U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

MSc

2.º
CICLO

FCUP
2019

U.PORTO

Too cold to shine? Does temperature influence sex differences in a plastic social ornament?

Rita Ferreira Pinto Mendes de Freitas

FC

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

MSc

Too cold to shine? Does temperature influence sex differences in a plastic social ornament?

Rita Ferreira Pinto Mendes de Freitas

Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto em
Ecologia e Ambiente

2019

MSc

Too cold to shine? Does temperature influence sex differences in a plastic social ornament?

Rita Ferreira Pinto Mendes de Freitas

Mestrado em Ecologia e Ambiente
Departamento de Biologia
2019

Orientador
Sandra Cristina de Sousa Trigo, Investigadora Científica, CIBIO-InBIO

Coorientador
Gonçalo Canelas Cardoso, Investigador Científico, CIBIO-InBIO

U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

Agradecimentos

Primeiro que tudo, o meu agradecimento mais importante vai para os meus orientadores, Sandra Trigo e Gonçalo Cardoso, que sempre demonstraram uma enorme disponibilidade e atenção. Obrigada por todos os conhecimentos novos transmitidos, pelas correções e sugestões sábias, por contribuírem para o meu crescimento académico e profissional, por sempre responderem às minhas questões com a máxima prontidão, por me fazerem sentir apoiada e segura durante este ano que passou e pelas conversas amigas e descontraídas nas pausas do trabalho. Não podia ter tido melhores orientadores (é um facto!). Obrigada pela oportunidade de trabalhar convosco e numa das áreas de que mais gosto, que é comportamento animal. Agradeço também ao resto do grupo *Behavioural Ecology*, ao Paulo, à Cristiana e à Ana Cristina, que deram igualmente um contributo importante para a concretização da minha tese.

Quero agradecer também aos meu pais, pelo excelente modelo que são, pela paciência, pelo apoio que sempre me deram ao longo do meu caminho, por tornarem possível eu estudar, tirar um curso, fazer uma tese de mestrado e chegar onde estou hoje. Por isto e por muito mais, obrigada. Ao meu avô, que esteve sempre a torcer por mim e por quem eu tenho uma admiração enorme por todos os conhecimentos que acumulou ao longo dos seus 91 anos e os quais partilha com muito entusiasmo. À minha avó, por ter sido uma inspiração de força, e que apesar de já não estar comigo fisicamente, estará sempre no meu coração. Foi a mulher mais forte que eu alguma vez conheci e vou ter presente, para sempre, a definição de força que ela me deixou para superar todos os obstáculos que encontrar ao longo da minha vida. Um obrigada também à minha irmã, Catarina, que me faz querer ser melhor todos os dias, porque é um exemplo de empenho, trabalho, força de vontade e dedicação.

Quero agradecer ainda ao meu namorado, Vasco, por todo o apoio, incentivo e força que me deu ao longo desta jornada, pela amizade, por ter estado sempre “lá” quando eu precisava, por me ter aturado nos meus dias piores e por sempre acreditar em mim.

Por fim, mas não menos importantes, gostava de agradecer aos meus amigos pelos jantares, pelos momentos de descontração no meio do stress de escrever a tese e pelas conversas animadas que sempre surgem quando estamos juntos.

Foi um caminho longo e, por vezes, um pouco custoso; teve os seus altos e baixos, mas, com a ajuda e o apoio de todos os que me são chegados, consegui

terminar esta que foi uma das etapas mais importantes da minha vida, pelo menos até agora.

Resumo

Alguns sinais e atributos em aves podem apresentar características plásticas. Geralmente, pensa-se que diferenças entre sexos em caracteres sexuais secundários, em espécies com papéis sexuais convencionais, são geneticamente determinadas. No entanto, estas diferenças podem dever-se a fatores ambientais, como o ambiente social, as condições climáticas, etc. Recentemente, foi descoberto que o bico-de-lacre-comum (*Estrilda astrild*) possui um ornamento social sexualmente dimórfico, o bico vermelho, cujas diferenças sexuais são totalmente plásticas. Foi sugerido que essas diferenças sexuais plásticas encontradas no bico do bico-de-lacre-comum poderiam ser devidas a causas ambientais, mais precisamente ao facto de temperaturas frias energeticamente stressantes afetarem o investimento das fêmeas na coloração do bico.

O objetivo do meu trabalho foi testar experimentalmente a hipótese de que a temperatura ambiente pode ser responsável por mudanças na saturação da cor do bico no bico-de-lacre-comum, e se esta afeta distintamente a cor do bico em fêmeas e em machos. Para isso, manipulei a temperatura dentro de gaiolas com escalfetas elétricas e mantive outras à temperatura ambiente num aviário exterior durante a época não-reprodutiva (inverno), e posteriormente troquei os tratamentos de temperatura. Em intervalos de tempo regulares, medi a saturação da cor do bico de todas as aves e quantifiquei também as taxas de alimentação e de movimento das aves em cada gaiola.

A taxa de alimentação e o índice de movimento alteraram-se com a mudança dos tratamentos de temperatura, confirmando que a manipulação de temperatura teve efeitos fisiológicos. No entanto, mudanças na média da saturação da cor do bico não diferiram significativamente entre as aves que passaram de temperaturas frias para quentes e as aves que passaram de temperaturas quentes para frias. Nas fêmeas, observei que flutuações na saturação da cor do bico diminuíram nas que passaram de temperaturas frias para quentes, comparando com as que passaram de temperaturas quentes para frias. Nos machos, não se verificou nenhum efeito da manipulação da temperatura na flutuação da cor do bico. Isto sugere que, em vez de investirem mais na cor do bico (a média da cor do bico não aumentou), as fêmeas beneficiaram de temperaturas mais quentes favoráveis para expressar uma saturação consistente da cor do bico.

Concluo, então, que aumentar as temperaturas durante o inverno, ou seja, aliviar o stress térmico e energético do inverno, reduz as oscilações na saturação da cor do bico das fêmeas nos bicos-de-lacre-comuns. Este efeito foi específico do sexo, uma

vez que a temperatura ambiente não teve efeito na cor do bico dos machos, indicando que o investimento das fêmeas na ornamentação é mais sensível que o dos machos às condições ambientais, nomeadamente, ao stress energético e à temperatura ambiente.

Palavras chave: bico-de-lacre-comum, diferenças sexuais, plasticidade fenotípica, saturação da cor do bico, stress energético, temperatura

Abstract

Some signals or traits in birds may have plastic characteristics. Sex differences in secondary sexual traits in species with conventional sex roles are usually thought to be genetically fixed but, instead, they may be due to environmental factors, such as the social environment, the climatic conditions, etc. Recently, it was found that the common waxbill (*Estrilda astrild*) has a sexually dimorphic social ornament, the red-coloured bill, whose sex differences are entirely plastic. It was suggested that the plastic sex differences found in the bill of the common waxbill may be due to environmental causes, more precisely due to energetically stressful cold temperatures affecting female investment in bill colour ornamentation.

The aim of my work was to test experimentally the hypothesis that ambient temperature may be responsible for changes in bill colour saturation in the common waxbill, and if it affects differently female and male bill colour. For this, I manipulated temperature inside bird cages with warming electric devices and maintained other cages at ambient temperature in an outside aviary during the non-reproductive season (winter), and then I switched the temperature treatments. At regular periods of time, I measured bill colour saturation of all birds and also quantified birds' feeding and movement rates in each cage.

Feeding rate and movement index changed when changing temperature treatments, confirming that temperature manipulation had physiological effects. But changes in mean bill colour saturation did not differ significantly between the birds passing from cold to hot temperatures and the birds passing from hot to cold temperatures. In females, I found that fluctuations in bill colour saturation decreased when passing from cold to hot temperatures, as compared to females passing from hot to cold temperatures. In males, there was no effect of the temperature manipulation on the extent of bill colour fluctuation. This suggests that, instead of investing more in bill colour (mean bill colour did not augment), females benefited from favourable warmer temperatures to express consist bill colour saturation.

I then conclude that warming temperature during winter, i.e. alleviating the thermal and energetic stress of winter, reduces oscillations in female bill colour saturation in common waxbills. This effect was a sex-specific effect, since ambient temperature did not affect males' bill colour, indicating that female investment in ornamentation is more sensitive than males' to environmental conditions, namely energetic stress and ambient temperature.

Keywords: bill colour saturation, common waxbill, energetic stress, phenotypic plasticity, sex differences, temperature

Table of contents

List of figures

b) and 3.9 (horizontal line in graphic c) are statistically significant. Nights (from sunset to sunrise) are represented by dashed lines and days (from midday until the following midday) by solid lines.

Figure A1 - Graphic with climatic data from IPMA (Portuguese Institute for Sea and Atmosphere): mean temperature over the months during the period 1971-2000, which gives us December, January and February as the coldest months. Obtained from http://portaldoclima.pt/pt/.

Introduction

Birds ornamentation and social and sexual selection

In an overall view, ornamentation is different in males and females as the role of ornamentation in males differs from females. Ornamentation in birds is known as the colouration patterns shown on their body (feathers, legs, beak, etc.), which in males is typically more developed than in females (Clutton-Brock, 2009; Darwin, 1871; McGraw & Ardia, 2003). Intense and exaggerated ornamentation usually indicates good physiological condition and health status (Aguilera & Amat, 2007; Baeta, Faivre, Motreuil, Gaillard, & Moreau, 2008; Dunn, Garvin, Whittingham, Freeman-Gallant, & Hasselquist, 2010; Hill, Hood, & Huggins, 2009), which is a key factor to females' mate choice (Hill, 2006). It is important to note that this female choice can vary along different environmental and social contexts of breeding, so different aspects of male condition and sexual ornamentation can be important to different females in different environments (Badyaev & Duckworth, 2003). Nevertheless, in general, male ornamentation is thought to indicate aspects of individual condition, so males with more conspicuous ornaments get more chances of mating and thus reproducing. Whereas the main role of ornamentation in males is to attract females and compete with other males for them (intrasexual competition), the role of ornamentation in females has a different dynamic. Based on several reviews (Clutton-Brock, 2007, 2009; Hare & Simmons, 2019; Tobias, Montgomerie, & Lyon, 2012), females use their secondary sexual traits mainly to get higher social status and to compete for ecological resources. Though, they can also compete with each other to get access to males and increase the chances of mating (female intrasexual competition to mate acquisition), in a way similar to males. The same way male ornamentation is important for females' mate choice, female ornamentation may be an indicator of fecundity or parental care capacity for males (Clutton-Brock, 2009). According to Clutton-Brock (2009) in monogamous species, where both sexes are brightly coloured or carry other ornaments, mutual mate choice may play an important role in the development of secondary sexual characters in both sexes. Nevertheless, this happens more commonly in species with no conventional sex roles (polyandrous and sex-role-reversed species) (Tobias et al., 2012).

Ornamentation is thus associated to sexual selection and, because sexual selection is stronger in males than in females in species with conventional sex roles, it generally evolves more in males. But ornamentation not only is involved in aspects of sexual selection (competition for fertilizations) it is also involved in social selection, as

seen above (competition among group members for social hierarchy, space and food resources). In fact, sexual selection may be considered a subset of social selection (Lyon & Montgomerie, 2012; Tobias et al., 2012). For example, intrasexual competition for mating with an individual of the opposite sex, besides sexually select them, also leads to socially select those individuals before the population, with the winning individual showing higher social status. In situations like this, ornamentation has a fundamental role. Or another example, some non-sexual social preferences between individuals of the same sex rely on the colour ornamentation (Cardoso et al., 2014b). So, herewith we can see that social competition occurs in a wide variety of contexts and that ornamentation can have important social functions besides being a sexual signal (reviewed in Lyon & Montgomerie, 2012).

Carotenoids and ornamental colouration in birds

Among the most important pigments in avian coloration are the melanin and carotenoid pigments (Hill et al., 2009). Carotenoids are the main component of the vivid red, orange and yellow colours in birds, such as the estrildid finches, and they can be found either in the beak, in the plumage or in the legs (McGraw & Schuetz, 2004).

Birds cannot synthetize carotenoids metabolically (Brush & Power, 1976; Weaver, Santos, Tucker, Wilson, & Hill, 2018), so they must be obtained from food (dietary carotenoids). Basically, there are two biochemical forms of making use of carotenoids: some species use them directly from their diet and other species have the capacity to chemically modify these dietary pigments and transform them into other forms to then use or deposit them where they are needed, for example, in ornamentation (Brush, 1990; Brush & Power, 1976; McGraw & Schuetz, 2004, and see Koch, McGraw, & Hill, 2016). Accordingly, carotenoid intake can limit or enhance ornament expression (Hill et al., 2009; Hill, Inouyet, & Montgomerie, 2002), being also a signal of foraging success as these pigments can be limited in nature (Endler, 1983; McGraw, Nolan, & Crino, 2006; McGraw & Schuetz, 2004). Parasites (Brawner, Hill, & Sundermann, 2000; Thompson, Hillgarth, Leu, & McClure, 1997) and nutritional condition (Hill, 2000), independent of carotenoid access, can also affect the expression of carotenoid ornamental colouration. Besides body colouration, carotenoids have some important functions in cellular pathways and in physiological systems (Chew & Park, 2004). They work as antioxidants, fighting oxidative stress, and as immunostimulants (Britton, 1995; McGraw & Ardia, 2003; Moller et al., 2000). They are also key elements for vitamin A synthesis, modulate T and B lymphocyte responses and antibody production, etc.,

helping in the immunocompetence of the individual (Chew & Park, 2004; Hill & Johnson, 2012; Peters, Denk, Delhey, & Kempenaers, 2004). Carotenoids may trade-off between ornamental colouration and physiological systems, like the immune system for example, and birds may take them from the ornaments and reallocate them into those systems when they need (Baeta et al., 2008; Rosenthal, Murphy, Darling, & Tarvin, 2012; Weaver et al., 2018). This way, the amount of carotenoids may signal current body condition, because if they are in great availability in secondary traits, it means they are not being necessary as immune factors and that the immune system is strong (Hill, 2011; Weaver et al., 2018). Summing up, higher foraging success leads to more carotenoid-rich food, which thus leads to a healthier individual and to an individual with brighter colours. Based on this, females may use carotenoid-based sexual signals to detect a male's immune vigour and reproducing ability by examining somehow these traits, as showed by Pike et al. (2007) and Peters et al. (2004).

As seen, carotenoid pigments have many functions, either at an ornamental level or at a physiological level. In addition to the functions mentioned, females also use carotenoids for reproduction (Blount et al., 2002a; Ewen et al., 2009). They may fetch these pigments from living tissues, like the bill for example, which may serve as a great carotenoid supply, to incorporate into the eggs, more specifically into the egg yolks (Hipfner, Dale, & McGraw, 2010). As carotenoids have antioxidant and immunostimulant properties, this investment is done with a view to protect the developing embryos against oxidative stress (mainly lipid peroxidation) and enhance their immune system function (Blount, Houston, & Moller, 2000; Blount, Surai, Houston, & Moller, 2002b; Saino, Ferrari, Romano, Martinelli, & Møller, 2003). But this reallocation of carotenoids has potential costs to the females' health because they need to maintain their own antioxidant activity and immune function as well (Blount et al., 2000; Blount et al., 2002a; Saino et al., 2003). During reproduction, females make use of a bigger amount of carotenoids than usual to ensure the reproduction success, so if they obtain those carotenoids from their body to invest into the offspring, their body condition will decrease. However, with the bill being a carotenoid storage, females can get carotenoids from there whenever they need and reallocate them either to invest into the eggs or to fight environmental stress, nutritional deficit or weak immunity. Depending on the availability of carotenoid-rich food the bill will be richer or poorer in carotenoids, which will also affect its colouration. Consequently, low carotenoid availability may not only limit ornament expression but also the egg-laying capacity of female birds (Biard, Surai, & Møller, 2005; Blount, Houston, Surai, & Møller, 2004).

Concluding, males may be able to invest more in colour ornamentation than females because females have the responsibility of investing in reproduction and generating healthy offspring, which males do not have. Therefore, it may be more difficult for females to invest carotenoids in bill colour, as they have to adjust the colour instead of investing almost everything into the bill or into other ornamental traits as males do. This may contribute to explain why males generally have a stronger colouration and are able to evolve more.

Signals/phenotypic plasticity

Phenotypic plasticity is the capability of a living being changing or adapting its physiological, morphological or behavioural characteristics when exposed to different environmental conditions or pressures. This feature enables individual signal adjustments which are crucial to survival. Signals or traits like reproductive timing or termination of hibernation in mammals (Boutin & Lane, 2014), size of seeds or biomass allocation (root: shoot ratio, for example) in plants (Sultan, 2000), bill colour (Karubian, Lindsay, Schwabl, & Webster, 2011) or seasonal timing of migration (Charmantier & Gienapp, 2014) in birds, may have plastic characteristics; of course each one within a different time range. Concerning birds, for example, Dorado-Correa et al. (2018) showed the presence of a particular form of signal plasticity, the Lombard effect, in mallards (*Anas platyrhynchos*), which allows the communication in noisy environments by increasing the vocal amplitude as the background noise increases. This study supports the hypothesis that this mechanism of signal plasticity is present in all extant birds. In its turn, Rosenthal et al. (2012) showed that in American goldfinches (*Spinus tristis*) the bill colouration, which is carotenoid-based, can change in a matter of hours, suggesting that this plastic signal may indicate physiological changes in 'real time'. Still regarding carotenoid-based colouration, Kelly et al. (2012) demonstrated that bill colour (a dynamic signal) and plumage colour (a static signal) may provide different information about phenotypic and immunological quality depending on the sex, concluding that in females both of these traits function as condition-dependent signals while in males they were poorly related to aspects of immunological condition and metabolic rate. Accordingly, these plastic structures, whether it is the bill colouration or other, "can play an important role in sexual and social communication because they can reflect short-term [and long-term] changes in physiological or motivational status" (Rosenthal et al., 2012, p. 1).

Recently, Funghi et al. (2018) showed that common waxbills (*Estrilda astrild*) have a sexually dimorphic social ornament, the red bill, but that its sex differences are

entirely plastic. It is generally thought that sex differences in ornamentation (sexual dichromatism) in species with conventional sex roles are genetically fixed because of the strong sexual selection over males as opposed to females. But, unlike sexual selection, some aspects of social selection don't need to be stronger in males than in females. Hence, it is plausible that some sex differences in the ornamentation of gregarious animals are not genetically fixed but are instead due to external constraints affecting differently males and females (Funghi et al., 2018). Although only using males, Eraud et al. (2007) showed that under cold exposure, an ambient constraint, zebra finches (*Taeniopygia guttata*) developed less red bills than when exposed at a warm temperature, either carotenoid-supplemented or not. Laczi et al. (2019) showed, in a great tit (*Parus major*) population in the Carpathian Basin, that more saturated carotenoid-based colouration during moulting is linked to warmer and dryer weather. Also, Moreno et al. (2019) showed that in the pied flycatcher (*Ficedula hypoleuca*) cold and rainy springs are responsible for decreases in the wing and forehead patches area in females and that there was no association between climate and wing patch in males. Despite that, cold pre-breeding season was associated with increases in female wing patch area and in male forehead patch area. In the end the authors conclude that males are differently affected by climate than females, with males not being so strongly affected by poor conditions during incubation and nestling rearing as females, because of the different efforts that males and females put in breeding (Moreno et al., 2019). These three studies indicate that ambient temperature may affect the expression of different ornaments in birds, with cold being, in general, an energetic constraint and warmer temperatures being a favourable factor.

An example of other environmental constraints that can affect the expression of birds' ornamental colouration is the study of Hill et al. (2009). Hill et al. (2009) manipulated three environmental conditions simultaneously: carotenoid access, parasite load and food access, to test the effects of their abundance or scarcity on the bill and plumage colouration of male American goldfinches. This study showed that all of the three factors shape the expression of bill colour (*ad libitum* food, high doses of carotenoids and protection from pathogens developed a more intense carotenoid-based colouration in the bill, comparing with the opposite treatments), while concerning plumage colour only high doses of carotenoids increased the carotenoid-based colouration. "The different responses of feather and bill colouration support the idea that bill and feather colouration are fundamentally different traits in songbirds, even if both are produced through carotenoid pigmentation" (Hill et al., 2009, p. 1231).

As, in several bird species, carotenoids are used either in different physiological functions and in colour pigmentation on a daily basis, carotenoid-based signals are excellent traits to study the effect of environmental stress on the maintenance of body colouration. Environmental stress, or more precisely low ambient temperature, will be the focus of my work, and one species that reunites key characteristics for that research is the common waxbill, which will be my study object.

The study species: the common waxbill (*Estrilda astrild*)

The common waxbill (*Estrilda astrild*) (Figure 1) is a small granivorous finch from the Estrildidae family and native from sub-Saharan Africa (Cardoso & Reino, 2018), which means that it is an alien species in Portugal. The common waxbill is one of the most successful invaders among estrildid finches and it is one of the few non-native bird species establishing wild populations in the Mediterranean Basin (Reino, 2005; Silva, Reino, & Borralho, 2002; Stiels, Schidelko, Engler, van den Elzen, & Rödder, 2011). This species has been introduced in many countries in different continents and was first introduced in Portugal around 1964 (Reino & Silva, 1998). Since then it has spread to many parts of the country and to Spain, currently showing a wide distribution (Cardoso & Reino, 2018; Reino, 2005; Sullivan, Davies, Reino, & Franco, 2012).

Fig. 1 - Common waxbill (*Estrilda astrild*). Photo by Russ Telfer. (Available in https://www.birdguides.com/gallery/birds/estrilda-astrild/480095/).

These birds are socially monogamous but extremely gregarious, moving in flocks and roosting conjointly, and also nesting close to each other (Clement, Harris, & Davies, 1993). They can be found in a wide variety of open habitats, being typically associated to areas near water and with rank vegetation. They also breed in different habitats as long as there is water available, proper vegetation to sustain the nest and abundance and variety of seeds in the site (Reino & Silva, 1998). The common waxbill is an opportunistic species, so it reproduces when there are good conditions for that. In the Iberian Peninsula, the breeding season lasts from early spring until autumn (Sanz-Aguilar, Carrete, Edelaar, Potti, & Tella, 2015).

The most prominent ornaments of common waxbills are their vividly-coloured red bill (in adults; juveniles have a black bill), a red mask (red stripe around the eye) and red

plumage in the breast extending down to the abdomen (Figure 1), both in males and females. It is noteworthy that in males the saturation of red colour is on average higher than in females (Cardoso et al., 2014b), as this is a species with conventional sex roles. However, the sexes overlap extensively in the extent and saturation of red colour, and there are large differences in ornamentation within each sex (Cardoso et al., 2014b). Besides, this red colour can vary with some other factors. For example, it is known that a better body condition is related to a redder bill colouration (Marques, Batalha, & Cardoso, 2016) and the absence of energetically-stressing cold nights was suggested to also be related to a redder bill in waxbills (Funghi et al., 2018). Stiels et al. (2011, p. 2) refers to some experiences with captive birds (Immelmann, Steinbacher, & Wolters, 1965; Nicolai, Steinbacher, van den Elzen, Hofmann, & Mettke-Hofmann, 2007) that showed that common waxbills "are sensitive to low temperatures (below 15ºC) and seem to suffer in particular from cold and wet weather conditions". So it makes sense that the absence of cold temperature conditions contributes to a more vivid red bill.

Background of this work

As I said before, the red bill of the common waxbill was found to be a plastic sexually dimorphic social ornament (Funghi et al., 2018; see also Cardoso et al., 2014b). This was the first time that sex differences in ornamentation were shown to be entirely plastic, rather than genetically fixed, in an animal with conventional sex roles.

In a previous experiment, the birds were captured from the wild and kept separately by sexes in bird cages, and a couple of months after being in captivity, females had augmented their bill colour saturation and bill sexual dichromatism had disappeared (Cardoso et al., 2014b). Funghi et al. (2018) also captured waxbills from the wild and kept them in same-sex groups in bird cages in the beginning. They observed that bill colour saturation in females and males fluctuated through time, and that bill sexual dichromatism gradually disappeared. The birds were then separated into mixed-sex groups and same-sex groups, but this difference in social context did not affect bill sexual dichromatism (Funghi et al., 2018).

Since the change in females bill colour saturation was not related to social hierarchies among individuals (individual differences in aggressiveness, which is a behaviour closely related to social dominance, did not predict changes in bill colour, neither did changing social context, i.e. from same-sex to mixed-sex groups), Funghi et al. (2018) concluded that living with males does not influence female investment in bill

colour (social constraint hypothesis). Instead, environmental conditions appear to have caused changes in female bill colouration (ecological constraint hypothesis) because female bill colour positively correlated with ambient temperature. While female bill colour correlated with temperature, male bill colour did not, suggesting that it affects female and male ornamentation differently and that females are more sensitive than males to the environment. Funghi et al. (2018) then concluded that when female waxbills are released from ecological constraints and exposed to favourable energetic conditions, like *ad libitum* access to food and protection from cold nights, they may increase the saturation of red in their bills. One of the main factors that is energetically stressful for small birds are cold temperatures, especially night temperatures, and based on the results of this study, those affect female investment in bill colouration.

Hereupon, there is evidence that the sexual plasticity found on the bill of the common waxbill is not due to social factors but instead appears to be related with females, and not males, disinvesting in ornamentation under periods of more stressful energetic conditions. However, the previous work on the effect of the environment on waxbill colouration was observational, and so there is a lack of strong experimental results linking temperature to bill colouration in this species.

Thesis goal

The aim of my work is to test experimentally if and how climatic and energetic stress (more specifically winter cold temperatures) influence the bill colouration of common waxbills and if it affects differently males and females of this species. I studied how temperature affects the plasticity of this social ornament by manipulating temperature inside the cages on the research aviary in the non-reproductive season (winter). During this temperature manipulation experiment, I regularly measured bill colour using reflectance spectrophotometry and also quantified movement and feeding rates of the birds, as an add-on to the research, to see if there were some physiological changes beside the colour of the bill.

Following previous correlational results (Funghi et al., 2018), I hypothesise that 1) females will be more sensitive to changes in ambient temperature than males, 2) bill colour saturation of females will increase with higher temperatures, because the energetic constraint (cold) disappears, and, if the experimental temperature manipulation effectively affects energetic stress, I also predict that 3) birds' feeding rate will decrease and birds' movement index will increase with increasing temperatures.

To test these predictions, I first tested if the temperature manipulation was sufficient to alter the physiological state of the individuals, by influencing birds' behaviour (movement indexes and feeding rates). Then, I tested whether temperature manipulation influenced the average bill colour saturation of males and females, or whether it influenced the extent of colour fluctuations throughout the experiment.

Materials and methods

Birds maintenance and social context

The waxbills (*Estrilda astrild*) were acquired from certified breeders in March 2018, approximately 10 months before my experiments. They were a total of 24 birds; 12 males and 12 females. All the birds were ringed with individually numbered metal rings for identification and housed in 6 metal cages (88.5 x 30 x 40 cm) with four long perches (Figure 2).

Fig. 2 - Cage example used in the experiment.

The birds were kept there throughout the experiment, in mixed-sex groups, with 4 birds in each cage (2 males and 2 females). One of the birds died before the end of the experiment, and so I continued with a sample size of 23 birds (12 males and 11 females). The cages were in a sheltered outdoor aviary with *ad libitum* water and food (Tropical Finches Prestige, Versele-Laga, a commercial mixture of seeds for exotic birds) and access to appropriate sand for birds in a small plastic plate placed on the floor of each cage (Figure 3b). Each cage had two water dispensers changed every 2 or 3 days and two long feeders that allowed all birds to feed simultaneously. Twice a week a bath tub filled with water was placed inside the cages for them to refresh. Although the aviary where the waxbills were kept was protected from wind and direct sunlight, one of the walls was entirely made of metal grid and plastic net so that the temperature and humidity inside the aviary were identical to the outside conditions. Natural illumination was supplemented with artificial lights in a cycle corresponding to the beginning and end times of natural light, to reinforce the natural period of daylight. These lights were programmed to turn on at half an hour to 45 minutes before sunrise and turn off at half an hour to 45 minutes after sunset, which are approximately the start and end times of natural light, as I mentioned before.

Temperature manipulation

This temperature manipulation experiment took place in the Research Centre in Biodiversity and Genetic Resources (CIBIO-InBio) of the University of Porto, in Portugal, and was conducted in winter, which is the non-breeding season (Sanz-Aguilar et al., 2015). It lasted two months and a half, starting on the 10th December 2018 and ending on the 20th February 2019.

Diverse methods for manipulating temperature have been used for experiments with other species, either birds or other animals, like rooms equipped with air-conditioning (Blahová, Dobšíková, Straková, & Suchý, 2007; Visser, Holleman, & Caro, 2009), nest boxes warmed by a small controlled electric resistor (Meijer, Nienaber, Langer, & Trillmich, 1999), temperature-controlled chambers (Nord & Folkow, 2018; Pendlebury, MacLeod, & Bryant, 2004; Salvante, Walzem, & Williams, 2007) and other climate-controlled aviaries (Schaper et al., 2012). In order to test how bill colour is affected by temperature, I manipulated temperature inside three cages (heated cages) and maintained the three other cages at ambient temperature (ambient temperature cages). For that, I chose a method that could warm individual cages, so that heated cages and ambient temperature cages could both remain in the same shared environment, without having to isolate or move some of the birds to a different environment where they wouldn't be accustomed to be or where, for example, they would not hear the usual sounds of the birds in the other cages.

In detail, I manipulated the temperature of the heated cages using two warming devices (24 x 27.5 x 7 cm electric foot warmers, Junex model 5768, with a 70 W warming plate protected by wooden frames) below each cage; one below each of the two floor panels of the cage (Figure 3a). Each floor panel, a metal grid, was covered with white paper for protecting the devices from droppings, dirt, etc. I made a styrofoam structure around each heater to avoid heat dissipation to the sides, the front or the back (Figure 3a). In addition, the top 26 cm of the front grid of each cage was covered with transparent cling film, to retain warm air within the cage; aeration of the cages was made by the 7 cm-high area in the bottom of the front of the cage without cling film (Figure 3a). The sides, back and top of each cage, made of metal plate, were covered on the outside by styrofoam plates to further retain heat. To record the temperature inside the cages, each of the six cages had a data logger (Elitech RC-5 USB Temperature Data Logger) hanging from the top of the cage, mid-way in the centre (Figure 3a). All the ambient temperature cages were subjected to the same experimental setup as the heated cages, but without the warming devices underneath.

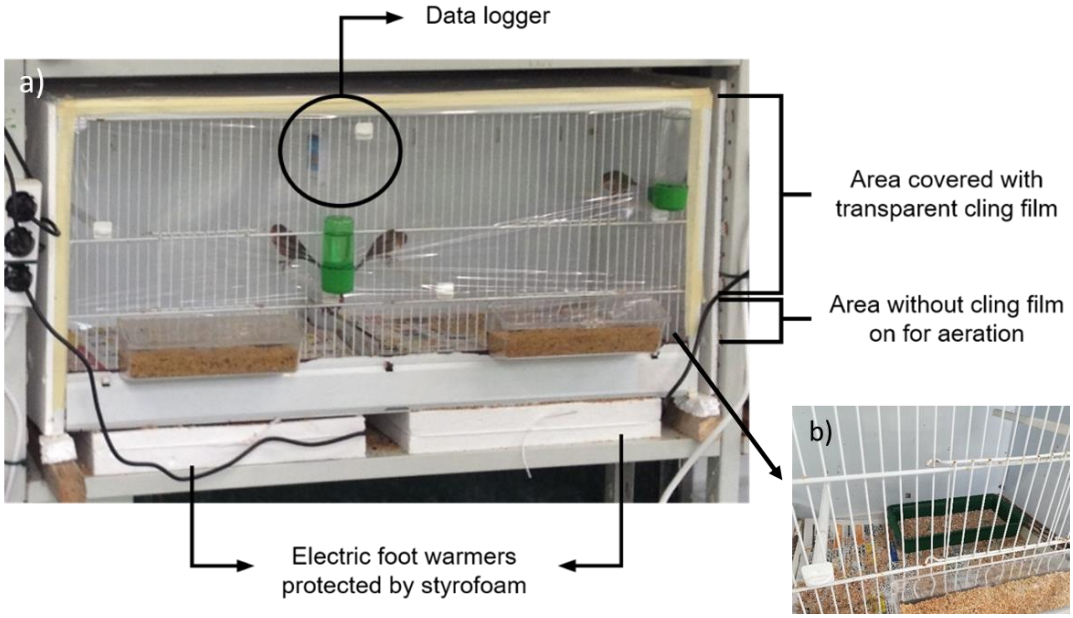

Fig. 3 - a) Cages experimental setup. b) Plastic plate with sand inside a cage.

This experiment was developed during December, January and February because those have been the coldest months of the year (climatic data from IPMA, Portuguese Institute for Sea and Atmosphere; see Appendix 1). I wanted to proceed with the experiment in the coldest months so that individuals in the ambient temperature cages experienced cold stress conditions within their natural variation. This way, I would get a stronger effect of warming on the individuals of the heated cages, protecting them against cold stress (especially cold night temperatures).

The total duration of experiments was ten weeks and the work was divided in two parts: the first part lasted four weeks (10th December 2018 - 9th January 2019), then there was a two-week interval, and the second part lasted other four weeks (21st January 2019 - 20th February 2019). In the first part of the experiment, I heated three cages and left the remaining three at ambient temperature, and in the second part, I reversed the temperature treatments: the ambient temperature cages became the heated ones and vice-versa, so that all birds experienced the two temperature regimes (cold and heated cages). For the onset of the hot treatment to be gradual, I turned on the warming devices on the first day of each part of the experiment but did not immediately apply the cling film, which I did to all six cages two days afterwards. Hereafter, I refer to the two groups of birds as those in the cold-hot treatment (starting at ambient temperature and then changing to heated), and those in the hot-cold treatment (starting in heated cages, which then changed to ambient temperature).

Temperatures were recorded every 30 minutes in each cage, as well as every 30 minutes in an additional data logger (EasyLog EL-USB-2-LCD+ Data Logger) in the

aviary outside the cages. Throughout the experiments, the ambient temperature cages had the same temperature as outside in aviary, and the temperature differences between these and the heated cages remained stable, with the heated cages around 5.7º Celsius warmer (Figure 5a in 'Results' section). In the second week of experiments, data loggers failed to record the temperatures inside the cages. To replace those missing data, I used the temperatures measured by the external temperature logger, located outside the cages in the aviary, to which I summed the mean difference between temperatures measured inside and outside the cages along the entire experiment (mean difference was 6.444ºC for the heated cages, and 0.679ºC for the ambient temperature cages). These reconstructed temperatures are represented by dashed lines in figure 5a.

Video recordings

During the experiment, a video system was set up in the aviary room to record the behaviour of the birds early in the morning, to observe feeding rates, and by the end of the afternoon, to observe the extent of birds' movement (i.e., to which extent they move more or less).

The video system included two webcams (HP HD 2300 webcam), connected to a laptop (Lenovo V330 14" V330-14IKB) with two software programmes (Debut Video Capture and Screen Recorder Software, and ManyCam Virtual Webcam) programmed to record from the webcams at specific times. The two webcams were tied to the metal grid of the front aviary wall and, together, they recorded frontal video image from all the cages (Figure 4). The recording schedule settled for the morning videos was from half an hour before sunrise (when the aviary lights turn on) until 2 hours later, and for the afternoon videos the schedule was 2 hours of recording ending half an hour after sunset (when the aviary lights turn off).

Fig. 4 - Video recording system with the two webcams tied to the metal grid and connected to the laptop.

Data collection: colour data

To obtain information about bill colouration, I used reflectance spectrophotometry, which allowed to quantify the saturation of the red colour of the bill. This saturation of the bill is the proportion of reflectance in the longer wavelengths of the visible spectrum (ca. 600-700 nm), which are perceived as red. I chose to measure colour saturation because it is related to the amount of carotenoids in the bill (Navara & Hill, 2003) and is the parameter that best reflects sexual dichromatism in waxbill bills (Cardoso, Batalha, Reis, & Lopes, 2014a). Besides, previous observations suggest that the difference in saturation between sexes changes depending on the environment (Funghi et al., 2018).

The measurements of scattered light reflectance were made using an Ocean Optics usb4000 spectrophotometer coupled to a PX-2 xenon light source. For each bird I took four measurements on the bill, two on each side of the upper mandible, because the upper mandible is the most visible part of the bill and has more homogeneous and on average more saturated red colour than the lower mandible (pers. obs.). These measurements were taken with the reflectance probe touching perpendicularly the bill surface and then enveloping the area with a black opaque velvet to prevent contamination from daylight. I measured all four birds in a cage before changing to the next, and the spectrophotometer was calibrated every time I changed cages using an Ocean Optics WS-1-SL white standard as well as a piece of black velvet. These measurements were taken always at the same time (around 2 p.m.) on the two Mondays in which the temperature treatments began (before turning on the heater devices), and then on the Wednesdays of the following four weeks in each of the two parts of the experiment.

For each reflectance spectrum I flattened reflectance lower than 1% to 1%, because small measurement inaccuracies near 0% reflectance can cause large arbitrary changes in some colour metrics (Cardoso & Gomes, 2015). I then $\log_{10}$-transformed reflectance spectra, which were originally in percentage reflection (from 0 to 100), because colour production and perception function on a ratio (i. e., logarithmic) scale, while linear reflectance scales underestimates brightness differences among dark colour compared to among bright colours (Cardoso & Gomes, 2015). For each spectrum I averaged $\log_{10}$ reflectance between 600 and 700 nm wavelengths, which is the interval where reflectance reaches a high plateau on these red bills (see Figure 1F[1] in Cardoso

[1] There is a typo in Figure 1 from Cardoso et al. (2014a); Figure 1F corresponds to bill reflectance and Figure 1E to breast reflectance.

et al., 2014a), and averaged $\log_{10}$ reflectance between 320 and 700 nm wavelengths, which is the bird-visible spectrum (Montgomerie, 2006). Following Funghi et al. (2018), I quantified the red colour saturation in waxbill bills by subtracting mean log-reflectance at 320−700 nm to the mean log-reflectance at 600−700 nm, and averaged this across all four measurements on the bill of each bird. Note that this subtraction is equivalent to widely used receiver-independent metrics of colour saturation based on the proportion of reflected light on selected wavelengths relative to the entire visible range (e.g. metric S1 in Montgomerie, 2006), because differences of log-transformed reflectance quantify ratios of linear reflectance (Cardoso & Gomes, 2015). I used this receiver-independent metric of colour saturation because reflectance spectra of waxbill bills have a fixed sigmoid shape, varying mostly in the height of the 600 and 700 nm reflectance plateau (Figure 1F in Cardoso et al., 2014a), and because using logarithmic reflectance quantifies differences in saturation according to Fechner law (visual receptors respond proportionally to the logarithm of light intensity) while avoiding uncertainties with implementing this law in models of avian vision (Cardoso & Gomes, 2015).

Behavioural data collection: video data

To visualize the videos, I used a native Windows 10 app named Movies and TV programmes. Data was collected using instantaneous observations at every 2 minutes of the video recording for the morning videos, and at every 4 minutes for the afternoon videos, along the entire 2 hours of video. These interval times were settled after preliminary observations to check that observations at these time intervals sufficed to capture representative differences of behaviour among birds. I watched one morning video recording and one afternoon video recording from each week of experiments, always made on Saturday, when there was no disturbance due to maintenance of the aviary or bird cages.

For the morning videos, every two minutes, I counted how many birds were feeding or at less than one body of distance behind the feeders. I then calculated an index of feeding rate for each cage, by computing the average number of birds feeding during the two hours. For afternoon videos, every four minutes, I counted how many birds were at each of 9 locations inside the cage (perches 1 to 4, partition in the middle of the cage, front grid of the cage, left side floor, right side floor and the small plastic plate) and computed an index of movement between locations. To do this, I calculated the absolute value of the difference between the number of individuals in a location and the number of individuals in that location in the following observation, then summed these

absolute values of differences for every location, and then averaged these sums across the two hours for each cage. The more the birds move between locations, the higher is this movement index for the cage.

To test the effect of the treatment in birds' physiology in the end of each part of the experiment, birds' body weight was also measured, on January 9th, 2019 and on February 20th, 2019 at around the same hour of the colour measurements (14 o'clock). These measurements were taken using a spring balance with a precision of ±0.05g.

Statistical Analyses

Effects of temperature manipulation on behaviour and body weight

To see if there was any effect of the temperature treatments in the behaviour or body weight of the birds, I made 3 independent samples t tests comparing the changes that occurred in the cold-hot treatment with the changes that occurred in the hot-cold treatment. I used, as statistical units, the changes in each individual (for body weight) or the changes in each cage (for feeding rate and movement index). To compute changes in body weight I subtracted the weight measured in the first part of treatment to the weight measured in the second part and then averaged this for each of the treatments. To compute changes in behaviour, I subtracted mean value in the first 4 weeks of the experiment from the mean value in the last 4 weeks and then averaged this for each of the treatments.

Effects of temperature manipulation on bill colour

To test if bill colour saturation changed differently in the cold-hot treatment and in the hot-cold treatment, I used independent samples t tests as above, using the changes in each individual as statistical units, but here I analysed males and females separately. Similarly to above, I computed changes in the bill colour saturation of each individual as the mean saturation in the last 4 weeks of experiment minus the mean saturation in the first 4 weeks.

Additionally, and in case bill colour takes a long time to change in response to temperature, I repeated these tests but computing changes in colour saturation using only measurements from the last 2 weeks of each 4-week period of temperature

manipulation: the mean saturation in the last 2 weeks of the experiment minus the mean saturation in the last 2 weeks of the first 4-week period of the experiment.

Finally, to test if the temperature manipulation affected the degree of fluctuation in bill colour saturation, I repeated these tests but using changes in colour fluctuation instead (i.e. changes in the standard deviation, rather than changes in the mean): the standard deviation in colour saturation across the last 4 weeks of the experiment minus the standard deviation in the first 4 weeks of the experiment.

Effects of measured temperature on behaviour and bill colour

I additionally tested whether bill colour saturation changed or fluctuated significantly throughout the experiment, by running a General Linear Mixed Model (GLMM) with individuals as subjects, bill colour saturation as the dependent variable, and sex and time point as factors. The time points are each one of the 10 colour saturation measurements taken along the experiment. This model tests if bill colour saturation differs between the sexes, and whether it changed significantly during the course of the experiment.

Finally, I tested if the real changes in temperature during the experiment (as measured by temperature loggers inside the cages; Figure 5a) predicted the changes of colour saturation in the waxbills bill by running a GLMM, separately for males and females, with individuals as subjects, bill colour saturation as the dependent variable and the mean temperature of the previous day as a covariate. I calculated this mean temperature counting from the mid-day of the day before the colour measurement to the mid-day of the measurement day. I ran another GLMM identical to this but replacing mean temperature of the previous day by the mean temperature of the previous night (counting from the time of sunset to the time of sunrise; times for Porto, Portugal; «Observatório Astronómico de Lisboa», 2014), because the colder night temperatures may be the most important stressor affecting bill colour. As it is not known exactly how long the bill colour takes to change in response to environmental factors, I also ran models similar to the ones above but using mean temperature across longer timespans: either the mean temperature across the 2, 3, 4, 5 or 6 previous days, or across the 2, 3, 4, 5 or 6 previous nights.

I tested as well if feeding rate and the index of movement were related to changes in the ambient temperature during the 10 weeks of the experiment. For that, I ran GLMM similar to the ones described above, but using either the feeding rate or the index of

movement as the dependent variable and cages as subjects. As before, I ran models using the mean temperature of the 1, 2, 3, 4, 5 or 6 previous days or nights. All statistical analyses were conducted in IBM SPSS Statistics 25.

Results

Effects of temperature manipulation on behaviour and body weight

Temperature outside the cages underwent dial oscillations, with colder nights and warmer days (black line in Figure 5a). By comparison, there was little variation in the mean temperature along the ca. 2 months of the experiment.

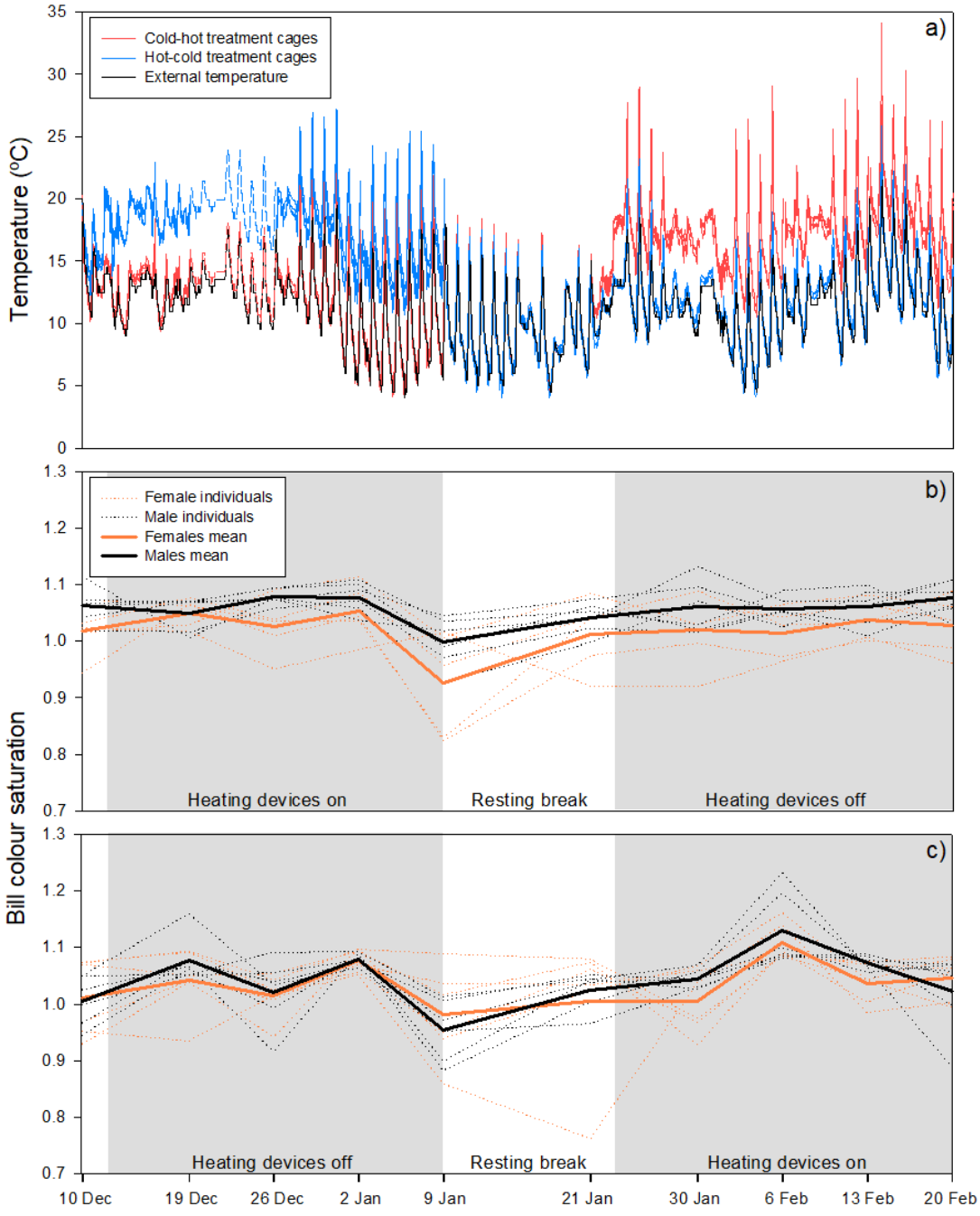

Fig. 5 - Fluctuations in temperature and bill colour. a) Real ambient temperatures along the experiment (dashed line portions correspond to the reconstructed temperatures during the second week of treatment; see 'Temperature manipulation' section in 'Materials and methods'; each red and blue entry is composed of 3 lines that correspond to the three cages in that treatment) and bill colour saturation measurements of the birds in b) hot-cold cages and c) cold-hot cages, for females (orange lines) and males (black lines).

Inside the cages I observed the same dial oscillations and, superimposed on those, cages with the heating devices on were on average 5.7ºC (± 0.7ºC) warmer than the other cages (coloured lines in Figure 5a).

In the cold-hot treatment, which started with lower temperatures and switched to higher temperatures, the feeding rate decreased from the cold period to the hot period, while in the hot-cold treatment feeding rate increased, and the difference between the two treatments was significant (t=-6.640; N=6 cages; p=0.003; Figure 6a). Movement in the cages also decreased in the cold-hot treatment, increased in the hot-cold treatment, and the difference between the two treatments was significant (t=-4.708; N=6 cages; p=0.009; Figure 6b). This means that with colder temperatures the birds eat and move more than with warmer temperatures.

Body weight increased in individuals on both temperature treatments and, although the increase in weight appears larger in the cold-hot treatment, the difference between treatments was not significant (t=0.726; N=23 birds; p=0.476; Figure 6c).

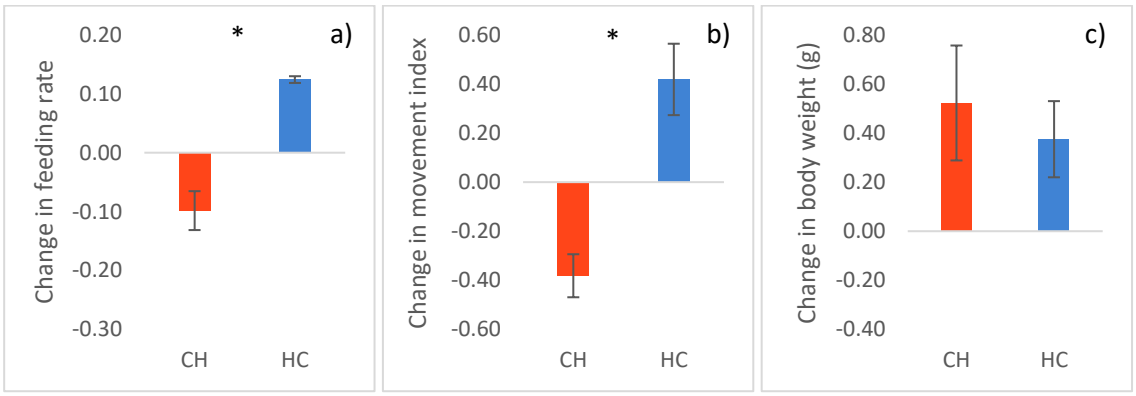

Fig. 6 - Changes in a) feeding rate and b) movement index, for cages, and in c) body weight, for birds, between the two temperature treatments (difference ± SE). CH: cold-hot treatment; HC: hot-cold treatment. Asterisks mark statistically significant differences.

Effects of temperature manipulation on bill colour

Comparing the change in bill colour saturation from the first 4 weeks to the last 4 weeks of the experiment (grey areas in Figure 5b, c), I found that changes in bill colour did not differ between the cold-hot treatment and the hot-cold treatment neither in females (t=-0.395; N=11 birds; p=0.702; orange bars in Figure 7a) nor in males (t=-1.135; N=12 birds; p=0.283; black bars in Figure 7a).

In case bill colour takes a long time adjusting to temperature, I decided to repeat these tests using data from the last 2 weeks of each 4-week period of temperature manipulation. Focusing on these last 2-week periods, the mean increase in female bill colour saturation in the cold-hot treatment was about 4 times higher than in the hot-cold treatment, but among-individual variation in these colour changes was large and the difference between treatments was not significant (t=1.198; N=11 birds; p=0.261; orange bars in Figure 7b). Also in males, the cold-hot and the hot-cold treatments did not differ in the extent of bill colour changes (t=0.010; N=12 birds; p=0.992; black bars in Figure 7b).

Within-individual fluctuation in female bill colour, computed as the standard deviation of colour saturation in each 4-week period, decreased in the cold-hot treatment and slightly increased in the hot-cold treatment, and the difference between treatments was significant (t=-2.539; N=11 birds; p=0.032, orange bars in Figure 7c). In males, changes in the extent of fluctuations in bill colour did not differ between treatments (t=0.174; N=12 birds; p=0.865; black bars in Figure 7c).

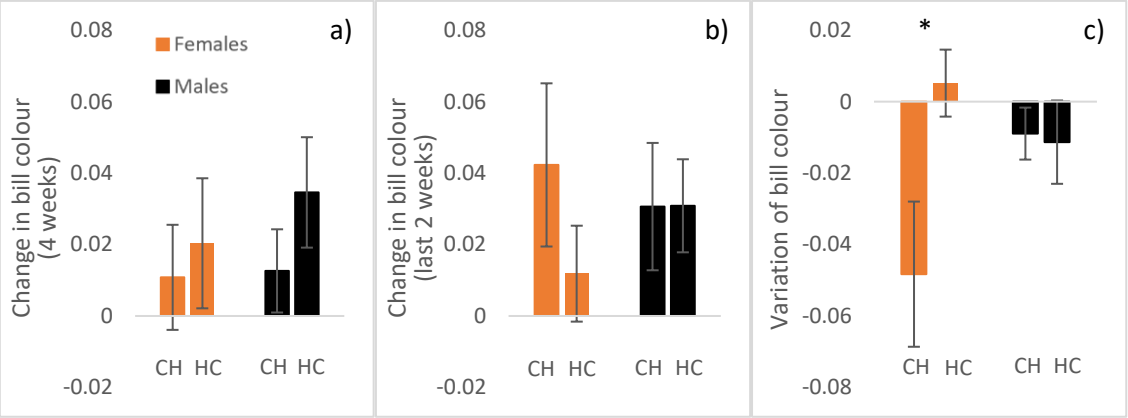

Fig. 7 - Changes in bill colour saturation using the difference between a) mean of all the 4 weeks of the first part of experiment and of the second part; b) mean of the last 2 weeks of the first part of experiment and of the second part; and c) standard deviation of all the 4 weeks of the first part of experiment and of the second part, for males (black) and females (orange). CH: cold-hot treatment; HC: hot-cold treatment. Asterisks mark statistically significant differences. Bars represent difference ± SE.

Effects of measured temperature on behaviour and bill colour

A GLMM on all colour measurements, using individual birds as subjects, showed that bill colour saturation differed significantly between sexes and across the different time points in the experiment (effect of sex: $F_{1,219}$=12.250, P=0.001; effect of time point: $F_{9,219}$=8.944, P<0.001; Figure 5b, c).

Temperature, as measured inside each cage, fluctuated strongly over the course of the experiment (Figure 5a). GLMMs relating behaviour to mean temperature over different periods of time (from 1 to 6 previous days of nights) found that feeding rate covaried significantly with temperature (all $F_{1,46}$>4.052, all P<0.05; Figure 8a), but the amount of movements did not (all $F_{1,46}$<4.052, all P>0.05; Figure 8b). Bill colour saturation also did not covary with the temperature measured inside the cages, for any period of time (from 1 to 6 days of nights prior to colour measurements), neither in males (all $F_{1,118}$<3,921, all P>0.05; Figure 8c) nor in females (all $F_{1,108}$<3,929, all P>0.05; Figure 8c).

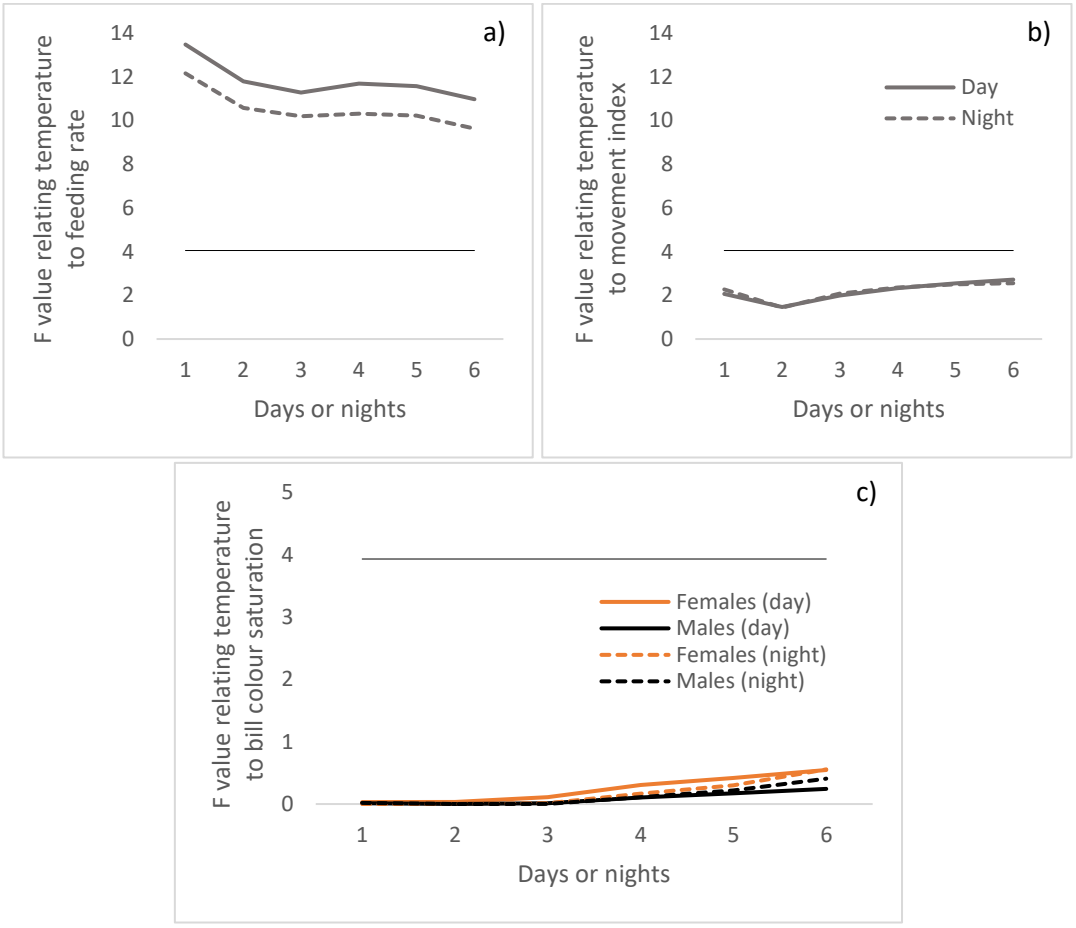

Fig. 8 - F values of General Linear Mixed Models relating temperature to a) feeding rate, b) movement index and c) bill colour saturation of males (black) and females (orange) at different time windows. F values above 4.1 (horizontal line in graphics a and b) and 3.9 (horizontal line in graphic c) are statistically significant. Nights (from sunset to sunrise) are represented by dashed lines and days (from midday until the following midday) by solid lines.

Discussion

Although plastic changes in colour or other ornamental traits have been studied in several bird species (e.g., Dorado-correa et al., 2018; Eraud et al., 2007; McGraw, 2004), phenotypically plastic sexual dichromatism was rarely documented in birds with conventional sex roles. In common waxbills, Funghi et al. (2018) observed that changes in female bill colour were correlated with ambient temperature, suggesting that females took advantage from favourable ecological conditions (warmer temperatures) to increase their bill colour and reach the male average bill colour. Funghi et al. (2018) proposed this as an explanation for the plastic disappearance of bill sexual dichromatism. In this work, I tested experimentally whether changing ambient temperature in winter affected female and male bill colour in waxbills. I found no evidence that sexual dichromatism decreases or disappears with warmer temperatures, as the non-experimental correlational results of Funghi et al. (2018) suggested. But I did find a sex-specific effect of temperature in bill colour, in that warmer temperatures made female bill colour (and not male's) more stable, with less oscillations over time.

More precisely, I found that bill colour saturation fluctuated less as females passed from the cold treatment to the hot treatment, as compared to females passing from the hot treatment to the cold treatment. Changes in the mean bill colour saturation, however, did not differ between these two treatments and mean bill colour saturation did not covary significantly with measured temperatures across the experiment. I also found significant changes in the birds' behaviour (feeding rate and movement index decreased when passing from the cold treatment to the hot treatment and increased, as compared to birds passing from the hot treatment to the cold treatment), indicating that the temperature manipulation was biologically meaningful. Plus, feeding rate covaried significantly with measured temperature along the experiment, although the movement index did not. Differences between treatments in body weight were non-significant.

Although my results did not support the hypothesis of females augmenting their bill colour saturation with favourable temperature conditions, they did show a sex-specific effect in the form of a stabilization of female bill colour fluctuations. Fluctuations in bill colour measured as the standard deviation, decreased notably in females from the cold to the hot period of temperature manipulation, as compared to a slight increase in females changing from the hot to the cold period. In contrast, males showed almost no difference in colour fluctuation between one treatment and the other. As I mentioned, in Funghi et al. (2018), female bill colour correlated with night temperatures but male's did not and also in my work, I found that more stressful and demanding temperatures (colder

temperatures) destabilize bill colour saturation in females, but not in males. Accordingly, there is an effect on females but not on males.

This result, albeit in a different manner than predicted, supports the hypothesis that females are more sensitive and more susceptible to changes in the environment than males (for example, Grzędzicka & Kubacka, 2018; Hipfner et al., 2010). This may happen because of female life history and physiological role that requires a balance in investing in ornamentation and in reproduction. The deposition of carotenoids for ornamentation may trade-off with the deposition of carotenoids for immunologic responses and other physiological functions (Rosenthal et al., 2012). Females, whose physiological requirements for reproduction are larger than males' (e.g., egg laying and investing nutrients into eggs), may need to allocate a larger amount of physiological resources to reproduction and, therefore, may not invest as many resources, such as carotenoids, in ornamentation. Investing more carotenoids into the eggs means generating higher quality eggs and thus offspring with more chances of survival (Blount et al. 2002a). Besides ornament pigmentation, carotenoids also work as antioxidants and immunostimulants (Peters et al., 2004; Weaver et al., 2018) and, thus, as females redirect carotenoids towards reproduction, they might be more prone to have less immunologic defences and be more easily affected by changes in the environment. Summing up, because of reproduction, and if the resources are limited, females may invest less in ornaments than males and may be less capable of maintaining the colour ornamentation stable.

Conversely, males may invest a higher amount of resources in ornamentation in order to attract females and thus ensure reproductive success, without being limited by the extra physiological demands of reproduction that females have. Herewith, and confirming my hypothesis that males wouldn't be as much affected as females by changes in temperature, the temperature manipulation I did had almost no effect on them. Comparing my study to other studies, Moreno et al. (2019) concluded that male and female pied flycatchers are differently affected by climate as well, with males not being so strongly affected by cold and rainy conditions during breeding season as females, because of the different efforts that males and females put in breeding. Similar to this, although in a different context, Kelly et al. (2012) also showed a sex-specific effect on carotenoid-based ornaments of American goldfinches, where female plumage and bill colour correlated with aspects of immune function and metabolic rate while males' poorly related to those functions. However, in the wild, waxbill males in worse body condition show a less saturated red bill (Marques et al., 2016), which consequently make them

more subject to adverse environmental conditions. Plus, Eraud et al. (2007) showed that zebra finch males exposed to cold temperatures displayed less red bills.

Interpreting results in greater detail, bill colour saturation of the different individuals, whether they were males or females, fluctuated over time, similar to what happened in the experiment of Funghi et al. (2018). Also, as expected (Cardoso et al., 2014b), males showed, on average, a higher bill colour saturation when compared to females (see Figures 5b and 5c). This is supported by the GLMM that showed that bill colour saturation differed significantly over time and between sexes. Regarding the temperature treatments, they did not have an effect on the mean colour saturation of the bill of both sexes, neither using all the 4 weeks of the two parts of the experiment nor the last two weeks of each 4-week period of the experiment. Although the interval time for changing bill colour should have been sufficient in the first situation, because bill colour in waxbills (Cardoso et al., 2014b; Funghi et al., 2018) and also in other species (Rosen & Tarvin, 2006; Rosenthal et al., 2012) can change rapidly (in a few days or even in a matter of hours) in response to short-term condition changes, I wanted to make sure that the time for birds to adapt to the new environmental conditions wouldn't affect the final result. Therefore, I used only the last two weeks of each part of the experiment and discarded the first two weeks, in case they were still adjusting bill colour to the new temperature regime in those first two weeks. In fact, considering this period of time (last two weeks of each temperature manipulation), there was a result closer to what I expected; the change in bill saturation of males did not differ between the two treatments and in females bill saturation increased considerably from the cold to the hot treatment, but this result was still non-significant.

Besides, females' and males' bill colour saturation did not covary with measured temperature fluctuations, contrary to what happened in Funghi et al. (2018), in which females' bill colour did covary significantly with temperature. One explanation for this discrepancy of results may be the time of the year in which the experiments were made. The time of the year may influence the way females invest in bill colouration since my experiment was made in December-February and the previous study in March-September, which corresponds to the breeding season (in the Iberian Peninsula it starts in early spring and extends until autumn; Sanz-Aguilar et al., 2015). In some bird species, males invest more in ornamentation in the breeding season, augmenting it or modifying it, in order to increase the reproductive success by being more conspicuous and, this way, competing with other individuals (Badyaev & Duckworth, 2003; Horrocks, Perrins, & Charmantier, 2009; Ivankina, Kerimov, Grinkov, & Bushuev, 2007). The same way, females may also invest more in ornaments (Clutton-Brock, 2009; Cornwallis & Birkhead,

2007), and perhaps that is why female colour augmentation was related to measured temperatures in Funghi et al. (2018) but not in my experiment.

The effectiveness of the temperature manipulation in my experiment is supported by the behavioural effects that temperature manipulation had on the birds (both in feeding rate and movement index). Small flying birds generally have few energetic reserves in the form of fat tissue and, therefore, "decrease in weight between the time they stop feeding in the evening and begin feeding in the morning and gain in weight during the daytime" (Kendeigh, 1969, p. 445). That is why the morning feeding is so important and so were the feeding observations from videos made early in the morning. This way, I could test if the feeding rate actually decreases with the increase of temperature, because of the decrease of energetic stress, or not. Results showed it does. The individuals in the heated environment were observed less at the feeders than in the cold environment. As I mentioned above, this result that feeding rate would decrease with an increase of temperature or increase with a decrease of temperature was expected because waxbills, and small birds in general, need to feed regularly to stay warm in cold environments. Cold is an important energetic stressor for these birds and especially in the nights they need to have energetic reserves to survive the fasting and the low temperatures (Polo & Bautista, 2006). Other environmental constraints beside temperature were demonstrated by Hill (2000), in which restricted food access reduced the expression of carotenoid-based plumage colouration and Hill et al. (2009), in which low doses of carotenoids, restricted food access and pulsed protection from parasites restricted the expression of carotenoid-based bill colouration.

Regarding the movement index, it also decreased with experimentally increased temperature. It could be expected that in a colder environment the birds would be closer to each other and move less so as to not lose much heat or energy, but they moved more than in a warmer environment. This may have happened because the birds need to feed more in colder environments and so they fly more to and from the feeders. Birds usually feed at two crucial times of the day: after sunrise and before sunset (bimodal routine; Bednekoff & Houston, 1994; Macleod, Barnett, Clark, & Cresswell, 2005; Mcnamara, Houston, & Lima, 1994) and thus, as the movement videos were made at sunset, it makes sense that if the birds eat more they also move more. Another hypothesis for this to have happened is the *ad libitum* water and food in the cage; as the birds don't need to be cautious about having food supplies, they don't need to worry about moving more and wasting energy, because they have everything they need at any time. Within bird's behaviour, although both rates (feeding rate and movement index) differed between treatments (cold-hot and hot-cold), only feeding rate covaried positively with temperature

measured in the previous 1 to 6 days and nights. Birds' movement did not have a correlation with ambient temperature.

Finally, the changes in body weight of the birds were also compared, but there were no significant differences between treatments. Even though the birds in cold temperatures were under bigger energetic stress than in hot temperatures, it is possible that the *ad libitum* food during the experiment prevented differences in body weight from developing.

Summing up, the main result I obtained was not what I had predicted based on previous work (a sex-specific increase in female bill colour saturation in warmer temperatures), but it was a different kind of sex-specific effect of temperature on female bill colour. Experimentally increasing temperature in winter was associated with reduced fluctuations of bill colour saturation in females. As the bill is a living tissue and its colour may be a condition-dependent signal (Eraud et al., 2007; Rosenthal et al., 2012), the oscillations that were observed in bill colour are expected to occur, for example, if conditions change or if individuals are not able to sustain the same level of investment in colour over time. Environmental stress, like cold temperatures, causes oxidative stress (Blagojevic, Grubor-Lajsic, & Spasic, 2011; Cong et al., 2018). Therefore, carotenoids might be needed to regulate the antioxidant machinery (Krinsky, 2001). Besides antioxidant functions, carotenoids could be beneficial for other physiological functions, as immune defence (Subba Rao & Glick, 1977). So, the trade-off of carotenoids enables the birds to retrieve carotenoids from the bill and replace them into the immune system or into the antioxidant machinery when there is a need. This way, bill colour fluctuations may be symptomatic of instability in the condition of the birds, or inability to sustain the same level of investment in bill colour. Stabilization of bill colour in experimentally elevated temperatures, therefore, suggests that the birds don't get into physiological deficit and thus don't need to take carotenoids from the bill, being able to maintain its saturation level.

Conclusion

Bill colour in the common waxbill was recently discovered to be a social ornament that presents plastic sexual dichromatism (Cardoso et al., 2014b; Funghi et al., 2018). Although female bill colour was suggested to be more dependent of environmental conditions than males in the common waxbill (Funghi et al., 2018), so far, experimental work testing this hypothesis was missing. This way, my work came to fill this gap. I demonstrated that the experimental warming of winter temperatures, instead of increasing female bill colour saturation, was associated with reducing oscillations in female bill colour saturation. This lets me conclude that alleviating the thermal stress of cold winter temperatures enabled females to have a better ability for sustained investment in bill colour, as opposed to having to readjust investment through time. With this experiment I also documented a sex-specific effect on bill colour, since the temperature treatment affected the stability of bill colour saturation in females but not in males. This confirmed that female bill colour is more sensitive to environmental conditions than male bill colour albeit, at least during winter and in our experimental conditions, in a different manner than previously hypothesised by Funghi et al. (2018). Too cold to shine? Yes, perhaps; at least to shine constantly.

References

Aguilera, E., & Amat, J. A. (2007). Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (Carduelis chloris). *Naturwissenschaften*, *94*, 895–902.

Badyaev, A. V., & Duckworth, R. A. (2003). Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. *Journal of Evolutionary Biology*, *16*, 1065–1076.

Baeta, R., Faivre, B., Motreuil, S., Gaillard, M., & Moreau, J. (2008). Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (Turdus merula). *Proceedings of the Royal Society B*, *275*, 427–434.

Bednekoff, P. A., & Houston, A. I. (1994). Avian daily foraging patterns : effects of digestive constraints and variability. *Evolutionary Ecology*, *8*, 36–52.

Biard, C., Surai, P. F., & Møller, A. P. (2005). Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia*, *144*, 32–44.

Blagojevic, D. P., Grubor-Lajsic, G. N., & Spasic, M. B. (2011). Cold defence responses: the role of oxidative stress. *Frontiers in Bioscience*, *3*, 416–427.

Blahová, J., Dobšíková, R., Straková, E., & Suchý, P. (2007). Effect of low environmental temperature on performance and blood system in broiler chickens (Gallus domesticus). *Acta Veterinaria Brno*, *76*, S17–S23.

Blount, J. D., Houston, D. C., & Moller, A. P. (2000). Why egg yolk is yellow. *Trends in Ecology and Evolution*, *15*, 47–49.

Blount, J. D., Houston, D. C., Surai, P. F., & Møller, A. P. (2004). Egg-laying capacity is limited by carotenoid pigment availability in wild gulls Larus fuscus. *Proceedings of the Royal Society B: Biological Sciences*, *271*, S79–S81.

Blount, J. D., Surai, P. F., Nager, R. G., Houston, D. C., Møller, A. P., Trewby, M. L., & Kennedy, M. W. (2002a). Carotenoids and egg quality in the lesser black-backed gull Larus fuscus: A supplemental feeding study of maternal effects. *Proceedings of the Royal Society B: Biological Sciences*, *269*, 29–36.

Blount, J. D., Surai, P. F., Houston, D. C., & Moller, A. P. (2002b). Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. *Functional Ecology*, *16*, 445–453.

Boutin, S., & Lane, J. E. (2014). Climate change and mammals : evolutionary versus plastic responses. *Evolutionary Applications*, *7*, 29–41.

Brawner, W. R., Hill, G. E., & Sundermann, C. A. (2000). Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *The Auk*, *117*, 952–963.

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, *9*, 1551–1558.

Brush, A. H. (1990). Metabolism of carotenoid pigments in birds. *The FASEB Journal*, *4*, 2969–2977.

Brush, A. H., & Power, D. M. (1976). House finch pigmentation: carotenoid metabolism and the effect of diet. *The Auk*, *93*, 725–739.

Cardoso, G. C., Batalha, H. R., Reis, S., & Lopes, R. J. (2014a). Increasing sexual ornamentation during a biological invasion. *Behavioral Ecology*, *25*, 916–923.

Cardoso, G. C., Leitão, A. V., Funghi, C., Batalha, H. R., Lopes, R. J., & Mota, P. G. (2014b). Similar preferences for ornamentation in opposite- and same-sex choice experiments. *Journal of Evolutionary Biology*, *27*, 2798–2806.

Cardoso, G. C., & Gomes, A. C. R. (2015). Using reflectance ratios to study animal coloration. *Evolutionary Biology*, *48*, 387–394.

Cardoso, G. C., & Reino, L. (2018). Ecologically benign invasions: the invasion and adaptation of common waxbills (Estrilda astrild) in Iberia. In *Histories of Bioinvasions in the Mediterranean* (pp. 149–169). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-74986-0_7

Charmantier, A., & Gienapp, P. (2014). Climate change and timing of avian breeding and migration: evolutionary versus plastic changes. *Evolutionary Applications*, *7*, 15–28.

Chew, B. P., & Park, J. S. (2004). Carotenoid action on the immune response. *Journal of Nutrition*, *134*, 257S-261S.

Clement, P., Harris, A., & Davies, J. (1993). *Finches and sparrows: an identification guide*. Princeton: Princeton University Press.

Clutton-Brock, T. (2007). Sexual selection in males and females. *Science Magazine*, *318*, 1882–1886.

Clutton-Brock, T. (2009). Sexual selection in females. *Animal Behaviour*, *77*, 3–11.

Cong, P., Liu, Y., Liu, N., Zhang, Y., Tong, C., Shi, L., Liu, X., Shi, X., Liu, Y., Tong, Z., & Hou, M. (2018). Cold exposure induced oxidative stress and apoptosis in the myocardium by inhibiting the Nrf2-Keap1 signaling pathway. *BMC Cardiovascular Disorders*, *18*, 1–8.

Cornwallis, C. K., & Birkhead, T. R. (2007). Experimental evidence that female ornamentation increases the acquisition of sperm and signals fecundity. *Proceedings of the Royal Society B*, *274*, 583–590.

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. London: Jon Murray.

Dorado-Correa, A. M., Zollinger, S. A., & Brumm, H. (2018). Vocal plasticity in mallards: multiple signal changes in noise and the evolution of the Lombard effect in birds. *Journal of Avian Biology*, *49*, 1–9.

Dunn, P. O., Garvin, J. C., Whittingham, L. A., Freeman-Gallant, C. R., & Hasselquist, D. (2010). Carotenoid and melanin-based ornaments signal similar aspects of male quality in two populations of the common yellowthroat. *Functional Ecology*, *24*, 149–158.

Endler, J. A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes*, *9*, 173–190.

Eraud, C., Devevey, G., Gaillard, M., Prost, J., Sorci, G., & Faivre, B. (2007). Environmental stress affects the expression of a carotenoid-based sexual trait in male zebra finches. *Journal of Experimental Biology*, *210*, 3571–3578.

Ewen, J. G., Thorogood, R., Brekke, P., Cassey, P., Karadas, F., & Armstrong, D. P. (2009). Maternally invested carotenoids compensate costly ectoparasitism in the hihi. *Proceedings of the National Academy of Sciences*, *106*, 12798–12802.

Funghi, C., Trigo, S., Gomes, A. C. R., Soares, M. C., & Cardoso, G. C. (2018). Release from ecological constraint erases sex difference in social ornamentation. *Behavioral Ecology and Sociobiology*, *72*, 67.

Grzędzicka, E., & Kubacka, J. (2018). Ambient temperature impacts the effect of experimental immunisation of Great Tit and Eurasian Blue Tit females on the PHA response of their offspring. *Journal of Ornithology*, *159*, 761–770.

Hare, R. M., & Simmons, L. W. (2019). Sexual selection and its evolutionary consequences in female animals. *Biological Reviews*, *94*, 929–956.

Hill, G. E. (2000). Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology*, *31*, 559–566.

Hill, G. E. (2006). Female mate choice for ornamental coloration. In *Bird Coloration, Volume 2: Function and Evolution* (pp. 137–200). Cambridge: Harvard University Press.

Hill, G. E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecology Letters*, *14*, 625–634.

Hill, G. E., Hood, W. R., & Huggins, K. (2009). A multifactorial test of the effects of carotenoid access, food intake and parasite load on the production of ornamental feathers and bill coloration in American goldfinches. *The Journal of Experimental Biology*, *212*, 1225–1233.

Hill, G. E., Inouyet, C. Y., & Montgomerie, R. (2002). Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society B: Biological Sciences*, *269*, 1119–1124.

Hill, G. E., & Johnson, J. D. (2012). The vitamin A - redox hypothesis: a biochemical basis for honest signaling via carotenoid pigmentation. *The American Naturalist*, *180*, E127–E150.

Hipfner, J. M., Dale, J., & McGraw, K. J. (2010). Yolk carotenoids and stable isotopes reveal links among environment, foraging behavior and seabird breeding success. *Oecologia*, *163*, 351–360.

Horrocks, N., Perrins, C., & Charmantier, A. (2009). Seasonal changes in male and female bill knob size in the mute swan Cygnus olor. *Journal of Avian Biology*, *40*, 511–519.

Immelmann, K., Steinbacher, J., & Wolters, H. E. (1965). *Vögel in Käfig und Voliere - Ein Handbuch für Vogelliebhaber: Prachtfinken, Vol 1: Astrilde*. Aachen: Hans Limberg.

Ivankina, E. V., Kerimov, A. B., Grinkov, V. G., & Bushuev, A. V. (2007). Structural and functional aspects of variation of the breeding plumage ornamentation in the male pied flycatcher Ficedula hypoleuca (Aves: Passeriformes). *Zhurnal Obshchei Biologii*, *68*, 278–295.

Karubian, J., Lindsay, W. R., Schwabl, H., & Webster, M. S. (2011). Bill coloration, a flexible signal in a tropical passerine bird, is regulated by social environment and androgens. *Animal Behaviour*, *81*, 795–800.

Kelly, R. J., Murphy, T. G., Tarvin, K. A., & Burness, G. (2012). Carotenoid-based ornaments of female and male American goldfinches (Spinus tristis) show sex-specific correlations with immune function and metabolic rate. *Physiological and Biochemical Zoology*, *85*, 348–363.

Kendeigh, S. C. (1969). Energy responses of birds to their thermal environments. *The Wilson Bulletin*, *81*, 441–449.

Koch, R. E., McGraw, K. J., & Hill, G. E. (2016). Effects of diet on plumage coloration and carotenoid deposition in red and yellow domestic canaries (Serinus canaria). *The Wilson Journal of Ornithology*, *128*, 328–333.

Krinsky, N. I. (2001). Carotenoids as antioxidants. *Nutrition*, *17*, 815–817.

Laczi, M., Hegyi, G., Nagy, G., Pongrácz, R., & Török, J. (2019). Yellow plumage colour of great tits Parus major correlates with changing temperature and precipitation. *IBIS - International Journal of Avian Science*. https://doi.org/10.1111/ibi.12761

Lyon, B. E., & Montgomerie, R. (2012). Sexual selection is a form of social selection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*, 2266–2273.

Macleod, R., Barnett, P., Clark, J. . A., & Cresswell, W. (2005). Body mass change strategies in blackbirds Turdus merula: the starvation-predation risk trade-off. *Journal of Animal Ecology*, *74*, 292–302.

Marques, C. I. J., Batalha, H. R., & Cardoso, G. C. (2016). Signalling with a cryptic trait: the regularity of barred plumage in common waxbills. *Royal Society Open Science*, *3*, 1–9.

McGraw, K. J. (2004). Winter plumage coloration in male American goldfinches: do reduced ornaments serve signaling functions in the non-breeding season? *Ethology*, *110*, 707–715.

McGraw, K. J., & Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *The American Naturalist*, *162*, 704–712.

McGraw, K. J., Nolan, P. M., & Crino, O. L. (2006). Carotenoid accumulation strategies for becoming a colourful house finch: analyses of plasma and liver pigments in wild moulting birds. *Functional Ecology*, *20*, 678–688.

McGraw, K. J., & Schuetz, J. G. (2004). The evolution of carotenoid coloration in estrildid finches: a biochemical analysis. *Comparative Biochemistry and Physiology*, *139*, 45–51.

Mcnamara, J. M., Houston, A. I., & Lima, S. L. (1994). Foraging routines of small birds in winter: a theoretical investigation. *Journal of Avian Biology*, *25*, 287–302.

Meijer, T., Nienaber, U., Langer, U., & Trillmich, F. (1999). Temperature and timing of egg-laying of European starlings. *The Condor*, *101*, 124–132.

Moller, A. P., Biard, C., Bount, J. D., Houston, D. C., Ninni, P., Saino, N., & Surai, P. F. (2000). Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian and Poultry Biology Reviews*, *11*, 137–159.

Montgomerie, R. (2006). Analyzing colors. In *Bird Coloration, Volume 1: Mechanisms and measurements* (pp. 90–147). Cambridge: Harvard University Press.

Moreno, J., Cantarero, A., Plaza, M., & López-Arrabé, J. (2019). Phenotypic plasticity in breeding plumage signals in both sexes of a migratory bird: responses to breeding conditions. *Journal of Avian Biology*. https://doi.org/10.1111/jav.01855

Navara, K. J., & Hill, G. E. (2003). Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behavioral Ecology*, *14*, 909–916.

Nicolai, J., Steinbacher, J., van den Elzen, R., Hofmann, G., & Mettke-Hofmann, C. (2007). *Prachtfinken Afrika - Handbuch der Vogelpflege*. Stuttgart: Eugen Ulmer.

Nord, A., & Folkow, L. P. (2018). Seasonal variation in the thermal responses to changing environmental temperature in the world's northernmost land bird. *Journal of Experimental Biology*, *221*, 1–10.

Observatório Astronómico de Lisboa. (2014). *Nascimento e Ocaso do Sol (Porto).* Obtained from http://oal.ul.pt

Pendlebury, C. J., MacLeod, M. G., & Bryant, D. M. (2004). Variation in temperature increases the cost of living in birds. *The Journal of Experimental Biology*, *207*, 2065–2070.

Peters, A., Denk, A. G., Delhey, K., & Kempenaers, B. (2004). Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *Journal of Evolutionary Biology*, *17*, 1111–1120.

Pike, T. W., Blount, J. D., Bjerkeng, B., Lindström, J., & Metcalfe, N. B. (2007). Carotenoids, oxidative stress and female mating preference for longer lived males. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 1591–1596.

Polo, V., & Bautista, L. M. (2006). Daily routines of body mass gain in birds: 1. An exponential model. *Animal Behaviour*, *72*, 503–516.

Reino, L. (2005). Variation partitioning for range expansion of an introduced species: The common waxbill Estrilda astrild in Portugal. *Journal of Ornithology*, *146*, 377–382.

Reino, L., & Silva, T. (1998). The distribution and expansion of the common waxbill (Estrilda astrild) in the Iberian Peninsula. *Biological Conservation Fauna*, *102*, 163–167.

Rosen, R. F., & Tarvin, K. A. (2006). Sexual signals of the male American goldfinch. *Ethology*, *112*, 1008–1019.

Rosenthal, M. F., Murphy, T. G., Darling, N., & Tarvin, K. A. (2012). Ornamental bill color rapidly signals changing condition. *Journal of Avian Biology*, *43*, 553–564.

Saino, N., Ferrari, R., Romano, M., Martinelli, R., & Møller, A. P. (2003). Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 2485–2489.

Salvante, K. G., Walzem, R. L., & Williams, T. D. (2007). What comes first, the zebra finch or the egg: temperature-dependent reproductive, physiological and behavioural plasticity in egg-laying zebra finches. *The Journal of Experimental Biology*, *210*, 1325–1334.

Sanz-Aguilar, A., Carrete, M., Edelaar, P., Potti, J., & Tella, J. L. (2015). The empty temporal niche: breeding phenology differs between coexisting native and invasive birds. *Biological Invasions*, *17*, 3275–3288.

Schaper, S. V., Dawson, A., Sharp, P. J., Gienapp, P., Caro, S. P., & Visser, M. E. (2012). Increasing temperature, not mean temperature, is a cue for avian timing of reproduction. *The American Naturalist*, *179*, E55–E69.

Silva, T., Reino, L. M., & Borralho, R. (2002). A model for range expansion of an introduced species: the common waxbill Estrilda astrild in Portugal. *Diversity and Distributions*, *8*, 319–326.

Stiels, D., Schidelko, K., Engler, J. O., van den Elzen, R., & Rödder, D. (2011). Predicting the potential distribution of the invasive common waxbill Estrilda astrild (Passeriformes: Estrildidae). *Journal of Ornithology*, *152*, 769–780.

Subba Rao, D. S. V., & Glick, B. (1977). Effect of cold exposure on the immune response of chickens. *Poultry Science*, *56*, 992–996.

Sullivan, M. J. P., Davies, R. G., Reino, L., & Franco, A. M. A. (2012). Using dispersal information to model the species-environment relationship of spreading non-native species. *Methods in Ecology and Evolution*, *3*, 870–879.

Sultan, S. E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, *5*, 537–542.

Thompson, C. W., Hillgarth, N., Leu, M., & McClure, H. E. (1997). High parasite load in house finches (Carpodacus mexicanus) is correlated with reduced expression of a sexually selected trait. *The American Naturalist*, *149*, 270–294.

Tobias, J. A., Montgomerie, R., & Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B*, *367*, 2274–2293.

Visser, M. E., Holleman, L. J. M., & Caro, S. P. (2009). Temperature has a causal effect on avian timing of reproduction. *Proceedings of the Royal Society B*, *276*, 2323–2331.

Weaver, R. J., Santos, E. S. A., Tucker, A. M., Wilson, A. E., & Hill, G. E. (2018). Carotenoid metabolism strengthens the link between feather coloration and individual quality. *Nature Communications*, *9*, 73.

Appendix 1

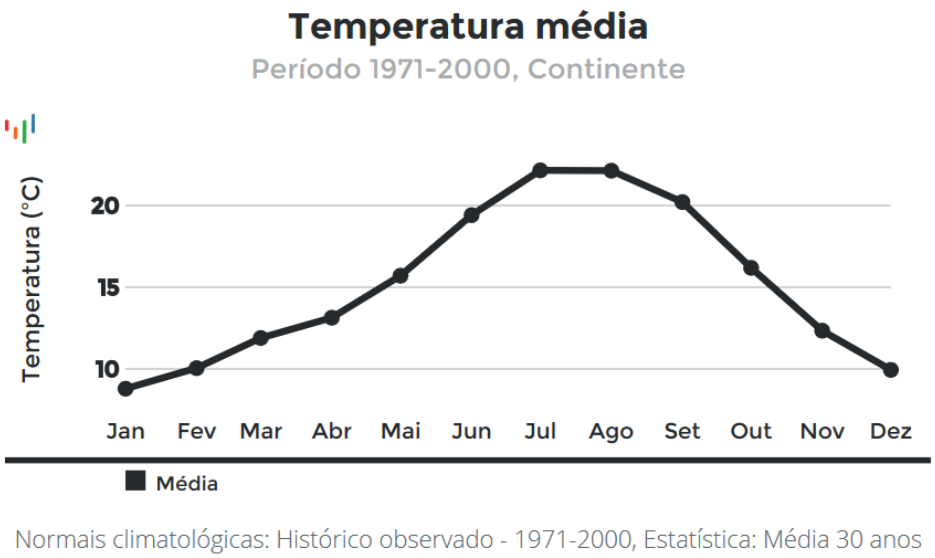

Fig. A1 - Graphic with climatic data from IPMA (Portuguese Institute for Sea and Atmosphere): mean temperature over the months during the period 1971-2000, which gives us December, January and February as the coldest months. Obtained from http://portaldoclima.pt/pt/.