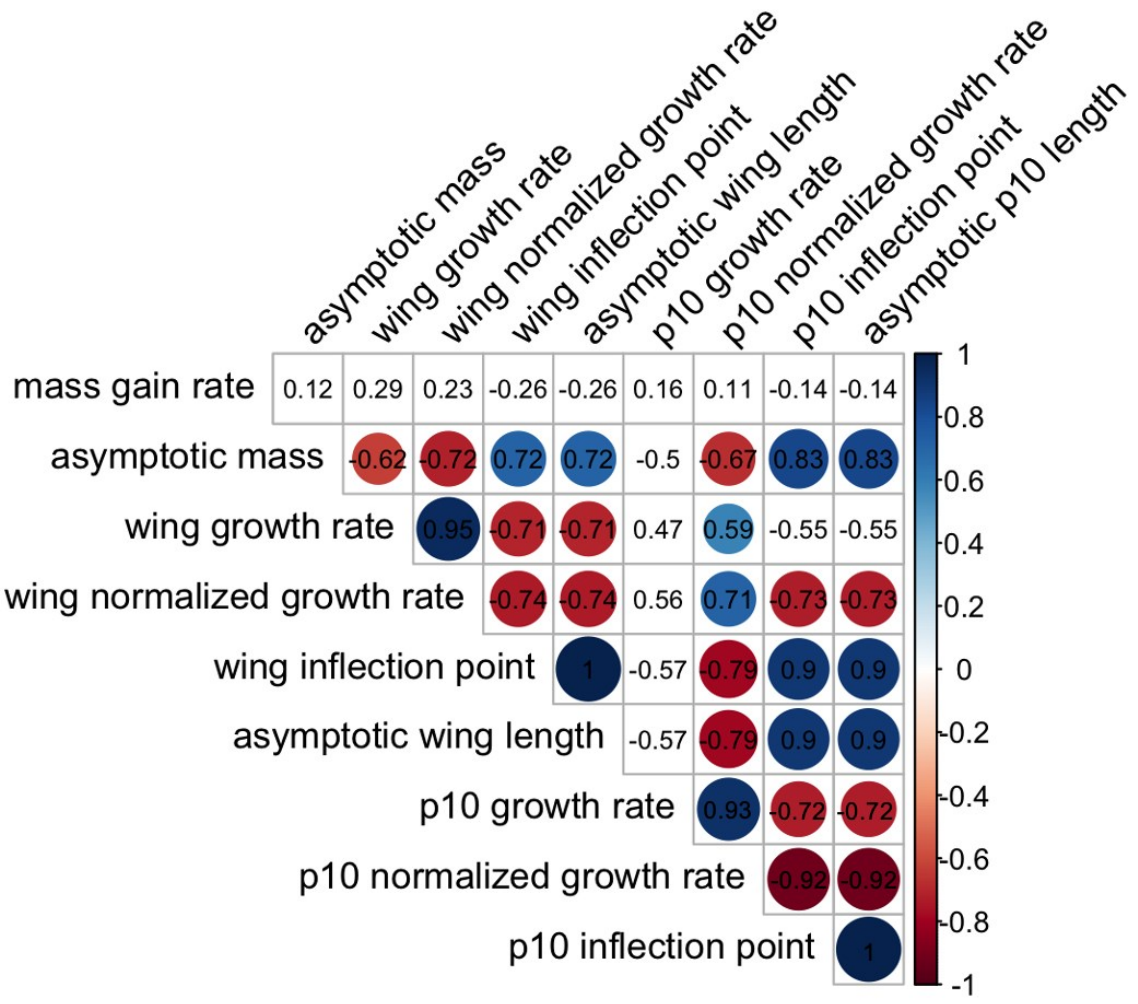
Each model was reduced using backward stepwise selection from a model including all main effect colour variables presented in Table 3.1, both hatch group delineations (timing and synchrony), and parental condition (Equations 9-20; Table 3.3). While hatch timing and synchrony were nonrandomly associated in both the maternal ($P = 0.0047$; Fisher's exact test) and paternal datasets ($P = 0.018$; Fisher's exact test), neither variable showed evidence of multicollinearity in any of the full models based on the GVIF values, so both were retained. I chose to exclude all two-way interactions to avoid overfitting the models (Babyak, 2004). Models were reduced based on *P* values reported in the 'summary' function, and a final model was achieved when all variables in the model were significant. Model assumptions (i.e., normal distribution and homoscedasticity of the residuals, presence of outliers) were evaluated for the final model by visually assessing R

diagnostic plots. *P* values of final models were adjusted for type 1 error with the false discovery rate correction (Benjamini & Hochberg, 1995).

Table 3.1 Colour variables included in full models for generalized linear regressions (hatch timing and synchrony) and linear regressions (chick growth)

| Sex | Colour Space | Variables retained |
|---|---|---|
| Male | chromatic | Upper mandible hue VIS, cere hue VIS, rosette achieved saturation, rosette hue VIS |
| | achromatic | Base of mandible, cere, and rosette brightness |
| Female | chromatic | Upper mandible achieved saturation, upper mandible hue VIS, cere hue VIS, rosette achieved saturation, rosette hue VIS |
| | achromatic | Cere and rosette brightness |

Table 3.2 Pearson's correlations between chick growth variables




Only correlations with a coloured circle are significant ($P < 0.05$). Note: Because the value at the inflection point is exactly half of the asymptotic value, the correlations between (normalized) growth rate and the inflection point are the same as the correlations between (normalized) growth rate and the asymptotic value.

Table 3.3 Equations for full models of generalized linear and linear regressions

| | Response variable | Predictor variables |
|---|---|---|
| 1 | Hatch timing | *Chromatic* variables + *Male* body condition |
| 2 | Hatch timing | *Achromatic* variables + *Male* body condition |
| 3 | Hatch timing | *Chromatic* variables + *Female* body condition |
| 4 | Hatch timing | *Achromatic* variables + *Female* body condition |
| 5 | Hatch synchrony | *Chromatic* variables + *Male* body condition |
| 6 | Hatch synchrony | *Achromatic* variables + *Male* body condition |
| 7 | Hatch synchrony | *Chromatic* variables + *Female* body condition |
| 8 | Hatch synchrony | *Achromatic* variables + *Female* body condition |
| 9 | Chick asymptotic mass | *Chromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 10 | Chick asymptotic mass | *Achromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 11 | Chick asymptotic mass | *Chromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |
| 12 | Chick asymptotic mass | *Achromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |
| 13 | Chick mass growth rate | *Chromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 14 | Chick mass growth rate | *Achromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 15 | Chick mass growth rate | *Chromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |
| 16 | Chick mass growth rate | *Achromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |
| 17 | Chick wing growth rate | *Chromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 18 | Chick wing growth rate | *Achromatic* variables + *Male* body condition + Hatch timing + Hatch synchrony |
| 19 | Chick wing growth rate | *Chromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |
| 20 | Chick wing growth rate | *Achromatic* variables + *Female* body condition + Hatch timing + Hatch synchrony |

## 3.4 Results

### 3.4.1 Body condition index

Body condition is given as the residuals from a regression with mass as the response variable. The final linear regression retained all three main-effect variables: wing length, Ordinal date of capture, and sex (Table 3.4). Mass was positively related to wing length (3.58 ± 1.05, $F_{1,35}$ = 28.42, $P < 0.001$; Figure B.1), but negatively related to Ordinal date (-0.50 ± 0.42, $F_{1,35}$ = 4.74, $P = 0.036$; Figure B.1). Mass was also higher in males compared to females (26.27 ± 8.96, $F_{1,35}$ = 8.60, $P = 0.006$; Figure B.1). The included variables were highly significant ($\alpha = 0.5$) and no evidence of multicollinearity was detected (VIF = 1.19, 1.17, 1.29, respectively).

Table 3.4 Analysis of variance table for body condition regression, 2022

| Predictor | Df | Sum Sq | Mean Sq | F | P |
|---|---|---|---|---|---|
| *Wing length* | 1 | 17177.6 | 17177.6 | 28.42 | <0.001 |
| *Ordinal date* | 1 | 2862 | 2862 | 4.74 | 0.0364 |
| *Sex* | 1 | 5199.9 | 5199.9 | 8.60 | 0.0059 |
| *Residuals* | 35 | 21154.1 | 604.4 | | |

### 3.4.2 Factors influencing hatch group

Male models contained 19 observations, each representing a unique chick-parent combination. Female models also contained 19 observations, but the full dataset contained 20 observations due to the presence of an unexpected female-female pair in a burrow. One of these females was caught by hand, while the other was captured by noose trap. Since both females are considered parents of the same puffling, I eliminated the individual caught by noose trap to avoid pseudoreplication and reduce uncertainty in paternity.

No variables were retained in any of the hatch timing or hatch synchrony models. Specifically, none of the male or female colour variables, nor adult condition, were significant predictors of hatch timing or hatch synchrony with prey availability.

### 3.4.3 Chick growth model selection

The preferred model differed between all three biometric measurements. The quadratic model was preferred for mass, the logistic model was preferred for wing length, and the extreme value function (EVF) model was preferred for p10 length (Table B.1; Figure 3.1). The EVF was the only model to appear as either the preferred or second-best model for all three measures of chick growth, but clearly was more representative of structural growth compared to mass gain (Figure 3.1).

Typically, a model is considered to perform significantly better than another model if the difference in $AIC_c > 2$. As expected, when the $\Delta AIC_c$ was small, as was the case for wing and p10 length, the probability that the best model was selected dropped well below 50%. Although considerable uncertainty exists in the model selection of wing and p10 growth rates, results are

only reported for values from the preferred models, since they likely yield similar results as the second-best models.

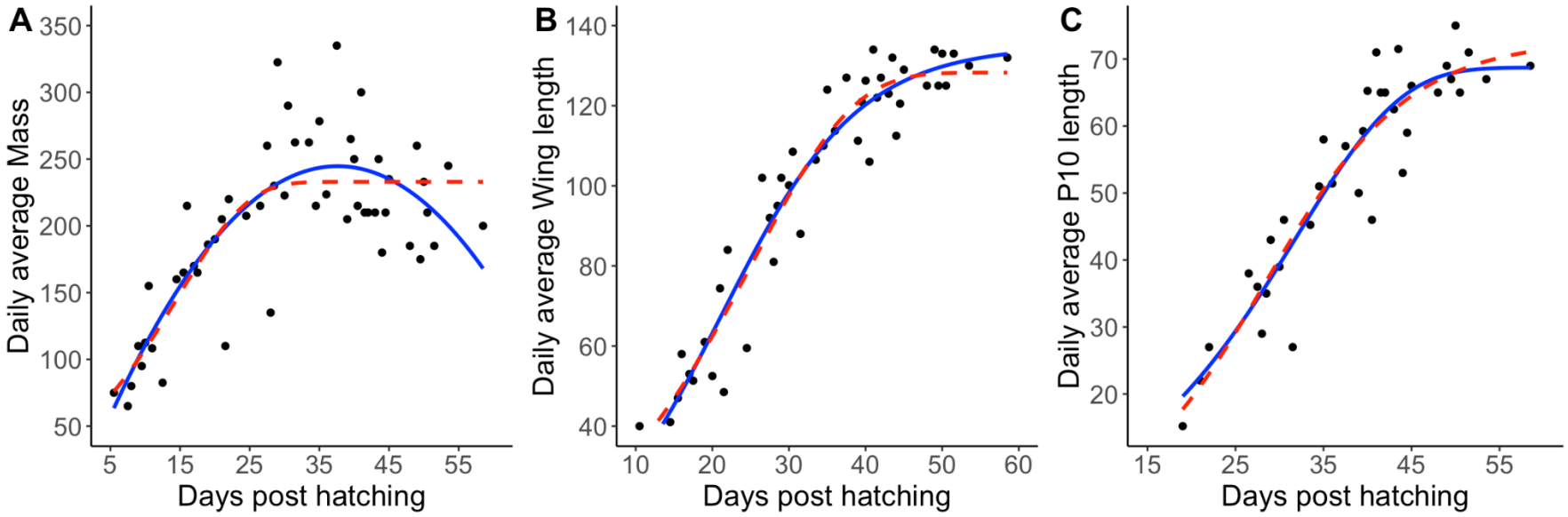



Figure 3.1 Growth models for chick biometrics. Blue solid line curves represent preferred models for each biometric, and red dashed curves represent second-best models for each biometric. A) mass growth curves with quadratic model as preferred and EVF model as second-best; B) wing length growth curves with logistic model as preferred and EVF model as second-best; C) p10 length growth curves with EVF model as preferred and logistic model as second-best.

### 3.4.4 Factors associated with chick growth

#### 3.4.4.1 Mass

Mass growth and asymptotic mass could be calculated for 18 pufflings, 14 of which had at least one captured female parent and/or at least one captured male parent. Therefore, both male and female mass models had sample sizes of 14 individuals. Due to the presence of a female-female pair, one mother-offspring datapoint was eliminated from the female dataset to ensure each puffling was only represented once. The individual caught by noose trap was removed for all chick growth models. The reported final models all met the assumptions of normality and homoscedasticity of the residuals.

Chick mass growth was not significantly influenced by any male parental characteristic, although rosette brightness approached significance ($t_{1,11} = -1.905$, $P = 0.081$). In contrast, hue VIS of the mother's upper mandible predicted offspring mass gain, such that pufflings with redder-billed mothers had higher growth rates. Timing or synchrony of hatch were not retained in any of the models predicting mass growth (Table 3.5).

Several variables were significant in the female chromatic model for chick asymptotic mass; achieved saturation of the upper mandible, hue VIS of the cere and rosette, and adult condition were all significant predictors (Table 3.5). Specifically, offspring of mothers in better condition, as well as those with a yellower (compared to orange) cere, a more orange (compared to yellow) rosette, and a more saturated bill had higher asymptotic masses (Figure 3.3). Timing of hatch was the only factor that was retained in all four asymptotic mass models (male/female and chromatic/achromatic). As depicted in Figure 3.2A, early hatching chicks achieved higher masses compared to late hatching chicks.

#### 3.4.4.2 Wing length growth

Wing growth metrics could be calculated for 17 chicks, 14 of which had at least one captured male parent and 13 of which had at least one captured female parent. Therefore, male wing growth models had a sample size of 14, whereas female wing growth models had a sample size of 13 chicks. After the removal of an influential outlier in both the male and the female dataset, all final models met the assumptions of normality and homoscedasticity of the residuals.

Hatch timing was the only significant predictor of normalized wing growth rate in three of the four models: male chromatic and achromatic models and the female achromatic model (Table 3.5; Figure 3.2). In contrast to asymptotic mass, late hatchers had higher normalized wing growth rates than early hatchers. Rosette hue VIS in the maternal chromatic model was the only significant parental characteristic. Again, the opposite trend compared to mass was observed; offspring of mothers with a yellower rosette had higher wing length growth rates.

Table 3.5 Final models predicting chick growth metrics from parental colour, condition, and hatch group. Only models with significant ($\alpha < 0.05$) or near significant ($\alpha < 0.1$) predictors are presented.

| Final model | Sex | A/C | Predictor variable | Estimate ± SE | t | P |
|---|---|---|---|---|---|---|
| *Mass growth* | M | A | Rosette brightness | -15.96 ± 8.38 | -1.905 | 0.094 |
| | F | C | Upper mandible hue VIS | -39.94 ± 12.67 | -3.153 | 0.016 |
| | | A | Rosette brightness | -10.55 ± 6.43 | -1.640 | 0.135 |
| *Asymptotic mass* | M | C/A | Hatch timing | -57.97 ± 23.61 | -2.455 | 0.042 |
| | F | C | Upper mandible achieved saturation | 118.85 ± 41.08 | 2.894 | 0.031 |
| | | | Cere hue VIS | 383.83 ± 100.76 | 3.810 | 0.015 |
| | | | Rosette hue VIS | -118.37 ± 46.86 | -2.523 | 0.043 |
| | | | Condition | 1.15 ± 0.27 | 4.317 | 0.009 |
| | | | Hatch timing | -78.50± 12.07 | -6.504 | 0.001 |
| | | A | Hatch timing | -66.52 ± 21.18 | -3.141 | 0.016 |
| *Wing growth* | M | C/A | Hatch timing | <0.001 ± <0.001 | 7.265 | <0.001 |
| | F | C | Rosette hue VIS | <0.001 ± <0.001 | 3.129 | 0.021 |
| | | | Hatch timing | <0.001 ± <0.001 | 4.856 | 0.004 |
| | | A | Hatch timing | <0.001 ± <0.001 | 3.286 | 0.016 |

M = males, F = females; A = achromatic, C = chromatic. *P* values corrected with the false discovery rate

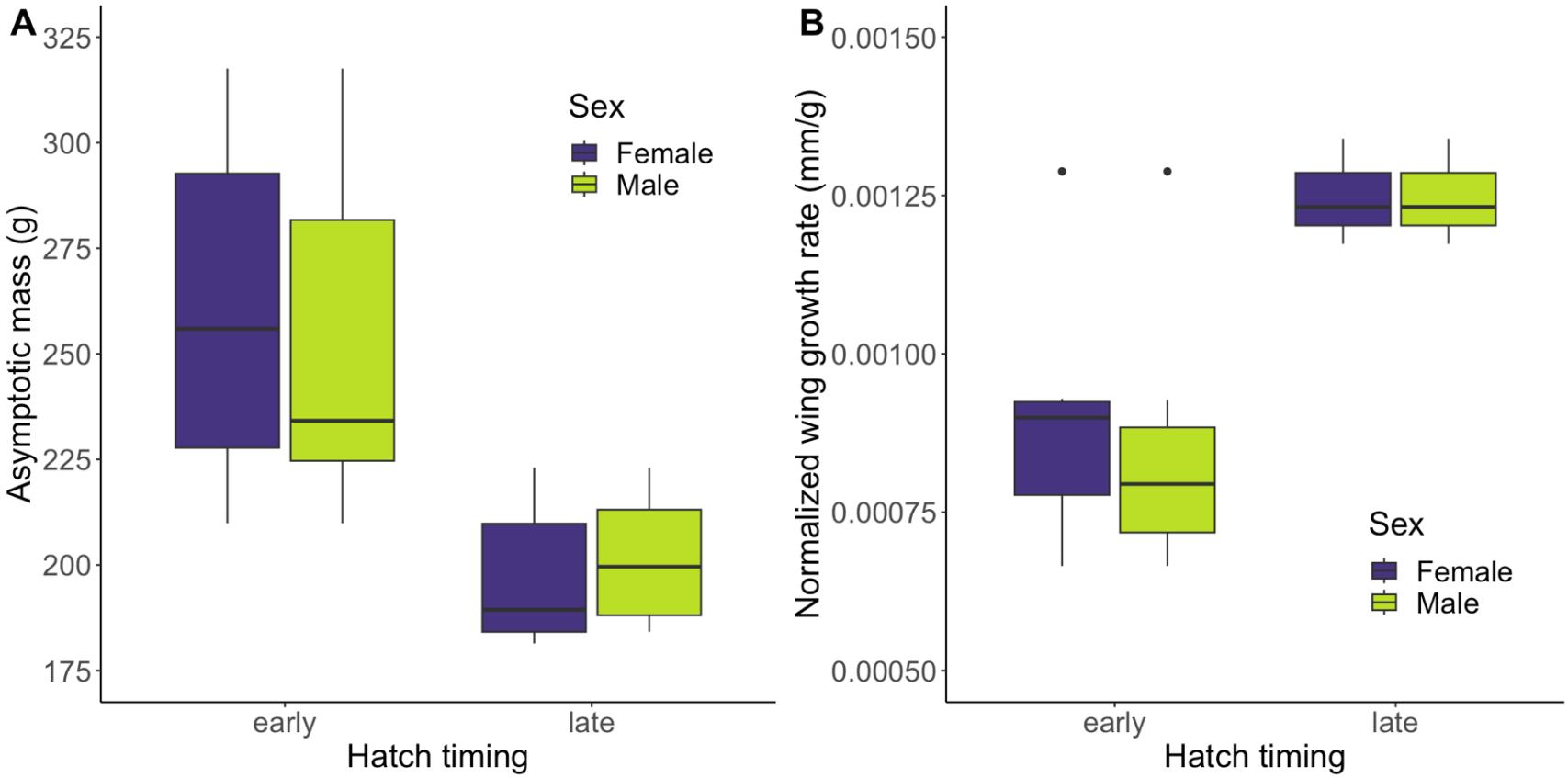



Figure 3.1 Timing of hatch significantly influences multiple aspects of chick growth. A) Early hatchers achieved higher asymptotic masses compared to late hatchers, and B) late hatchers have higher normalized growth rates compared to early hatchers.

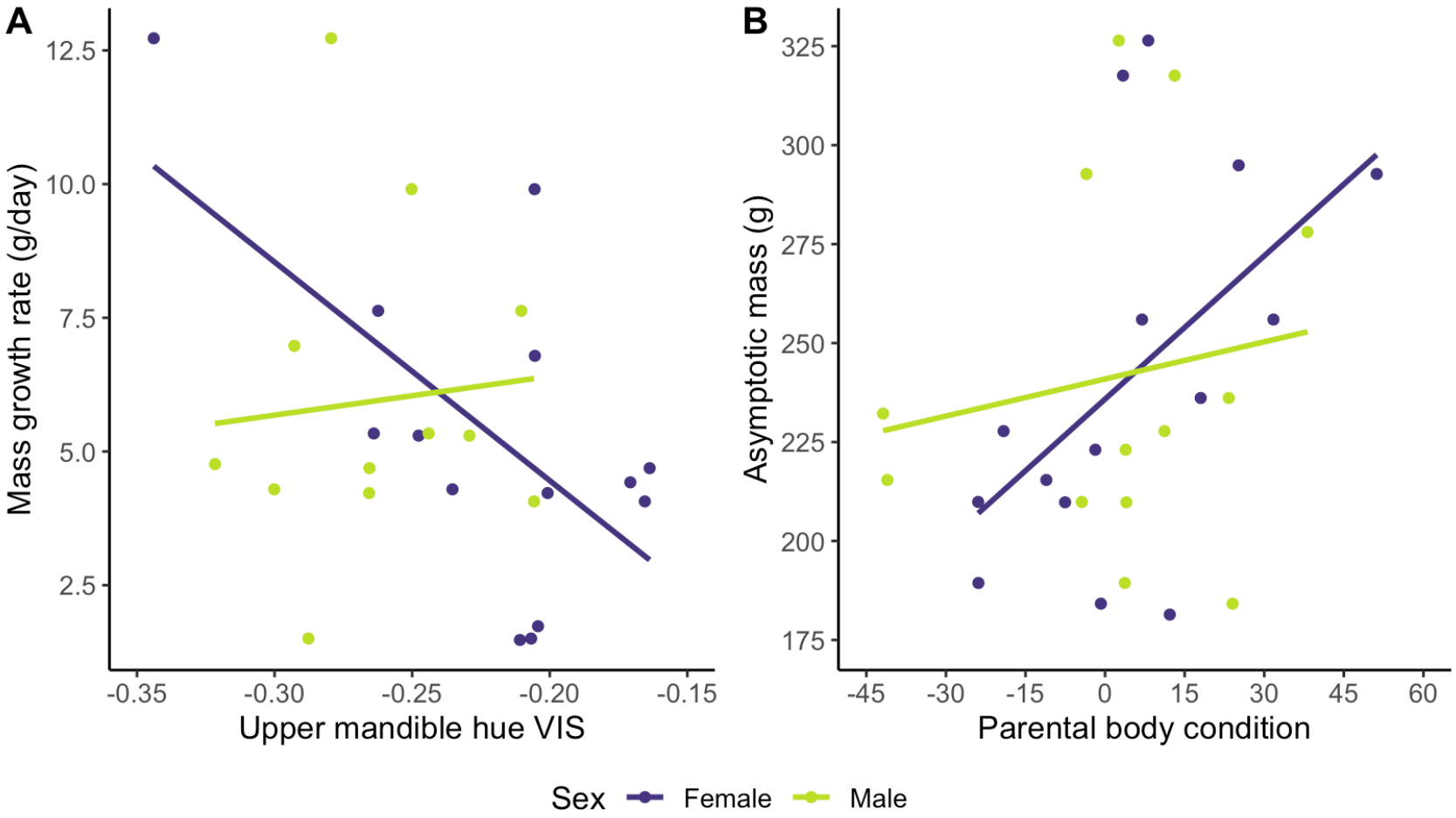



Figure 3.3 Maternal features, but not paternal features, significantly predicted chick growth outcomes. Two examples are illustrated in this figure: A) maternal upper mandible hue VIS predicted puffling mass growth, such that redder-billed mothers had faster growing chicks, and B) maternal body condition predicted asymptotic mass, such that mothers in better condition had chicks with higher peak weights.

## 3.5 Discussion

The primary aim of this study was to assess whether Atlantic puffin bill colouration reflects parental quality, as assessed by offspring hatch date, mass gain and structural growth. Some features of bill, cere and rosette colouration predicted chick growth, but no aspect of colouration explained timing or synchrony of hatch with local capelin availability. Instead, hatch timing itself predicted several measures of chick growth and, as such, seems to be one of the key factors influencing puffling growth patterns (in accordance with Nettleship, 1972).

However, the importance of parental colour should not be discounted; the hue and saturation of several maternal features significantly predicted peak mass, mass gain rate, and wing growth rate. In most cases when colour was retained in the final model, the relationship with chick growth was in the predicted direction; redder, more saturated colours were associated with better chick outcomes. This aligns with hypotheses linking carotenoid colouration and individual quality, where higher quality individuals can invest more carotenoids in a structure and produce a more saturated, redder-orange ornament as a result (e.g., red crossbills *Loxia curvirostra,* Cantarero et al., 2020; red grouse *Lagopus lagopus scoticus,* Mougeot et al., 2007; great black-backed gulls *Larus marinus,* Kristiansen et al., 2006). Indeed, higher quality mothers (i.e., those that could raise a heavier chick) were also those that produced more pigmented features in my study sample.

In no case did *paternal* colour or condition predict offspring growth, perhaps indicating that maternal bill colour more honestly reflects ability to provide parental care. This is puzzling considering that puffins exhibit obligate bi-parental care, with females and males providing near-equal contributions across the breeding season. Both parents are critical to successfully raising a chick; those that are raised by a single parent either die or develop much more slowly (Harris, 1978). However, there are a few important differences in investment between the sexes. Females

establish the initial investment in the breeding attempt, as they are responsible for egg production and accordingly the transfer of important nutrients and metabolites to the developing embryo (i.e., yolk composition; Price, 1998). This aspect of investment is especially intriguing considering the demonstrated link between metrics of egg quality, such as size or hormonal content, and enhanced chick growth in some avian species (e.g., androgens in black-headed gulls *Larus ridibundus*, Eising et al., 2001; size in thick-billed murres, Hipfner, 2000; egg size across avian taxa, Krist, 2011; androgens in Eastern bluebirds *Sialia sialis*, Navara et al., 2005; testosterone in European starlings *Sturnus vulgaris*, Pilz et al., 2004; testosterone in canaries *Serinus canaria,* Schwabl, 1996). There is even some evidence that the transmission of maternal carotenoids may be linked to offspring growth, as in yellow-legged gulls (*Larus michahellis,* Saino et al., 2008), but the benefits may vary according to offspring sex and timing of hatch (Romano et al., 2008) and more often extend to offspring immune function rather than growth (e.g., blue tits, Biard et al., 2007; barn swallows *Hirundo rustica,* Saino et al., 2003). Hormonal cycles of sex steroids in females may also affect both reproductive investment and carotenoid colouration, as high oestradiol levels in the early breeding season are critical to successful yolk and egg formation, while low oestradiol levels in the late breeding season permit the production of redder, more saturated integuments (Romero-Diaz et al., 2022). Females also contribute more to direct care of the young, even though males spend more time on the colony and are more involved in nest maintenance and defense (Creelman & Storey, 1991). Specifically, females incubate eggs longer, provision chicks more frequently, and provide more high quality nutritional items than males (Creelman & Storey, 1991; Fitzsimmons, 2018). Unlike males, females dynamically shift their feeding rate depending on foraging conditions, potentially indicating that females have a more flexible pattern of provisioning than males

(Fitzsimmons, 2018). Taken together, females seem to have more opportunities to adjust their investment across the breeding attempt than males.

While I was unable to explore whether females and males differentially invest in this study, it is interesting to consider how the aforementioned axes of sexually dimorphic investment may influence signaling behaviour (Creelman & Storey, 1991). In the context of differential allocation, it would only be informative for females to signal parental investment, since their status dynamically changes while that of males remains stable. Yet, by the same logic, males should be unreceptive to signals indicating changes in maternal investment. However, perhaps males would respond if female bill or rosette colour drastically changed in a way that reflected a marked decrease in parental quality. Future studies should investigate whether female bill colour has the potential to act as a signal of parental quality between mated pairs by experimentally manipulating female bill colouration during the breeding season. In puffins, the direction of the response may depend on when the decrease in partner quality occurs, and thus how much investment has already been made in the current offspring (Harris & Uller, 2009; Ratikainen & Kokko, 2010). If maternal investment in egg composition is minimal, and this is reflected in her bill colouration, then a male partner might be predicted to incubate/provision less or abandon the breeding attempt altogether (i.e., positive differential allocation). In contrast, declines in maternal quality, and thus ability to provision, observed close to fledging may result in a brief spike in paternal investment (i.e., negative differential allocation). While such temporal changes in investment strategy have not been previously documented in species with a single offspring, several studies demonstrate fluctuations in parental investment across multiple broods (i.e., Grüebler, 2007; Robinson et al., 2010).

Since my measure of parental quality did not consist of a direct assessment of investment (i.e., egg composition, provisioning rate), I cannot exclude the possibility that colouration reflects

another aspect of individual quality. Foraging ability is one measure of quality that could potentially explain the link between maternal bill colouration and enhanced chick mass gain (per the good parent hypothesis, Hoelzer, 1989; as in blue tits, García-Navas et al., 2012; Senar et al., 2002). However, this would require that 1) diet influences bill colour expression in females only, and/or 2) paternal provisioning has a weak influence on offspring growth compared to maternal provisioning. While food availability undoubtedly influences avian offspring growth, genetics are also likely to affect a chick's metabolism and ability to gain weight. This idea has received particular attention in the commercial poultry industry, where genetic selection for enhanced growth and development has been successful (Buzała et al., 2015; Emmerson, 1997). It is therefore plausible that maternal colouration simply reflects genetic quality, and the association between colour and offspring health is a result of the direct benefits she provided.

Another possibility is that females with redder, more saturated features are paired to males of higher parental or genetic quality. While annual survival is high and divorce rate is low in this species, significant mate changeover can still occur (22% of cases where both birds were banded, 9.3% of cases where both birds were present the next year, Creelman & Storey, 1991). Female bill colouration could be used by males to decide whether to stay with their mate or search for a new mate prior to breeding. Males typically solicit females in this species and may attempt to copulate with multiple females, but unreceptive females can evade or repel copulation attempts (i.e., no forced extrapair copulation; Creelman & Storey, 1991). It is difficult to ascertain which sex is "choosier" in this scenario, and in mutually ornamented species with bi-parental care, both sexes likely exhibit some degree of choosiness (Amundsen, 2000; Burley, 1977). Hence, it is possible that male bill colouration reflects an aspect of quality that I did not measure, and that bill colouration is important in mate choice for both sexes.

While parental colouration certainly played a role in predicting chick growth, hatch date proved to be the most consistent explanatory variable. Specifically, *timing* of hatch was significant in nearly every model, but *synchrony* of hatch was never retained. This indicates that there is more of a distinction between early hatching and late hatching chicks compared to synchronously and asynchronously hatching chicks. However, it is also possible that my measure of synchrony did not accurately reflect match/mismatch with capelin availability, as peak capelin abundance in nearshore waters tends to occur after spawning is first recorded (Nakashima, 1996). Nevertheless, timing of hatch was clearly an important factor in determining chick growth. Early hatching pufflings generally have higher fledging success, and may therefore be expected to have higher growth rates (Harris, 1980; Nettleship, 1972), but this did not hold across all growth metrics in my sample. Individuals that hatched early achieved higher peak masses, but also had lower normalized wing growth rates. This may reflect a trade-off between mass gain and structural growth, with early hatchers allocating more energy to mass gain and late hatchers investing more in wing and feather growth. Indeed, food-stressed pufflings prioritize growth of skeletal structures like the head and wings over body mass gain (Øyan & Anker-Nilssen, 1996). One possibility is that late hatchers invest in rapid structural growth so they can successfully fledge and hunt on their own at a younger age. The length of the chick rearing period is flexible in puffins, but there is likely an upper limit to this flexibility, as parents tend to reduce feeds and/or abandon the attempt after the young have reached a certain age (Erikstad et al., 1997; Johnsen et al., 1994). Puffin parents may be even less flexible toward the end of the breeding season, since both successful and unsuccessful breeders tend to leave the colony around the same time, if not the same day (Harris & Wanless, 2011). Therefore, late hatching chicks may not be able to prolong their rearing period and gain the nutrients necessary to attain the same fledging weights as early hatchers. This may be compounded

by increased predation of chicks and kleptoparasitism of provisioning adults by great black-backed gulls and herring gulls (*Larus argentatus*) during the late breeding season, when both adult and juvenile gulls are foraging at the colonies. Consistent with this, Nettleship (1972) found that late hatching chicks fledge at a younger age on more level habitat, where chicks are more exposed to gull predation and adults are more vulnerable to kleptoparasitism. Studies on this phenomenon have repeatedly found a reduced rate of predation and kleptoparasitism in gull-reduced or gull-free areas, but no resulting impact on offspring survival (Finney, 2002; Finney et al., 2001; Rice, 1985). Alternative growth strategies between early and late hatching chicks, where late hatchers develop quickly and fledge at younger ages but still maintain high rates of survival, may partially explain this phenomenon. A follow-up to this work could investigate whether early and late hatchers truly have differential predation/kleptoparasitism pressure, and whether these alternative strategies have long-term impacts on adult survival and/or reproductive success.

Overall, this study provides new insights on the factors most relevant to puffling growth. Several aspects of maternal colouration predicted offspring growth in our sample, providing support for female bill colour as an indicator of parental quality. This serves as the first glimpse into a potential explanation for the adaptive significance of the Atlantic puffin's colourful bill and supports the notion that female ornamentation is not merely a byproduct of selection on males, but instead may play an important signaling role in its own right. However, timing of hatch seemed to be even more important in shaping offspring growth. The advantages of early vs. late breeding demonstrate a clear trade-off between weight gain and structural development, which may result from differential predation and kleptoparasitism across the breeding season. Whether the relationship between hatch timing and offspring growth holds in the face of environmental instability remains unknown but would provide a fruitful area of future research.

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# CHAPTER 4 Summary, limitations, and future directions

## 4.1 Introduction

Colourful displays are produced in species throughout the animal kingdom and serve a variety of roles in inter- and intraspecific signaling. In birds, plumage or bare part colouration may be used to recognize conspecifics, decide whether to engage in costly aggressive interactions, or choose the best mate (Hill & McGraw, 2006b). Carotenoid-based colouration is one common mechanism of colourful trait production and is responsible for many of the red, orange, and yellow features found across avian species (Hill & McGraw, 2006a). There is considerable theoretical and experimental research on the signaling potential of this type of colouration, mostly due to several distinct properties of carotenoids. Carotenoids are biomolecules that must be acquired through an animal's diet, play an important role as immune-enhancers and antioxidants, and often are converted from their dietary form to produce a pigmented feature (Hill & McGraw, 2006a). These properties have led to three main hypotheses linking carotenoid-pigmented features to aspects of individual quality: the foraging hypothesis (Endler, 1980; Hill, 1992), the trade-off hypothesis (Lozano, 1994; Moller et al., 2000), and the shared pathway hypothesis (Hill, 2011). As a result, carotenoid-pigmented features may be expected to fluctuate with respect to changes in individual foraging ability, immune function, or overall health, depending on the mechanism.

Atlantic puffins display several conspicuous carotenoid-based features, including the red-orange bill, bright orange gape rosette, and orange feet. These features are only produced during the breeding season, sloughing off or diminishing in colour and size as the winter non-breeding season approaches. Males and females are sexually monochromatic from an avian visual perspective (Chapter 2; Doutrelant et al., 2013), representing a classic example of mutual

ornamentation. Prior work demonstrated that bill colouration positively correlates to current body condition, but overall, the adaptive function of this colourful integument remains unexplored.

This thesis had two primary goals, which I address in each of my data chapters. In Chapter 2, I thoroughly investigated the properties of the colourful bill to generate informed hypotheses on the potential function of the colourful bill and rosette. In Chapter 3, I used this information to examine one potential adaptive explanation; specifically, that the colourful bill reflects parental quality.

## 4.2 Summary of main results

### 4.2.1 Signaling properties

The primary aim of Chapter 2 was to determine whether the properties of bill colouration more closely align with a signal of quality or a signal of identity. In a mutually ornamented, social species like the Atlantic puffin, these are two of the most likely candidates, resulting from either mutual sexual selection or selection for recognition. Quality and identity signals overlap somewhat in their characteristics but are distinct along two axes: 1) quality signals are labile, whereas identity signals are stable over time, and 2) only quality signals are predicted to be condition-dependent. To explore the lability of bill colour, I employed both a cross-sectional and longitudinal approach. For the cross-sectional approach, I used a dataset of hundreds of individuals to examine broad trends in colour metrics across the population. For the longitudinal analysis, I sampled 41 individuals twice within a breeding season to ascertain whether bill colour changes within individuals in a way that would be perceptible to conspecifics.

The cross-sectional dataset gave me insight on how the colourful bill changes as the end of the breeding season draws near. Specifically, I found that 1) the bill becomes more red-orange and

the rosette becomes more orange-yellow, 2) the rosette decreases in UV reflectance while the cere increases in UV reflectance, 3) the lower mandible and the cere become more saturated, and 4) the rosette increases in brightness while the bill decreases in brightness. These patterns mirror ethological descriptions of adult puffins' transition from the breeding to nonbreeding season, as the rosette becomes yellower and paler and the keratinized plates of the bill peel off, revealing a bright red bill tip and black mandible base (Harris, 2014). While none of the puffins I sampled had nonbreeding plumage, it is likely that some individuals sampled toward the end of the breeding season were already preparing for these substantial shifts in colouration. In this case, colour change within individuals would be predicted, which I investigated with the longitudinal dataset.

My main finding from the longitudinal data was that the bill, cere, and rosette all perceptibly fluctuated within individuals across the breeding season, although the rosette was the most predictably labile. The time scale of these changes differed between regions, with fleshy structures like the rosette and cere seeming to change on more rapid time scales than the bill (i.e., days compared to weeks). The fact that these features can change in just a matter of days excludes the possibility that colouration could function as a reliable identity signal.

However, none of the regions that I measured were condition-dependent, contrasting with the predictions of a quality signal and the results from Doutrelant et al's (2013) previous study. It may be the case that bill colouration is related to some other aspect of individual quality that I did not measure, such as foraging ability or ability to provide parental care. It is also possible that bill colour does not relate to any genetic or phenotypic characteristic, but this then begs the question of why puffins continue to produce such a conspicuous display year after year.

### 4.2.2 Predictors of chick growth

Based on my conclusions from Chapter 2, I explored whether colouration might reflect an aspect of individual quality other than current body condition. Puffins produce the colourful bill during the breeding season, during which both parents work together to successfully raise a single nestling. At every stage of the reproductive process, from defending a burrow to incubating the egg and provisioning the growing chick, individuals in a mated pair must coordinate their activities and make informed decisions about time and energetic investment into the breeding attempt (Griffith, 2019). Too little investment could result in an undernourished or dead chick, while too much investment could lead to a severe decline in adult body condition that ultimately jeopardizes future chances of survival (Trivers, 1972). Any signal honestly indicating a partner's ability to provide investment would be theoretically useful between mated pairs in deciding how much care to provide. I investigated whether that signal could be bill colouration, such that colour honestly reflects an individual's quality as a parent, evaluated in terms of when the chick hatches and how well they develop.

I found that multiple aspects of maternal bill colouration predicted the peak mass that offspring achieved. Females in better body condition, with more saturated bills, yellower ceres, and more orange rosettes, raised heavier chicks. Mass gain and wing growth were also linked to maternal colouration, such that mothers with redder bills produced offspring with higher mass gain rates and mothers with yellower rosettes had chicks with higher wing growth rates. Interestingly, neither paternal colouration nor condition predicted any aspect of offspring development, discounting the idea that both male and female colouration serve as indicators of parental quality in the same way.

Perhaps even more important than colour in determining chick growth was hatch date. During the year of my study (2022), mean hatch date happened to coincide with local availability

of puffins' preferred prey, capelin. Whether chicks hatched before or after this date seemed to matter more than whether chicks hatched synchronously (i.e., ± 3 days of mean hatch date) with peak capelin abundance, providing support for the timing hypothesis over the synchrony hypothesis. Those that hatched prior to the mean hatch date reached higher masses but had slower rates of wing development. The opposite was found for those that hatched after the mean date (i.e., higher wing growth rates, reduced mass). This aligns with previous work demonstrating a trade-off between mass gain and structural growth, with those under nutritional stress favoring head and wing growth maintenance over mass gain (Øyan & Anker-Nilssen, 1996). Taken together, this indicates that late hatchers may have reduced access to food throughout the chick rearing period. Although hatch timing was not reflected in parental colouration, it is possible that another unmeasured aspect of individual quality or ability (i.e., age, experience) may dictate when egg-laying occurs, and thus, when the chick hatches.

## 4.3 Limitations

The main limitation of this work is that it is correlational; more definitive conclusions on the signaling value of the bill and its potential role in mediating parental investment can only be drawn from experimental manipulation (Sheldon, 2000). My findings support hypotheses concerning the adaptive value of the colourful bill but cannot exclude alternative hypotheses that are consistent with my observations.

Another key limitation is my choice to focus on the late breeding season and constrain my studies to the chick-rearing period. This prevented me from assessing how colour changes across the entirety of the breeding season. It also precluded me from exploring other opportunities for sexual selection to act on the colourful bill, including mate choice and decisions to divorce, extra-

pair copulations, courtship displays, and investment in other parental activities such as burrow maintenance/defense and incubation duties. However, this was a necessary decision to avoid adverse effects of investigator disturbance. Puffins can be very sensitive to disturbance, especially prior to chick hatch, when disturbed adults are more likely to desert eggs (Harris & Wanless, 2011; Rodway et al., 1996). Confining my study to the nestling period not only reduces the likelihood of abandonment, but also minimizes the chances that my results are confounded by investigator-related disturbance impacts.

For similar reasons, sample size is another limitation to consider in some parts of my thesis. For the longitudinal analysis in Chapter 2, I was only able to sample 41 individuals twice within a breeding season over the course of three years. In Chapter 3, I was only able to obtain full parent-offspring data on a small subset of individuals. In both cases, the challenge in obtaining sufficient sample size arose because of difficulties targeting specific individuals. The traditional method of puffin/puffling capture is "grubbing," which involves a researcher reaching an arm into a burrow and extracting the individual inside. This works well sometimes, but is not a consistently reliable method of capture. Zabala Belenguer & Bitton (2022) found that approximately a third of burrows are deeper than the average human arm length, and additional burrows without depth limitations may be otherwise hard to access (i.e., narrow, sharp angles, etc.). Over the course of the breeding season, adults will often continue to dig additional entrances or new connections between tunnels, making it difficult to assess which burrow belongs to whom. This behaviour also seems to be a common response in puffins that have already been captured, making it challenging to re-capture individuals or target the partner in a mated pair. In other cases, puffins are not in their burrows at night, which seems to occur particularly often on warm nights (pers. observ.). Burrow microclimate is important in several cold-climate seabird species (e.g., little auk *Alle alle,* Kulaszewicz &

Jakubas, 2018; Wilson's storm petrel *Oceanites oceanicus,* Michielsen et al., 2019), where warmer temperatures or otherwise favorable nest features lead to increased chick growth and survival. If the ambient temperature is sufficiently warm, then chicks may not have to rely on their parents' body heat to develop quickly. As summer temperatures rise due to climate change, adult absence from the burrow is likely to increase in frequency. Noose traps are a useful tool to complement hand grubbing, but they become increasingly inefficient as the chick rearing season progresses and adults return to provision less frequently. Until alternative methods of capture have been explored and reported for this species, reliable targeting of individuals remains elusive.

Finally, there were some aspects of this thesis that could have benefitted from additional or more fine-scale data, especially in Chapter 3. For the offspring growth analysis, I did not determine offspring sex, despite evidence that growth curves are different for males and females in several seabird species (i.e., common terns, Becker & Wink, 2003; limited support in thick-billed murres *Uria lomvia*, Cameron, 2003; Adélie penguins *Pygoscelis adeliae*, Jennings et al., 2016; African penguins *Spheniscus demersus*, Spelt & Pichegru, 2017; wandering albatross *Diomedea exulans*, Weimerskirch & Lys, 2000). However, one study in an Atlantic Canadian colony did not find any sex differences in puffin offspring mass, tarsus, or wing length development during the linear phase of growth (Cameron, 2003). Additionally, it would have been intriguing to consider potential sex bias in offspring investment, as has been documented in several species (i.e., common murres; Cameron-MacMillan et al., 2007; Adélie penguins, Jennings et al., 2016; brown songlarks *Cinclorhamphus cruralis*; Magrath et al., 2007; wandering albatross, Weimerskirch & Lys, 2000), but, again, there is no evidence of sex-biased provisioning in Atlantic puffins (Cameron, 2003). In both hatch group and offspring growth analyses, I was only able to consider hatch group as two-level categorical variables (timing and synchrony) in my models. While I believe this was the best

approach considering the reduction in sample size that would result from eliminating crude estimates, it may be useful to consider hatch date as a continuous variable in cases of less uncertainty.

## 4.4 Future directions

Future work should be directed at addressing the three key limitations identified in Section 4.3. The first of these limitations is the lack of experimental study. Several studies, and now this thesis, have examined the properties of the colourful bill at length and drawn links between colour and aspects of quality such as body condition and parental investment. To further our understanding of the adaptive function of the colourful bill and rosette, I recommend carefully controlled experimental manipulation in a natural setting. An experimental design could include direct manipulation of colouration (e.g., paint application), or indirect manipulation (e.g., change in diet/carotenoid availability to induce changes in colouration). The drawback to indirect manipulation, however, is that changes in partner investment may not be associated with change in colouration *per se*, because body condition cannot be excluded as a factor that individuals are cueing on (Hill, 2006). Therefore, direct manipulation of colour features would be preferential to assess how individuals in a mated pair respond specifically to changes in bill colouration. Based on my findings from Chapter 3, it would be most interesting to manipulate female bill colouration and evaluate resulting changes in partner provisioning effort or chick growth outcomes. If maternal colour honestly reflects parental quality, then maternal provisioning effort and chick growth outcomes should remain unchanged. However, if males use female colour to decide how much to invest in parental care, then males may alter provisioning rate in response to female manipulation,

leading to differential chick growth. Only careful experimental manipulations like this can disentangle the effects of adult colouration, parental investment, and chick health.

Another avenue of future research would be to explore whether the colourful bill might be useful in other breeding contexts. Although puffins tend to mate with the same individual each year, bill colour could play several roles at the onset of the breeding season. Mated pairs engage in distinct pair bonding behaviour before breeding, including head jerks, wing flutters, and most curiously, billing (i.e., knocking bills broadside together). Bouts of billing are prone to drawing in crowds of neighboring onlookers, who may engage with the billing pair or amongst each other (Harris & Wanless, 2011). Such a social display involving the colourful integument itself deserves further attention. While mate fidelity and adult survival are typically high in puffin populations, divorce and death still occur. Billing could be an opportunity for the unsatisfied to show off a flashy ornament to potential suitors, or for an onlooking widower to find a new attractive mate. Additionally, little is known about how young sexually mature puffins choose a lifelong partner. Although initial mate choice does not fully explain the continual production of a colourful display like the bill, it seems likely that some aspect of the puffins' conspicuous breeding plumage could be used to pick a partner from the crowd.

While this is certainly an exciting area of research, the negative effects of investigator disturbance must be considered before conducting such studies. While puffins are the least susceptible to abandonment during the chick rearing period, it may be feasible to study bill colouration prior to egg laying as well. Egg laying does not seem to be affected by early burrow inspections (Ashcroft, 1979; Nettleship, 1972), but this may not be the case for all colonies (Harris & Wanless, 2011), and does not consider the additional impact of handling. Therefore, it is recommended that researchers handling puffins prior to the chick rearing season exercise caution

and monitor burrows in a control plot to understand and account for the impact of their activities (Rodway et al., 1996). Such studies should only be carried out if the effects on breeding success are minimal.

Another possibility is that bill colour provides information on an individual's age, such that puffins could use bill colouration to choose an appropriately aged mate, either at initial mate choice and/or after divorcing/widowing. Maturation and senescence of carotenoid-based displays has been shown to occur in male common crossbills (*Loxia curvirostra*), where red plumage colouration increases during the first two years of life and decreases thereafter, independent of changes in body mass (Fernández-Eslava et al., 2021). It is notoriously challenging to follow puffin chicks into adulthood, as they exhibit a highly variable version of natal philopatry, with the majority staying at their natal colony but anywhere from 8% to 57% of individuals choosing to breed away from where they were reared (Breton et al., 2006; Harris & Wanless, 2011) For this reason, research dedicated to genetically aging these birds would be beneficial, as has been done in short-tailed shearwaters (*Ardenna tenuirostris*, De Paoli-Iseppi et al., 2019). A simple way to gauge approximate age would allow us to ask whether bill or rosette colouration corresponds to age, and whether birds might use this signal to preferentially mate with older, younger, or age-matched birds.

The final suggestion for future research is to expand this study to include more individuals across different populations. Atlantic puffins exhibit varying morphologies and phenologies in different parts of the North Atlantic, and were even classified into three taxonomic subspecies along a latitudinal gradient at one point (Harris & Wanless, 2011). Recent whole-genome analyses instead suggest four population clusters, with Canadian puffins as one set of distinct puffins (Kersten et al., 2021). Therefore, it is unclear how broadly applicable my findings are to other populations. The ecologies of different populations are also variable, with different foraging

regimes and predation pressures depending on gull and/or rodent presence (Harris & Wanless, 2011). It would be interesting to investigate whether my findings on the trade-off in growth between early and late hatchers is broadly applicable to other populations, or whether ecology and environment somehow alter this relationship. Hatch synchrony seemed to play less of a role in this population but could be more important in areas with declining prey abundance and increasing mismatch of peak availability, as is the case in northeastern populations (BirdLife International, 2018). The relative importance of hatch timing or hatch synchrony in "good" vs. "bad" foraging years is another outstanding question. Multiple years of data could shed light on whether hatch timing is consistently predictive of chick growth across fluctuating environmental conditions.

## 4.5 References


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# APPENDIX A Chapter 2

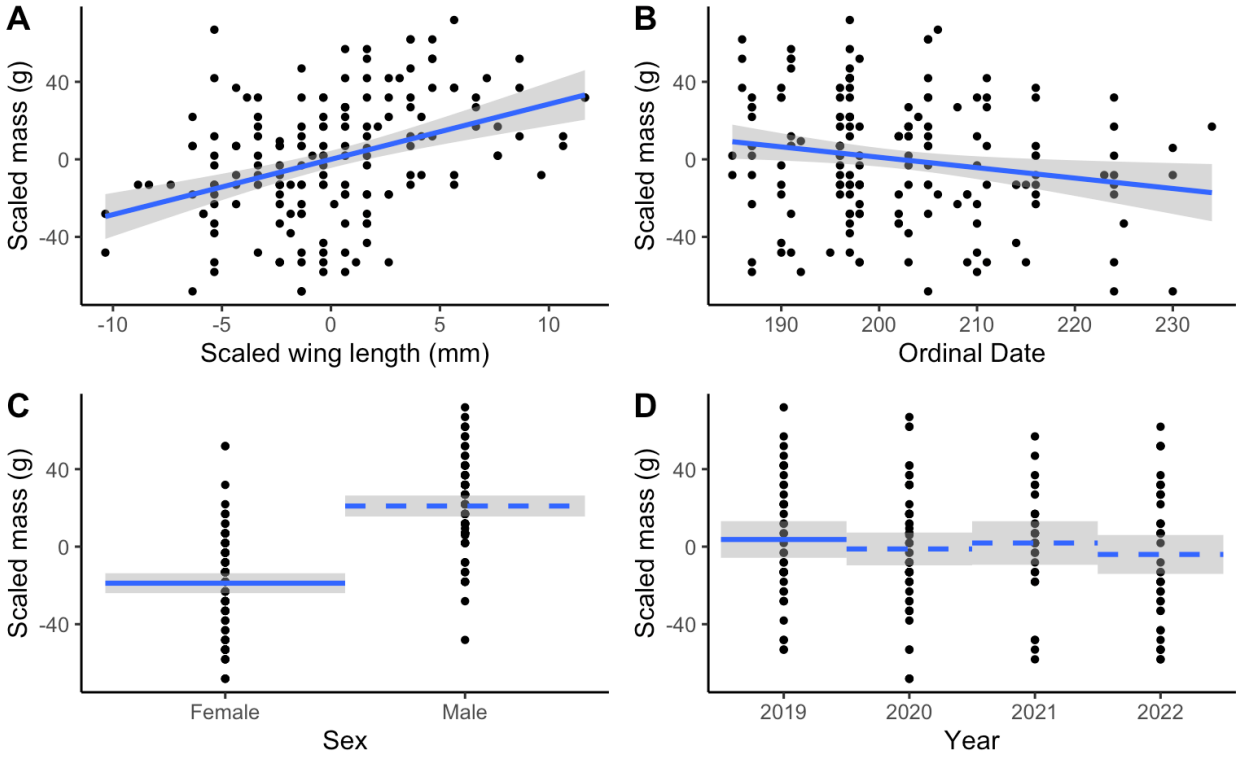


Figure A.1 The final body condition linear model for the colour analysis included mass as the response variable and four significant main-effect predictor variables. Mass was A) positively related to scaled wing length, B) negatively related to Ordinal Date, C) higher in males than females, and D) lower in 2022 compared to 2019 and 2020.

Table A.1 Glossary of visual modelling terms

| Term | Definition |
|---|---|
| Violet-sensitive (VS) | Avian visual system in which the peak sensitivity of the long wavelength photoreceptor is in the violet portion of the spectrum (i.e., 410 nm) |
| Quantum catch value | The number of photons absorbed by the photopigment molecules of a photoreceptor |
| Cone catch images | Images containing quantum catch information; generated with knowledge of animal visual system and environmental lighting conditions |
| Tetrahedral colourspace | 3-dimensional space of perceivable colours, where the central point is the achromatic center, and each vertex is the absolute stimulation of one of the four single cones |
| Hue VIS | Azimuth angle to the colour vector in a tetrahedral colourspace; the contribution of the visible spectrum (red-blue) to perceived colour |
| Hue UV | Elevation angle to the colour vector in a tetrahedral colourspace; the contribution of violet and UV to perceived colour |
| Chroma | Magnitude of the colour vector from the achromatic center; the saturation of a colour |
| Single cone | Photoreceptor type responsible for perception of chromatic information (i.e., colour) |
| Double cone | Photoreceptor type responsible for perception of achromatic information (i.e., brightness) |

Table A.2 Proportion of discriminable changes in colouration

| ROI | Discriminable | Non-discriminable | Percent Discriminable (%) |
|---|---|---|---|
| *Upper mandible* | 25 | 16 | 61.0 |
| *Lower mandible* | 14 | 27 | 34.1 |
| *Cere* | 21 | 20 | 51.2 |
| *Rosette* | 33 | 8 | 80.5 |
| *Total (JND = 1)* | 93 | 71 | 56.7 |
| *Total (JND = 2)* | 36 | 128 | 22.0 |
| *Total (JND = 3)* | 14 | 150 | 8.5 |

Discriminability percentages for distinct regions were calculated at a threshold of JND = 1.

Table A.3 Proportion of discriminable changes in brightness

| ROI | Discriminable | Non-discriminable | Percent Discriminable (%) |
|---|---|---|---|
| *Upper mandible* | 32 | 9 | 78.0 |
| *Lower mandible* | 24 | 17 | 58.5 |
| *Base of mandible* | 30 | 11 | 73.2 |
| *Cere* | 25 | 16 | 61.0 |
| *Rosette* | 36 | 5 | 87.8 |
| *Total (JND = 1)* | 147 | 58 | 71.7 |
| *Total (JND = 2)* | 87 | 118 | 42.4 |
| *Total (JND = 3)* | 52 | 153 | 25.4 |

Discriminability percentages for distinct regions were calculated at a threshold of JND = 1.

Table A.4 Variation in colour variables with time between sampling dates

| ROI | Response: colour variable | Predictors | Estimate | Std. Error | t | P |
|---|---|---|---|---|---|---|
| *Upper mandible* | Hue vis | Sampling interval | 1.72E-04 | 1.25E-03 | 0.137 | 0.892 |
| | | Ordinal date | -2.80E-03 | 1.01E-03 | -2.776 | 0.074(*) |
| | Hue UV (3 outliers) | Sampling interval | -1.35E-03 | 2.25E-03 | -0.598 | 0.775 |
| | | Ordinal date | 6.78E-03 | 1.73E-03 | 3.926 | 0.014* |
| | Achieved saturation (2 outliers) | Sampling interval | 4.07E-04 | 2.31E-03 | 0.176 | 0.887 |
| | | Ordinal date | -1.27E-03 | 2.81E-03 | -0.452 | 0.814 |
| | Brightness (2 outliers) | Sampling interval | -1.02E-02 | 4.69E-03 | -2.167 | 0.126(*) |
| | | Ordinal date | 3.40E-03 | 3.11E-03 | 1.091 | 0.521 |
| *Lower mandible* | Hue vis | Sampling interval | -4.42E-04 | 1.92E-03 | -0.229 | 0.869 |
| | | Ordinal date | -6.25E-04 | 1.55E-03 | -0.403 | 0.814 |
| | Hue UV (1 outlier) | Sampling interval | -2.89E-03 | 3.00E-03 | -0.963 | 0.544 |
| | | Ordinal date | 7.66E-03 | 3.06E-03 | 2.503 | 0.107(*) |
| | Achieved saturation (3 outliers) | Sampling interval | -3.90E-03 | 1.87E-03 | -2.089 | 0.126(*) |
| | | Ordinal date | -1.09E-03 | 1.79E-03 | -0.608 | 0.775 |
| | Brightness | Sampling interval | -1.07E-03 | 2.99E-03 | -0.358 | 0.816 |
| | | Ordinal date | 2.38E-03 | 2.41E-03 | 0.985 | 0.544 |
| *Mandible base* | Brightness (2 outliers) | Sampling interval | -5.70E-03 | 2.37E-03 | -2.402 | 0.108(*) |
| | | Ordinal date | 7.28E-04 | 1.58E-03 | 0.461 | 0.814 |
| *Cere* | Hue vis (3 outliers) | Sampling interval | 0.28 | 0.13 | 2.153 | 0.126(*) |
| | | Ordinal date | 0.013 | 6.79E-03 | 1.852 | 0.182 |
| | | Sampling interval x Ordinal date | -1.42E03 | 6.67E-04 | -2.147 | 0.126(*) |
| | Hue UV | Sampling interval | -6.60E-04 | 2.42E-03 | -0.273 | 0.860 |
| | | Ordinal date | 1.78E-03 | 1.95E-03 | 0.913 | 0.559 |
| | Achieved saturation (3 outliers) | Sampling interval | -6.10E-03 | 2.16E-03 | -2.819 | 0.074(*) |
| | | Ordinal date | 5.22E-03 | 1.86E-03 | 2.805 | 0.074(*) |
| | Brightness (2 outliers) | Sampling interval | -8.04E-03 | 3.82E-03 | -2.106 | 0.126(*) |
| | | Ordinal date | -3.53E-03 | 2.54E-03 | -1.392 | 0.379 |
| *Rosette* | Hue vis | Sampling interval | -2.54E-03 | 1.83E-03 | -1.388 | 0.379 |
| | | Ordinal date | -1.84E-03 | 1.47E-03 | -1.249 | 0.452 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Hue UV | Sampling interval | 2.78E-03 | 1.35E-03 | 2.054 | 0.126(*) |
| | | Ordinal date | 1.10E-03 | 1.09E-03 | 1.011 | 0.544 |
| | Achieved saturation (3 outliers) | Sampling interval | -3.45E-03 | 2.90E-03 | -1.192 | 0.469 |
| | | Ordinal date | 1.32E-03 | 3.07E-03 | -0.430 | 0.814 |
| | Brightness | Sampling interval | -2.48E-02 | 1.00E-02 | -2.465 | 0.107(*) |
| | | Ordinal date | -3.17E-03 | 8.10E-03 | -0.392 | 0.814 |

*P* values were corrected with the false discovery rate method. (*) indicates significance prior to the false discovery rate correction, and * indicates that the variable remained significant after applying the correction.

Table A.5 Relationship between colour variables and body condition

| ROI | Response variable | Estimate | Std. Error | t | P |
|---|---|---|---|---|---|
| *Upper mandible* | Hue VIS | 2.87E-04 | 1.77E-04 | 1.620 | 0.85 |
| | Hue UV | 1.10E-04 | 3.33E-04 | 0.329 | 0.97 |
| | Achieved saturation | 9.01E-06 | 2.86E-04 | 0.031 | 0.99 |
| | Brightness | 4.99E-06 | 3.13E-04 | 0.016 | 0.99 |
| *Lower mandible* | Hue VIS | 4.63E-04 | 1.98E-04 | 2.344 | 0.35(*) |
| | Hue UV | -2.49E-04 | 3.24E-04 | -0.769 | 0.94 |
| | Achieved saturation | 2.45E-04 | 2.95E-04 | 0.829 | 0.94 |
| | Brightness | -1.82E-05 | 3.17E-04 | -0.057 | 0.99 |
| *Base of mandible* | Brightness | 2.11E-04 | 1.80E-04 | 1.171 | 0.85 |
| *Cere* | Hue VIS | -1.03E-04 | 1.95E-04 | -0.530 | 0.96 |
| | Hue UV | 2.23E-04 | 2.87E-04 | 0.779 | 0.94 |
| | Achieved saturation | 5.12E-04 | 4.25E-04 | 1.205 | 0.85 |
| | Brightness | 1.38E-04 | 3.78E-04 | 0.365 | 0.97 |
| *Rosette* | Hue VIS | -1.10E-04 | 2.20E-04 | -0.498 | 0.96 |
| | Hue UV | 1.77E-04 | 1.53E-04 | 1.156 | 0.85 |
| | Achieved saturation | 2.07E-04 | 3.57E-04 | 0.580 | 0.96 |
| | Brightness | 4.99E-06 | 3.13E-04 | 0.016 | 0.99 |

Statistical significance at $\alpha = 0.05$. (*) indicates a significant *P* value before application of the false discovery rate.

# APPENDIX B Chapter 3

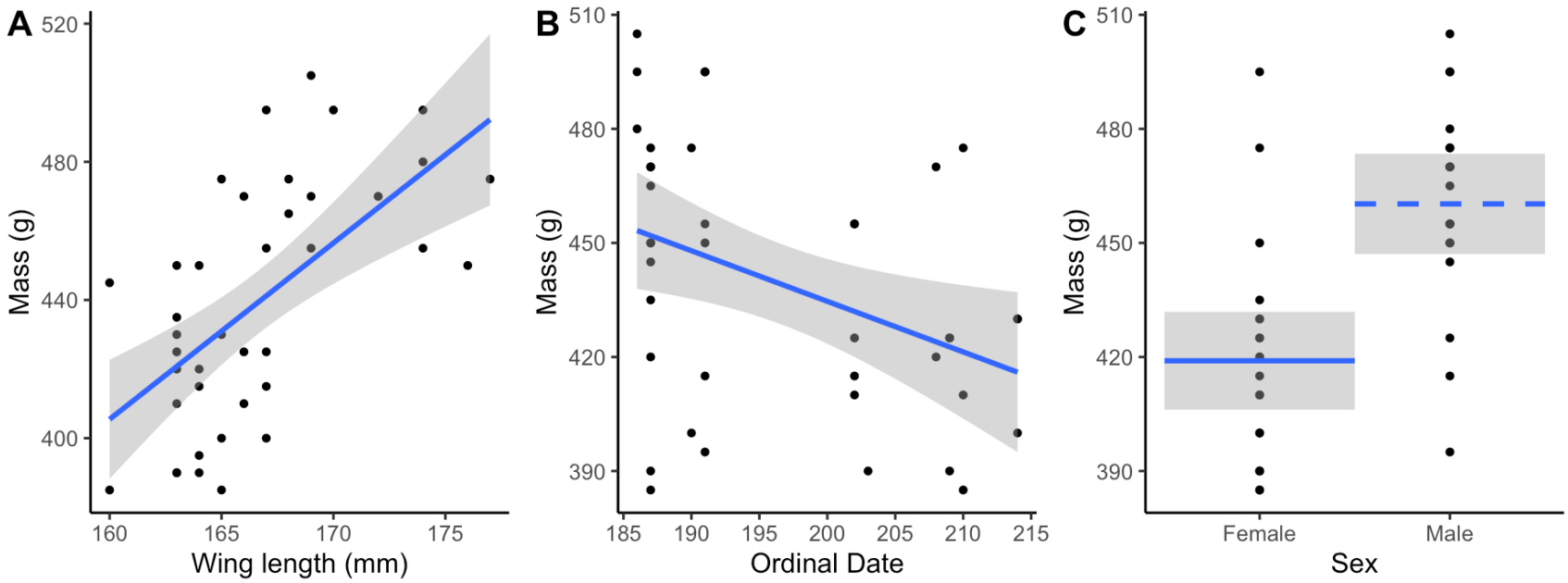


Figure B.1 The final body condition linear model for the chick growth analysis included mass as the response variable and three significant main-effect predictor variables. Mass was A) positively related to scaled wing length, B) negatively related to Ordinal Date, C) higher in males than females.

Table B.1 Chick growth model selection based on corrected AIC values

| Biometric | *Rank* | Model | AICc | AICc weight | ΔAICc | Prob. correct |
|---|---|---|---|---|---|---|
| *mass* | 1 | Quadratic | 541.29 | 0.70 | -- | 82.75% |
| | 2 | EVF | 544.81 | 0.12 | 3.51 | |
| | 3 | Logistic | 545.73 | 0.08 | 4.43 | |
| | 4 | Gompertz | 546.45 | 0.05 | 5.16 | |
| | 5 | von Bertalanffy | 546.85 | 0.04 | 5.56 | |
| | 6 | Linear | 570.66 | 0.00 | 29.37 | |
| *wing length* | 1 | Logistic | 378.46 | 0.54 | -- | 29.40% |
| | 2 | EVF | 379.15 | 0.38 | 0.70 | |
| | 3 | Gompertz | 382.91 | 0.06 | 4.45 | |
| | 4 | von Bertalanffy | 385.72 | 0.01 | 7.26 | |
| | 5 | Quadratic | 388.63 | 0.00 | 10.17 | |
| | 6 | Linear | 418.06 | 0.00 | 39.60 | |
| *p10* | 1 | EVF | 237.80 | 0.29 | -- | 11.77% |
| | 2 | Logistic | 238.05 | 0.25 | 0.250 | |
| | 3 | Gompertz | 238.90 | 0.17 | 1.10 | |
| | 4 | Quadratic | 238.99 | 0.16 | 1.18 | |
| | 5 | von Bertalanffy | 239.35 | 0.13 | 1.55 | |
| | 6 | Linear | 248.14 | 0.00 | 10.34 | |

*Δ*AICc calculated relative to the first ranked (preferred) model. The probability that the selected model was the correct model was calculated in relation to the second-best model only.