

# Coping with the heat

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Effects of simulated heatwaves on behaviour,  
maintenance and reproduction  
in the polymorphic Gouldian finch

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*Para o Xico e a Xica  
com saudades...*



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## Abstract

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For decades, evolutionary biologists have sought to understand the evolution of individual behaviour, physiology and ecology allowing organisms to cope to environmental change. One of the main challenges of current climate change is the unprecedented rate of temperature increase, as well as the increased occurrence of extreme heat events. Inter-individual response variability opens a whole new area of opportunities to understand how individual phenotypic traits are linked to individual response differences. In colour polymorphic species, colour honestly reflects an individual's life-history strategy, and each morph may, therefore, represent an alternative life-history strategy. As such, colour polymorphic species, such as the Gouldian finch (*Erythrura gouldiae*), may be good models to assess how different strategies between morphs are linked to their respective responses to environmental variations. However, polymorphic species have mainly been disregarded for that purpose. In this context, the main aim of this thesis was to understand how the two morphs of the Gouldian finch respond through phenotypic plasticity to simulated heatwaves reaching thermocritical temperatures, and whether such differential responses may help to identify a 'winner' and a 'loser' morph in the light of climate change. To address these issues, we used an integrative approach including measurements of behavioural (**Study 1**), physiological (**Study 2**), and reproductive (**Study 3**) parameters. The novelty of our approach was to assess the immediate behavioural and physiological response variation of individuals of the two morphs longitudinally across different thermal conditions, as well as the postponed effects of this thermocritical heatwave exposure on their reproductive performance. In this study, although the behavioural responses generally did not differ between morphs or according to temperature intensity, the physiological and reproductive parameters differed in response to morph and temperature intensity. Black-headed females, in particular, seem highly sensitive to thermocritical heatwaves, as they exhibited decreased body mass and increased oxidative damage during the thermocritical heatwaves, and advanced breeding initiation after these conditions, whereas these variables remained mostly unaffected in black-headed males and red-headed individuals. However, despite some response differences between morphs, both invested similarly in reproduction following intense heatwaves, and the offspring of both morphs were similarly affected. Based on these results, no morph therefore seems to appear more disadvantaged than the other following an intense heatwave, and red- and black-headed Gouldian finches may both be considered as climate stress 'losers'.

# PART I

## Introduction

## Climate change as a new selection pressure

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Climate affects several biological systems in plants and animals, from the supply of energy, the diversity and distribution of species, to the activity of organisms (Erwin, 2009). Climatic variation includes daily and seasonal cycles, extreme annual changes such as ‘El Niño’, as well as long-term changes such as ‘the Pleistocene glaciations’ (deMenocal, 2004). All these climatic variations create punctual selective pressures on individuals’ phenotype, such as on morphological (e.g. colour patterns, body sizes), physiological (e.g. homeostasis change, mass balance), and behavioural (foraging and breeding alterations) traits (Parmesan et al., 2000; Moreno & Møller, 2011). Such phenotypic changes may affect life-history traits, individual survival and *in fine* alter population structure and dynamics. A recent study revealed that 7% of the animal population decline can be associated to current climate change (Monastersky, 2014). One aspect of climate change is the rate of temperature increase. In contrast to previous climate variations, Earth’s mean temperature has drastically risen since the mid-19th century, mainly as the consequence of significant increases of atmospheric CO<sub>2</sub> (+ 40%) and methane levels (+ 150%) since the industrial revolution in 1750 (IPCC, 2013). The main challenges of current temperature increase for animals are threefold : (i) its rapid intensity due to an increase in the mean of climatic variables (e.g. temperature), (ii) the duration and frequency of episodes of extreme climate events (e.g. heatwaves) due to a change of the variance of climatic variables (IPCC, 2013; Meehl et al., 2000), and (iii) the low predictability of climatic events due to the rapid rate of environment variability. All these challenges may lead to a decrease in the capacity to recover and successfully acclimate following these changes. The species more likely to persist are those for which individuals will successfully cope with these challenges. Most studies which assess the effects of temperature increase on organisms have focused primarily on the consequences of high temperatures while neglecting extreme heat events (Altwegg et al., 2017; Solow, 2017).

Extreme climatic events are rare by definition but are among the most prominent consequences of climate change (Van de Pol et al., 2017). The simultaneous occurrence of climate extremes on both sides of the Earth in early 2019 was a perfect example of opposing effects of extreme events: the polar coldwave in Chicago with temperatures dropping down to -40°C led to costs estimated at 5 billion dollars to the US economy (Roach, 2019), whereas the heatwave in Australia with temperatures reaching +49°C caused sudden mass mortality among

various animal populations, such as in flying foxes (*Pteropus sp.*; Akst, 2019; Nguyen and McKinnell, 2019). Extreme heat events can be defined in many different ways (see Table 1 of Van de Pol et al., 2017), but they are most commonly defined as a prolonged period of temperature exceeding the mean temperature predicted at that time by ecological studies (Smith, 2011; Ummenhofer and Meehl, 2017). Consequently, long-lasting extreme heat events may extend the period of coping by causing organisms to shift into an ‘emergency life-history stage’ where energy is mainly allocated to self-maintenance to promote individuals’ direct survival, which at the same time decreases their opportunities for foraging and breeding (Wingfield, 2003; Wingfield et al., 2011). Furthermore, higher frequency of extreme events decreases potential recovery periods, affecting response performance and adaptation success (Ma et al., 2017). Finally, the additional energetic demands under higher temperatures (e.g. due to the activation of thermoregulation processes; duPlessis et al., 2012; McKechnie and Wolf, 2010)) can be a major burden and lead to rapid mortality for species with a narrow thermoneutral range<sup>1</sup> and/or already living close to their upper thermal tolerance (e.g. tropical species, (Gardner et al., 2017; Reside et al., 2015; Şekercioğlu et al., 2012a). Thus, higher energy allocated to self-maintenance (i.e. survival and thermoregulatory processes) under such extreme events reduces energy allocation to reproduction, and so the impact on total fitness is the result of such trade-offs. That is why the higher occurrence of extreme heat events may lead to increased frequency of reproductive failure and reduction of survival rates, and is therefore expected to become one of the main causes of species extinction due to current climate change (Chown et al., 2010; Stenseth et al., 2002), and consequently be partially responsible for the 6<sup>th</sup> mass extinction crisis (Barnosky et al., 2011; Monastersky, 2014). The ecological outcome at the species or populational level will depend on the strength of selective pressures acting on the different individuals forming these species or populations and their respective response capacity to these pressures. Yet, our understanding of individuals’ and species’ responses to extreme heat events is scarce, emphasising the necessity for multidisciplinary research to quantify the vulnerability of biodiversity to heat extremes (Harris et al., 2018; McKechnie et al., 2012).

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<sup>1</sup> Thermoneutrality is the range of temperature where basal metabolic rate (i.e. energy needed for baseline metabolic functions) is constant and minimal (Kingma et al., 1975). Temperatures beyond this thermoneutral range trigger thermoregulatory processes, such as shivering or panting.

## **Intra- and inter-individual response variability**

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The ability of organisms to cope with these extreme events and the constraints that they pose is the basis of natural selection and evolution. To keep up with the novel challenges derived from extreme heat events, individuals have two options: to move (i.e. moving to better ranges of individual tolerance) or to adapt. The first evidence of geographical shifts due to climate change was recorded during the Pleistocene glaciation (Cabanne et al., 2016; Longino et al., 2008; Lyons, 2003). Studies also already showed clear evidence of range expansion and shifts of whole populations under current climate change (e.g. butterflies, moths and birds; Mccarty, 2001; Parmesan et al., 1999; Parmesan et al., 2000). A typical example is the 19-km northward breeding expansion of several bird species in Great Britain over the past 50 years due to current climate change (Thomas and Lennon, 1999). Besides movement and micro-evolutionary adaptation<sup>2</sup> (Chevin et al., 2010), individuals can also display phenotypic plasticity to respond to environmental variations (Stearns, 1989). Intra-individual phenotypic plasticity is ‘the ability of an organism to express different phenotypes depending on the environment’ (Pigliucci, 2001). Organisms can express varying phenotypes throughout their lifecycle and in response to extreme events, such as morphological (e.g. changes in body size, diapause or hibernation; Chevin and Hoffmann, 2017), behavioural (e.g. phenology shifts, changes in habitat use; Funghi et al., 2019; Ma et al., 2019; Marrot et al., 2017) or physiological (e.g. endocrine and metabolic rate changes; Norin and Metcalfe, 2019; Wingfield et al., 2017a) changes. As such, physiological trait regulation, and the associated behavioural response, are key buffers to environmental change, as they allow individuals to respond rapidly to environmental stimuli. Phenotypic plasticity may therefore be referred to as ‘plastic rescue’ under environmental change (Snell-Rood et al., 2018), as it may allow individuals to respond within a much shorter time and to minimise some deleterious effects of rapid environmental change when movement is impossible (e.g. ocean, mountain chains) and evolutionary adaptation is insufficient (Canale et al., 2010). In an extinction risk model based on organismal response with and without plasticity, a recent study has shown that plasticity reduced extinction risk by 72% under climate warming (Riddell et al., 2018). Thus, inter-individual differences in physiology and behaviour may likely explain the response variability (i.e. the survival and coping capacity) to extreme events (see Box A).

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<sup>2</sup> Changes in the genetic composition across generations in response to selection (Pulido & Berthold, 2004)

Parents may not only optimise their own phenotype to environmental conditions but also that of their offspring via transgenerational effects<sup>3</sup> (e.g. variation in parental care, maternal hormone transfer; (Donelson et al., 2018; Salinas and Munch, 2012). Indeed, parents may increase their fitness by adjusting the phenotype of their offspring so that it matches the expected environment (Grootuis and Schwabl, 2008; Hayward and Wingfield, 2004; Uller, 2008) (i.e. anticipatory transgenerational affect, Marshall and Uller, 2007). However, such transgenerational adjustments may be costly, especially when the subsequent environment is not matching the predicted one (Costantini, Monaghan & Metcalfe, 2014). In such heterogenous and unpredictable conditions, the parents may maximise their overall fitness at the expense of their current offsprings' survival and fitness (i.e. selfish transgenerational effect, Marshall and Uller, 2007). In either case, such transgenerational plasticity may be adaptive if the given phenotypic plasticity increases the overall parental fitness and if the trait is selected in several consecutive generations (i.e. adaptive phenotypic plasticity; West-Eberhard, 2003). A typical example is the advancing laying date of birds in response to increasing and advancing spring temperatures (Both and Visser, 2001; Marrot et al., 2017; Marrot et al., 2018).

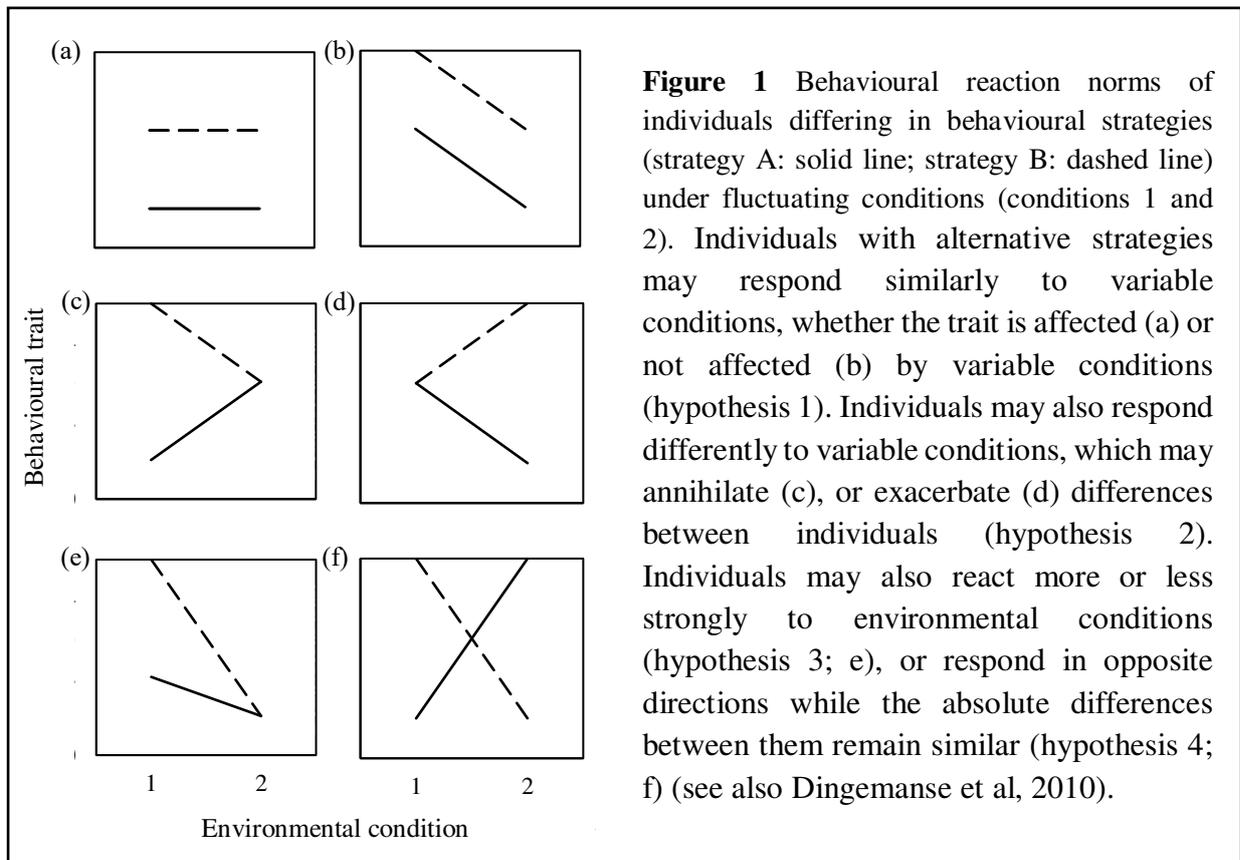
Within species, all individuals are not equally plastic. Some individuals show high plastic responses, while others show little or no plastic responses, resulting in a variety of reaction norms<sup>4</sup> between individuals (Fig. 1). Differences between individuals' reaction norms underlie not only differences in life histories (e.g. differential allocation to self-maintenance, reproduction and growth), but also differences in their behavioural (e.g. foraging and breeding behaviour) and physiological (e.g. metabolic rate) response capacity (Box A). For instance, while some individuals may have better physiological thermoregulatory abilities, others may be able to regulate their temperature through behavioural adjustments (Beaulieu et al., 2015; Wolf, 2000). For instance, during heatwaves in Australia, the mortality of juvenile and female flying foxes following heat exposure was higher than that of males (Welbergen et al., 2008). Such plastic capacity differences may arise, among others, from differences in body condition, age, social status and life-history strategies, and explain why, under extreme events, some individuals enter the 'emergency life-history stage' more rapidly than others. Thus, phenotypic patterns of 'winners' and 'losers' within species may be observable if specific phenotypic traits

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<sup>3</sup> Transgenerational effects are changes in offspring phenotype due solely to parental influence without any change in offspring DNA sequence, and visible in several consecutive generations (Dictionary of biology, 6<sup>th</sup> edition, Oxford University press).

<sup>4</sup> Reaction norms are defined as the expression of a gradient of expressed phenotypes by the same genotype across a range of environmental values (Dictionary of biology, 6<sup>th</sup> edition, Oxford University press).

confer some individuals higher advantages to cope with environmental change than other traits (Hoffmann et al., 2011).

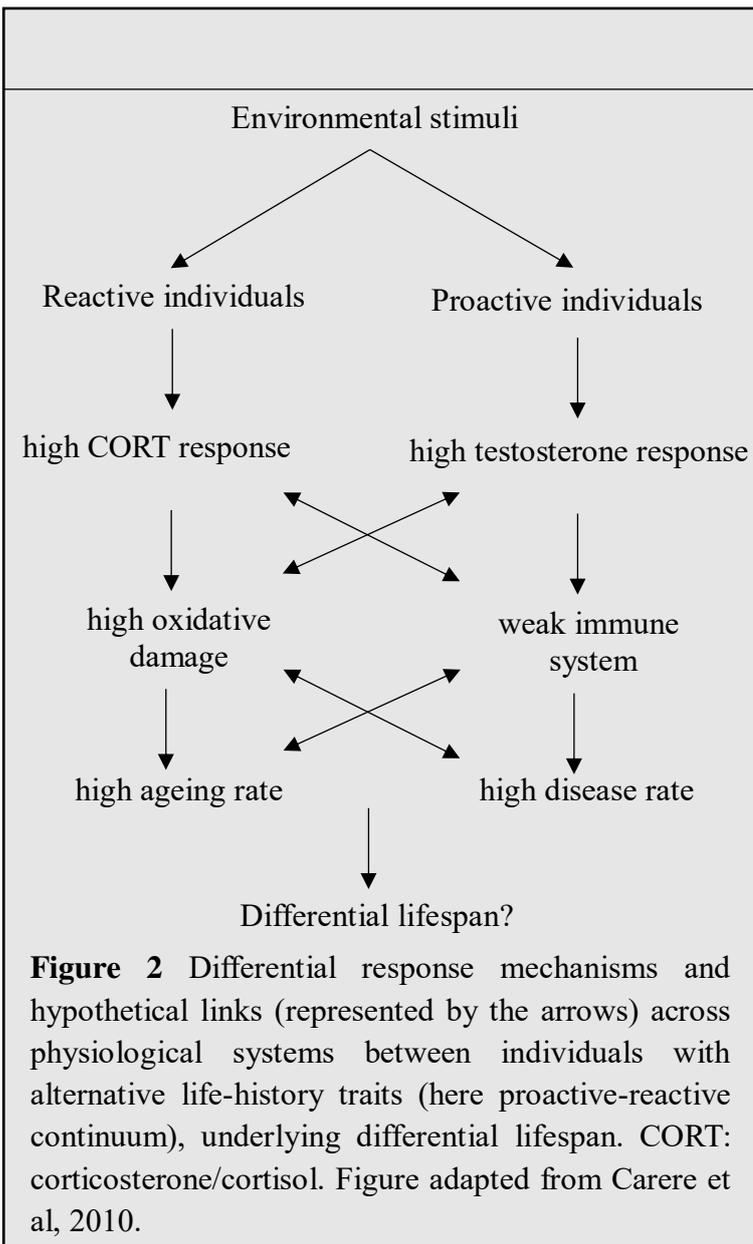


## Box A Pace of life syndromes: Concept linking behaviour, physiology and life history

Some phenotypic traits are better suited to some environments, whereas other traits to other environments. How a certain life-history strategy, and thereby a set of physiological and behavioural traits, confers an adaptive advantage, and under which condition, may help us to understand inter-individual response differences. Several researchers have recognised that behavioural, physiological and life-history traits may have coevolved in response to environmental conditions (see review Dammhahn et al., 2018). The correlations between these three components, referred to as ‘pace-of-life syndrome’, differ between individuals, resulting in a continuum of individual responses to environmental conditions. Despite the strong effect that temperature has on biological systems, thermal physiology and behaviour still needs to be examined within this syndrome framework (Goulet et al., 2017).

For example, in the proactive-reactive continuum, proactive individuals are behaviourally more aggressive, more active, more exploratory and bolder, but invest less into reproductive behaviours, such as courtship and parenting, whereas reactive individuals show the opposite behavioural traits. These behavioural characteristics are correlated with distinct physiological traits, such as steroid hormones (e.g. corticosterone and testosterone) (Arnold et al., 2016; Wingfield and Romero, 2000). As such, proactive individuals show lower corticosterone responses and higher testosterone levels than reactive individuals. High levels of testosterone increase individuals’ reproductive output at the expense of

parental care and survival rates (McGlothlin and Ketterson, 2008; Reed et al., 2006). Moreover, proactive individuals also faster in activating the HPA axis (responsible for the fight/flight response) and in regulating body temperature in response to stimuli (Carere and van Oers, 2004; Carere et al., 2010; Hau et al., 2010). Aside from hormonal differences, individuals along the proactive-reactive continuum also differ in their oxidative profile, either through metabolic rate differences or differences in stress responsiveness (Costantini et al., 2012). Plasmatic oxidative profiles (e.g. antioxidant capacity and oxidative damage) are largely used in ecophysiological studies as key mediators for life-history trade-offs (Costantini, 2008; Metcalfe and Alonso-Alvarez, 2010). Indeed, lower antioxidants and higher oxidative damage may be linked to higher aggression in several animal species, such as mice, lizards and mammals (Costantini et al., 2008; Garratt and Brooks, 2015). Low neophobic greenfinches (*Carduelis chloris*) show higher antioxidant defences and lower damage (Herborn et al., 2011). Similarly, low neophobic and highly exploratory blue tits (*Cyanistes caeruleus*) had low antioxidant defences (Arnold et al., 2015). Furthermore, oxidative profile may also vary between individuals of different social status, as dominant females of the white-browed sparrow weaver (*Plocepasser mahali*) significantly reduced their antioxidant defences after breeding, whereas subordinate females did not vary their antioxidant profile (Cram et al., 2015).



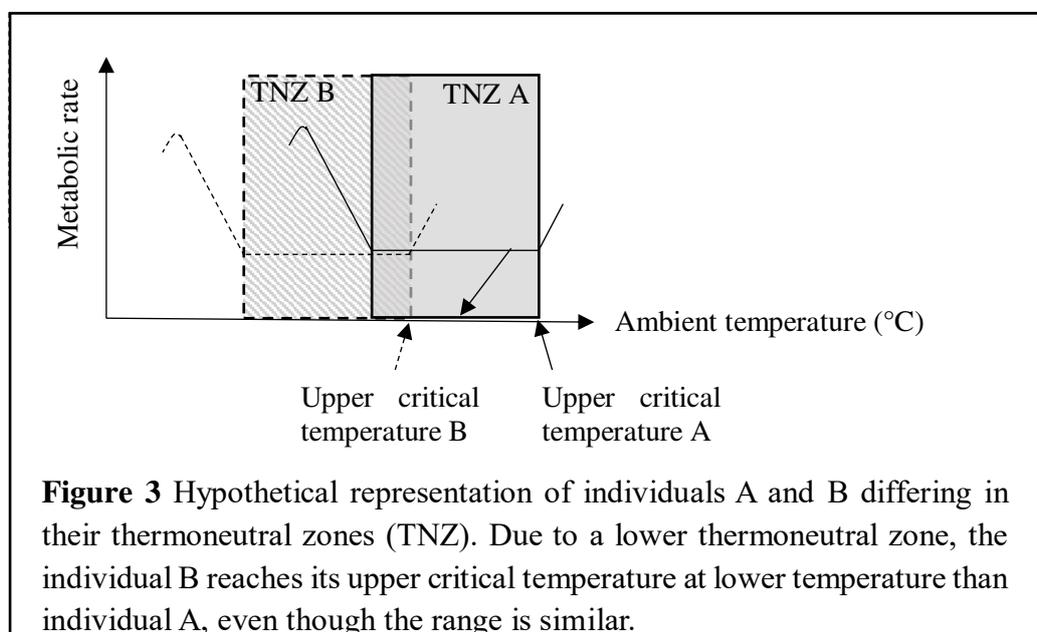
Sometimes, this continuum of reactions within species are visible between individuals differing in colouration. Indeed, due to the function of melanin on hormones (Hill and McGraw, 2006), high-melanic individuals are commonly more aggressive than paler conspecifics, and may therefore better cope with competitive interactions due to invading species that expand their range in northern latitudes and at higher altitudes

(Roulin, 2014; Almasi et al., 2010; Ducrest et al., 2008; Roulin, 2014; Roulin et al., 2008).

The general view is that reactive individuals react to a challenging condition with high 'stress hormones' (i.e. higher steroid hormones, higher oxidative damage), whereas proactive or fast exploratory individuals, that engage fast with challenging conditions show high levels of testosterone levels (Fig. 2). How such response differences affect *in fine* an individual's survival capacity, lifespan and fitness remain to be examined. Knowing which and how specific individual trait patterns are adaptive to certain conditions and another pattern adapted to another environmental condition, may allow to extrapolate how species, or even entire populations, may respond in the light of climate change. To conclude, differences in hormones, oxidative profile, behaviours and life histories between individuals may show higher or reduced sensitivity to environmental change. Such differences in sensitivity may partially explain the response outcome variability within species. Despite their importance, the effects of glucocorticoid, behavioural, or genetic responses to environmental variations remain largely unstudied (see Creel et al., 2013).

## Polymorphism to understand intraspecific response variability

Back in 1859, Darwin had already recognised that individuals within species differ in size, mass or colour, which may reflect their own quality (Wilson and Nussey, 2009), but also in thermoneutral ranges (Fig. 3). Such intraspecific differences may arise due to extrinsic (e.g. early-life environment, social environment) or intrinsic (e.g. genetic, transgenerational effects) factors, leading to a variety of life-history strategies within species. Thus, intraspecific phenotypic variability may result in a variety of maintenance strategies within the same species (Kempnaers et al., 2008; Schwartz and Bronikowski, 2013). To reduce such interindividual physiological differences, animals may differently alter their behaviour. For instance, individuals unable to adjust their physiology to extreme temperatures may still be able to keep maintenance parameters stable by reducing their overall physical activity (Beaulieu et al., 2015a; Wolf, 2000). The importance of high intraspecific variability has significantly increased in evolutionary ecology studies, especially in the light of climate change, as it may allow for broader ecological niche expansion, higher levels of establishment and higher species response capacity (Ducatez et al., 2017; Forsman, 2016; Forsman and Wennersten, 2015; Forsman et al., 2008; Table 1).



In most colour polymorphic species<sup>5</sup>, colour differences directly or indirectly reflect genetic (e.g. differences in melanisation; Emaresi et al., 2014; Roulin, 2004), behavioural (e.g. mating strategies; Tuttle, 2003; Van Rhijn, 1973) and physiological (e.g. metabolic rate or thermoregulation; Dreiss et al., 2016; Küpper et al., 2016; Boyles et al., 2011) differences. One of the most studied examples is the genetically-determined breeding strategies of male ruffs (*Philomachus pugnax*). Male ruffs display a wide range from ornamental plumes from white, rust, brown to black, and each colour morph honestly signals its breeding strategy (Van Rhijn, 1973). In general, most intraspecific colour variation can therefore be understood as information cues from ‘signalers’ to ‘receivers’, e.g. information that honestly reveals their quality, attractiveness, genetic compatibility or breeding strategy (Hill and McGraw, 2006). As a result, individuals of different morphs may display alternative life-history strategies with equal fitness outcomes, one being more adapted under given environmental conditions whereas the other being better adapted under other conditions. The degree to which polymorphism provides ecological and evolutionary advantages in the light of rapid environmental change is still under debate (Barrett and Schluter, 2008; Forsman et al. 2008; Bolton et al. 2015; Forsman 2016; Bolton et al. 2016). On the one hand, polymorphism may confer more advantages under unpredictable and rapid environmental change than monomorphism when higher genetic and phenotypic variation promotes the use of broader niches, leading to better colonisation success and range expansion, and alternative life-history strategies reduce competition risk within populations (Table 1). On the other hand, strong correlations between traits (e.g. in behavioural syndromes; see Box A; Sih et al., 2004) and the importance of social structure for the dynamics of the polymorphic population may make them more vulnerable to any environmental and social variation (Table 1). If individuals of different morphs show similar reaction norms to the same environmental stimuli, such responses are rather environmentally-driven (Fig. 1a-b), whereas if individuals of different morphs show different reaction norms, then these morph-specific responses to the environmental are genetically-driven (Fig. 1c-f). Such response differences between morphs may affect not only morph proportion, but also the overall interaction dynamics within populations, species and habitats. Despite the advantages and disadvantages of colour polymorphism under rapid temperature increase, colour-polymorphic species are quite rare compared to monomorphic species (e.g. 3% in birds; Galeotti et al., 2003).

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<sup>5</sup> Polymorphism is the occurrence of two (or more) distinct genetic forms of a species together in the same habitat, in such a proportion that the rarest morph cannot only be maintained by recurrent mutation (Ford 1940). Polymorphism are either transient, with morph frequencies within the population changing over time, eventually leading to monomorphism (i.e. transient polymorphism), or represent morph frequencies that are stable over time (i.e. balanced or fixed polymorphism) (Ford, 1945).

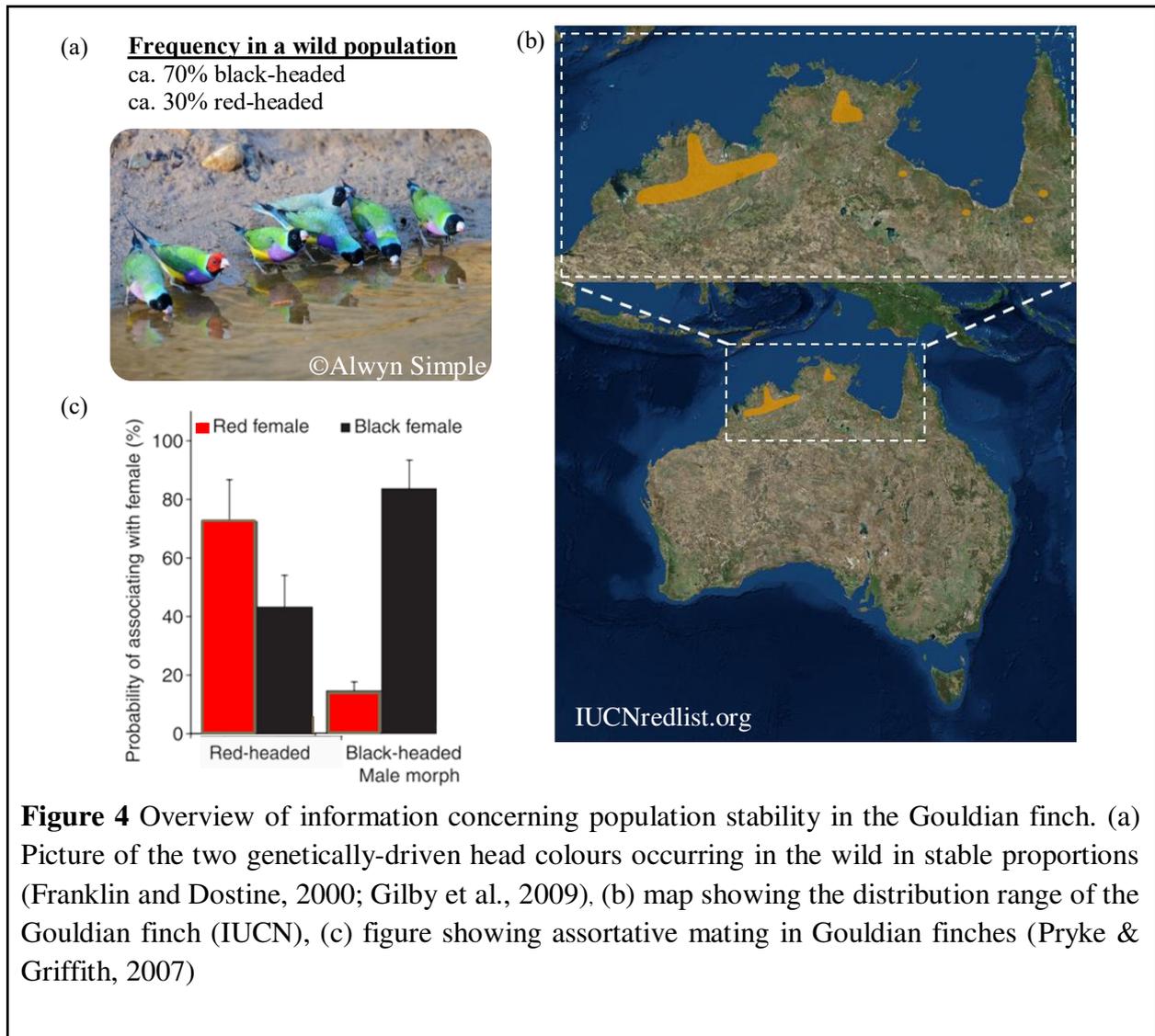
This may be due to the fact that the equilibrium proportion at which colour morphs achieve the same fitness only rarely persists during very long periods of time, and that disruptive selection between morphs can rapidly lead to speciation (Hugall and Stuart-Fox, 2012; Roulin, 2004). For example, within the genus *Erythrura* including approx. 12 species, only the Gouldian finch (*Erythrura gouldiae*) shows distinct, genetically-determined colour morphs. Because (i) the part due to genetically-driven changes and due to phenotypic plasticity are easily distinguishable, (ii) life-history strategy differences between morphs may confer them higher advantages under rapid environmental change and (iii) some polymorphic species may be on the edge of speciation, colour polymorphic species represent ideal objects to study climatic-induced response differences.

**Table 1** Compilation table of morph-specific traits conferring adaptive advantages in polymorphic species. Exp: experimental approach; Obs: observations of wild populations; Phyl comp: Phylogenetic comparison; Theo: theoretical approach; Rev: Review

<b>Advantage of polymorphism for ecological and evolutionary success of species and populations</b>	Pygmy grasshopper <i>Tetrix subulata</i>	Exp	higher establishment success	Forsman et al., 2012; Wennersten et al., 2012
	Fruit flies <i>Drosophila cardini</i> group;	Phyl comp	larger range sizes	Brisson et al., 2006; Roulin and Wink, 2004
	Birds of prey <i>Accipitridae</i> Australian frog taxa	Phyl comp	polymorphism was significantly associated with lower extinction risk	Forsman and Hagman, 2009
	Snakes <i>Serpentes</i>	Phyl comp	polymorphic species are older than monomorphic ones	Pizzato and Dubey, 2012
		Rev	decreased vulnerability to environmental change; increased evolutionary potential; decreased extinction risk; higher exploitation of resources	Forsman et al., 2008
<b>Disadvantage of polymorphism for ecological and evolutionary success of species and populations</b>		Rev	decreased vulnerability to environmental change; increased evolutionary potential; increased population growth rate	Hughes et al., 2008
		Rev	high inter-morph interactions leading to a unique response to environmental changes	Bolton et al., 2015; Bolton et al., 2016
	Viper <i>Vipera berus</i> and <i>Vipera aspis</i>	Obs	polymorphic species have a narrower niche than monomorphic counterparts	Broennimann et al., 2014
	Gouldian finches <i>Erythrura gouldiae</i>	Theo	Trait correlations and density dependence between morphs negatively affect population persistence	Kokko et al., 2014

The Gouldian finch is a known Australian polymorphic species, showing two main distinct head colour morphs in both sexes, the genetically-dominant red- ( $Z^R$ ) and the recessive black-headed ( $Z^r$ ) morph (Fig. 4a; Franklin and Dostine, 2000). However, colour differences between red- and black-headed Gouldian finches occur only over a minor surface of their body (approximately 4%). Thus, the phenotypic differences between the two morphs are unlikely to be directly mediated by thermoregulatory differences due to pigmentation *per se*, but rather due to pleiotropic effects of the sex-linked gene responsible for colour polymorphism. Despite genetic dominance, the red morph is less frequent than the black morph within populations in the wild (30 vs. 70%, respectively; Gilby et al., 2009; Evans and Fidler, 2005). Only recently, a study suggested that colour polymorphism is maintained by the use of alternative life-history strategies (i.e. by balancing selection) in the Gouldian finch (Kim et al., 2018; Kim et al., 2019). Besides, red males are socially dominant over black-headed birds, with red-headed individuals more frequently initiating aggressive interactions (Pryke, 2007; Pryke and Griffith, 2006), and having priority access to high-quality nest and foraging sites (Brazill-Boast et al., 2013). In accordance, red-headed individuals show higher levels of testosterone than black-headed birds under different conditions (Pryke et al., 2007). Furthermore, red-headed individuals show some higher plastic capacity than black-headed individuals, as both red- and black-headed males increase spermatozoa midpiece under high frequency of red males, but only red-headed males vary their spermatozoa length under high frequency of black males (Immler et al., 2010). Moreover, red-headed females preferentially mate with red-headed males, whereas black-headed females do not discriminate between morphs (Pryke and Griffith, 2007). Males of both morphs, however, mate assortatively (Fig. 4c; Pryke and Griffith, 2007). Despite apparent advantages, especially in a sexual selection context, red-headed birds show higher glucocorticoid levels (Pryke et al., 2007) and poorer reproductive outcome in high frequencies of the red morph (i.e. under higher competitive conditions; Pryke and Griffith, 2009; Pryke et al., 2012). In contrast, black-headed birds remain unaffected by competition variation. Finally, Gouldian finches are tropical finches, living exclusively in the North of Australia (Fig. 4b; Evans and Fidler, 2005). Therefore, Gouldian finches are enduring and will have to endure current and future extreme events related to climate warming *in situ*, as they are not migratory birds and are surrounded by physical barriers (the sea in the North and the desert in the South). Similar to many tropical species, their tolerance to thermal variation is very limited (i.e. thermoneutral range is from 31.8°C to 38.0°C; Burton and Weathers, 2003). Consequently, with an estimated increase in temperature of 3-4°C expected in the North of Australia in the future years (IPCC, 2015; Evans and Fidler, 2005), they will inevitably be confronted to temperatures

exceeding their thermal tolerance. Furthermore, as the natural population currently consists of only 2400 adult individuals (IUCN Red list, 2016) and they have a very low capacity of evaporative cooling at high temperatures compared to other small tropical seed-eaters (Burton and Weathers, 2003), Gouldian finches are particularly sensitive and vulnerable to environmental change.



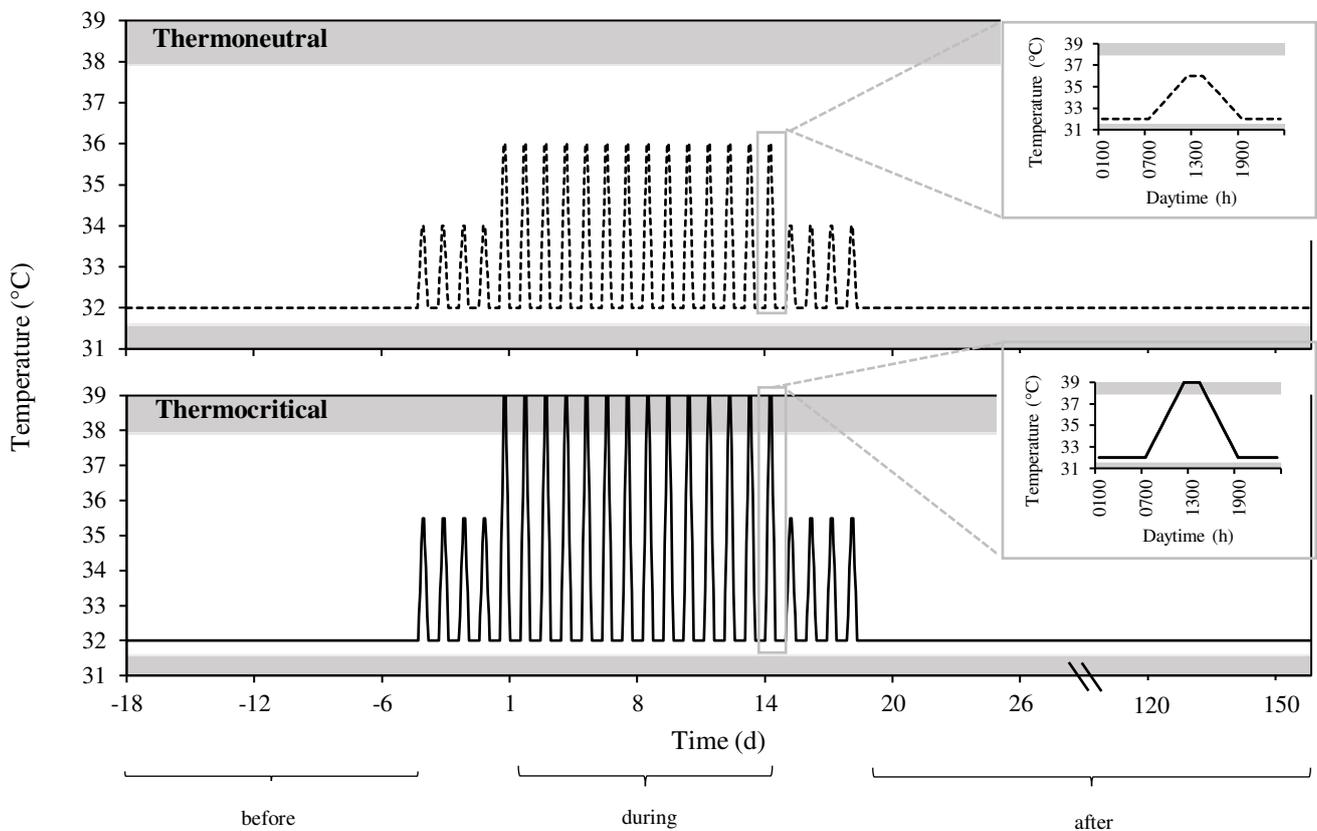
## Aims and Methods of the thesis

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High intraspecific variability, such as in polymorphic species, may be important to cope with extreme heat events, especially for tropical species having narrow thermotolerance and already living close to their upper thermal limits. However, how individuals of different morphs respond to the same environmental stimuli, whether one morph appears more advantaged than the other, and how their respective responses affect the maintenance of intraspecific variability (e.g. reduction or exacerbation of inter-individual differences) remain to be studied. To have a thorough understanding of the different coping mechanisms under environmental fluctuations between individuals of different morphs, it is necessary to examine morph-specific responses (i) at the mechanistic level (i.e. genetic differences, metabolic flexibility and/or sensitivity), and (ii) at the populational level (i.e. dynamics between morphs, interactions, population processes etc). Only then, it is possible to understand whether polymorphism can remain stable.

The first part of my thesis therefore focuses on the examination of the immediate behavioural (**Study 1**) and physiological (**Study 2**) effects of a thermally-challenged heatwave in red- and black-headed Gouldian finches. Towards this end, we adopted an experimental approach using captive, young (approx. 1 year-old) Gouldian finches. Pairs of similar morphs were randomly formed, and each couple was separated physically, but not visually nor phonetically (considering that the courtship song of red-headed males differs from the one of black-headed males; see Box S1). Temperatures initially remained stable throughout the day at 32°C (i.e. within thermoneutrality of Gouldian finches; Burton and Weathers, 2003) for two weeks, where behavioural and physiological traits were measured to assess baseline values (Fig. 5). I then experimentally exposed birds to different thermal conditions: either a thermally-challenging condition, with temperatures above thermoneutrality (i.e. thermocritical heatwave) or a sham- controlled condition, under which birds experienced temperature changes within thermoneutrality (i.e. thermoneutral exposure) for two weeks, with a temperature maximum (39°C for thermocritical and 36°C for thermoneutral conditions) for two hours in the middle of the day (Fig. 5). This experimental set-up was used to mimic as closely as possible long heatwaves as projected in the next years in Australia (Frich et al., 2002; Perkins et al., 2012). Under these thermal conditions, the same behavioural and physiological traits as during the initial phase were measured again. This approach finally allowed me to assess behavioural and

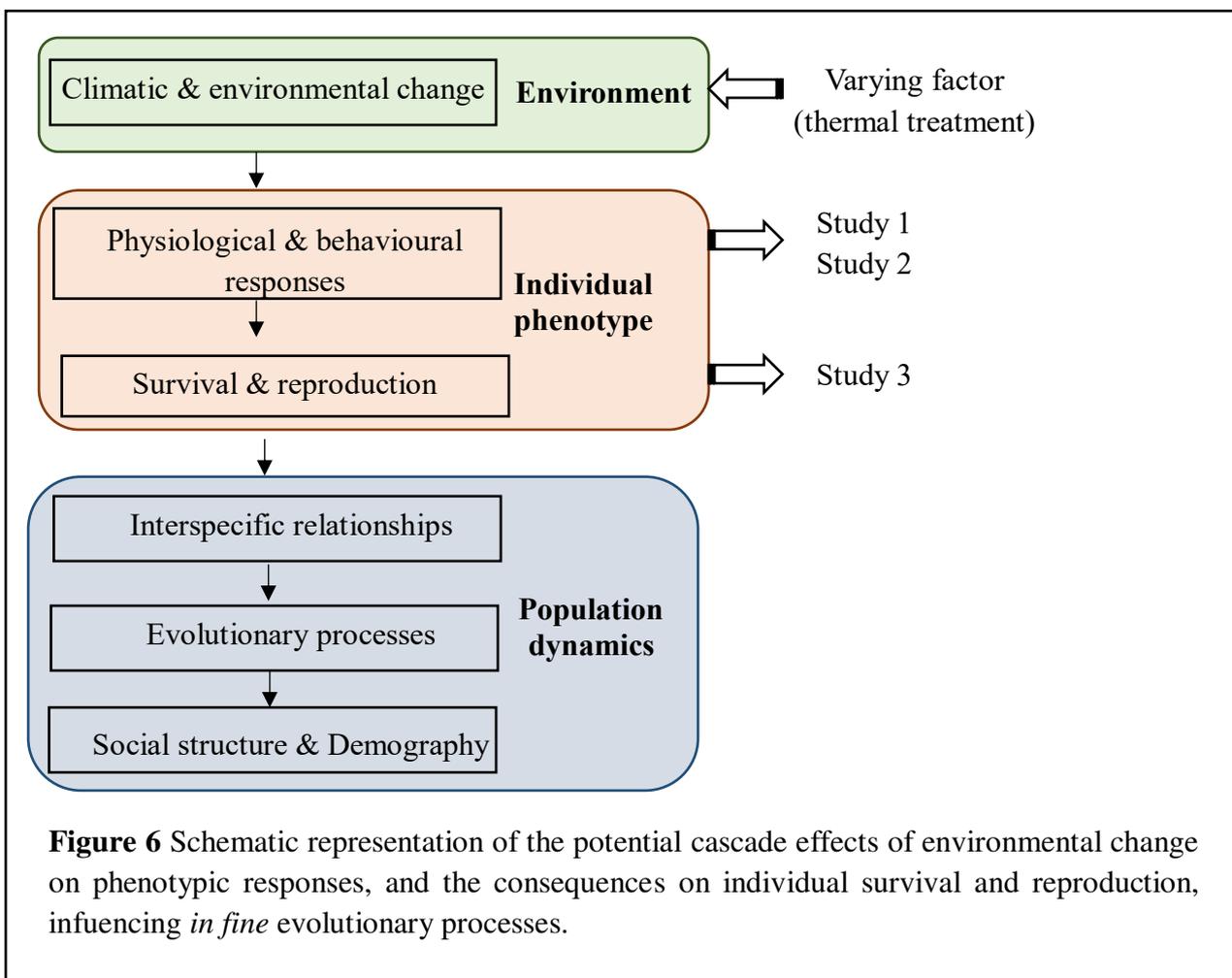
physiological response variation between these two thermal conditions, but also between the two morphs.



**Figure 5** Chronogram of the experiment conducted under thermoneutral (a, dashed lines) and thermocritical (b, solid lines) conditions. Time is represented in relation to the onset of the heatwave exposure. The grey area corresponds to temperatures outside the thermoneutral zone of Gouldian finches (Burton and Weathers, 2003). The insets represent the temperature variation within a day, with the maximum temperature occurring for two hours in the middle of the day.

The immediate phenotypic responses to extreme conditions are important for species survival, but the reproductive capacity following these conditions are as important. However, even though the immediate effects of high temperatures were primarily assessed in ecological studies, their postponed effects have been mostly neglected. Therefore, the second part of my thesis consists in examining the postponed effects of thermocritical heatwaves on the reproductive performance of Gouldian finches, and the subsequent consequences on their offspring (**Study 3**). The main aim was to understand whether phenotypic response differences between morphs under thermally-challenging condition explain subsequently intra-specific differences in reproductive success and fitness components. In this part, ambient temperature was set back at 32°C (Fig. 5), the L:D cycle was changed to 13:11 L:D, and each cage was

supplemented with a nest box, nest material, germ sprouts and egg food to stimulate breeding initiation (Evans and Fidler, 2005). During these four months of reproduction monitoring where birds could freely breed, nest boxes were inspected every morning to keep track of the birds' breeding phenology and outcome. This approach allowed me to assess any postponed effects of the thermocritical heatwave (i.e. thermally-challenged condition) on the reproductive performance of birds relative to a temperature change within thermoneutrality (i.e. sham-controlled condition). Finally, at fledging, we measured the same behavioural and physiological traits in fledglings as those measured in adults, which allowed me to assess the carry-over or transgenerational effects of heatwaves.





# PART II

## Results and Discussion

## Synthesis of the results

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How species respond to environmental variation is essential for their persistence. The capacity of individuals to display phenotypic plasticity will determine the possible winners and losers under rapid environmental change. This thesis presents work that aimed to examine intraspecific variation in response to simulated heatwave reaching thermocritical temperatures, by comparing the immediate behavioural and physiological responses of red- and black-headed Gouldian finches, as well as their reproductive performance after exposure to these heatwaves. In accordance with previous studies, I found behavioural and physiological differences between red- and black-headed Gouldian finches (Table 1) which support the hypothesis of distinct life-history strategies between morphs, with red-headed birds having a faster pace-of-life than black-headed individuals. Despite such differences in life histories, the overall behavioural responses did not differ between morphs nor according to temperature intensity. Indeed, females of both morphs decreased their time spent on feeding and individuals of both morphs decreased their response to threat when exposed to temperature change, irrespective of the intensity. The only behavioural parameters that varied differently to temperature change was their response to novelty, with only red-headed individuals decreasing their time spent close to a novel object during both thermal conditions (Table 2). The immediate effects of thermocritical heatwaves between morphs were mostly visible in the physiological parameters. More specifically, black-headed females decreased body mass and increased oxidative damage during the thermocritical heatwave, whereas these parameters remained unchanged from initial values in red-headed birds (Table 2). Despite black-headed females initiating breeding earlier following exposure to thermocritical heatwaves, the exposure to a thermocritical heatwave affected the reproductive performance of both morphs similarly, mostly by increasing their reproductive investment and by decreasing, at least temporarily, the quality of their offspring (Table 2).

**Table 2** Summary table of the differences observed between thermal treatments, morphs (red- (R) and black-headed (B) birds) and morph-specific responses to different thermal treatments observed in this study. + : positive effects of thermocritical treatment; ++ : positive effect of both thermocritical and thermoneutral treatment; / : no effect observed; -: negative effect of thermocritical treatment; -- : negative effect of both thermocritical and thermoneutral treatment. In bold are the parameters for which the response to environmental variations differed between morphs.

	Phenotypic trait	Effect of treatment	Effect of morph	Effect of treatment*morph
<b>Behaviour</b>				
Diurnal feeding	Feeding duration	females: - ; males: /	males: R > B; females: /	/
Response to threat	Aggressiveness	--	B females > B males & R	/
	Capture time	--	B females > B males & R	/
	Docility	/	/	/
Response to novelty	Neophilia/neophobia	/	R > B	R: -- ; B: /
<b>Physiology</b>				
Body condition	Body mass	--	/	R: -- ; B: -
Oxidative status	Antioxidant capacity	/	/	/
	Oxidative damage	/	/	R: -- ; B: females +, males /
Telomere dynamics	Telomere length	/	R males < B males & females	/
<b>Reproduction</b>				
Traits	Sperm morphometry	-	/	R: - ; B: /
	Clutch initiation	-	/	R: / ; B: -
	Clutch size	/	/	/
Outcome	Provisioning rate	+	/	/
	Hatching success	/	/	/
	Fledging success	/	/	/
	Brood size	/	/	/
	Overall productivity	/	/	/
	Chick's sex-ratio (% males)	-	/	/
	Chick's traits	Aggressiveness	-	/
	Docility	/	/	/
	Breathing rate	-	/	/
	Body mass	/	R < B	/
	Antioxidant capacity	-	/	/
	Oxidative damage	-	R > B	R: / ; B: + ; R > B (TN)

## Discussion

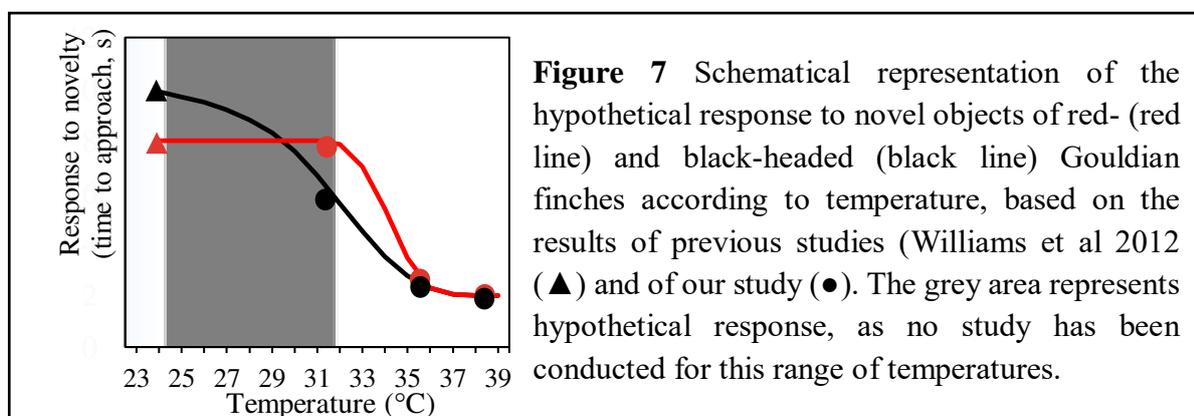
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### **Independent effect of heatwave intensity and morph**

Previous studies highlighted consistent physiological and behavioural differences between red- and black-headed Gouldian finches, suggesting life-history strategy differences between the two morphs. In this study where morphs were isolated, I found that red-headed males eat significantly more (indicating a faster metabolism), are more explorative, and have reduced telomere length (indicating shorter lifespan; Bize et al., 2009) compared to black-headed males (Studies 1 and 2). Together with previous findings showing that red-headed individuals are also behaviourally dominant and more aggressive (Pryke, 2007; Pryke and Griffith, 2006; Williams et al., 2012), the results of this study suggest that red-headed individuals have a faster pace-of-life than black-headed individuals (see Fig. 2), which might consequently explain the reduced telomere length of red-headed males. However, the behavioural response to temperature variations, irrespective of the intensity, generally did not differ between morphs. Indeed, females of both morphs decreased their time spent on feeding, and individuals of both morphs decreased their response to threat during high temperatures. In contrast, only red-headed individuals decreased their response to novelty (Study 1). Such differences in plasticity regarding the response to novelty may be explained by the fact that red-headed birds initially have a higher behavioural capacity compared to black-headed birds. Then, during challenging conditions, the time that red-headed birds spent close to a novel object drops to values similar to black-headed birds. Thus, higher behavioural plasticity may explain why red-headed birds keep their self-maintenance processes similar to baseline levels, whereas black-headed individuals show a higher response in terms of maintenance (Study 2, Table 2). However, with this study's approach, I could not disentangle whether behavioural plasticity is the cause or the consequence of the self-maintenance status of individuals, as the different behavioural and physiological parameters inform us about different time windows of the state of the organism. Indeed, markers of self-maintenance inform us about the response process through the homeostatic state of individuals within a short time, whereas behavioural measurements inform us about the behavioural response outcome following the heatwaves.

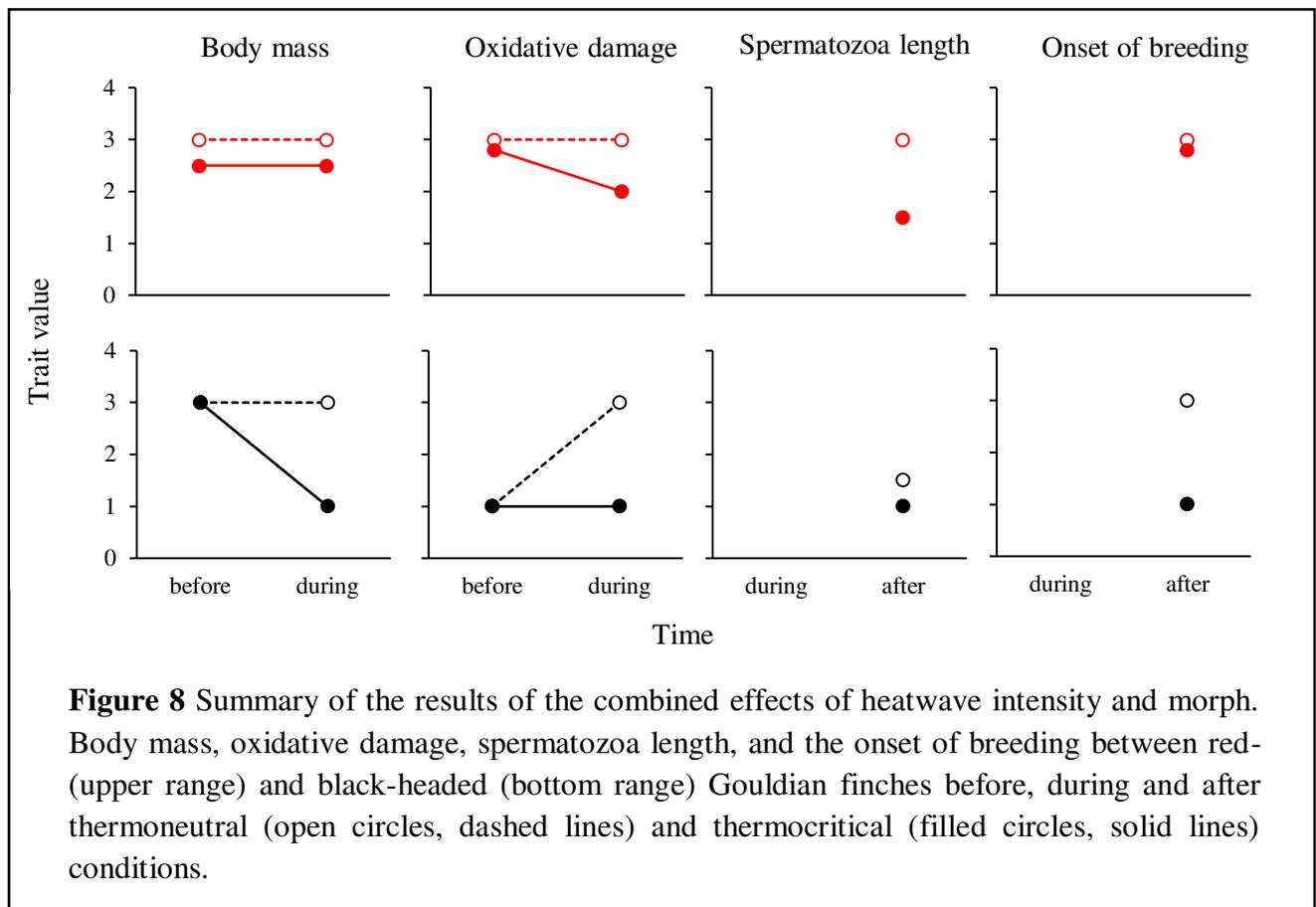
Despite the behavioural parameters not differing between thermal treatments in my study, I found differences between morphs depending on thermal conditions if I compare with previous studies on Gouldian finches having different experimental set-up (Fig. 7, see also

discussion in Study 1). Indeed, 75% of birds visited a novel object during the neophilia test in the study by Williams et al. (2012) conducted at 24°C, while in our study, 54% and 20% of birds did so at 32°C and during the two thermal treatments (at 36°C and 39°C), respectively. Moreover, the time taken by black-headed birds to approach a novel object differed between previous studies and ours, with black-headed birds approaching a novel object more rapidly in previous studies than black- and red-headed birds in our study, whereas the time to approach of red-headed birds was exactly the same in our study compared to the previous study (Study 1, Williams et al. 2012). Taken together, these findings from different studies suggest that black-headed birds become gradually less exploratory with higher temperatures whereas red-headed birds seem to become abruptly less exploratory after a threshold of 32°C (if we assume the absence of social effects; Fig. 7). This decrease in exploratory behaviour with high temperature would result in no morph differences during heatwaves in our study. In zebra finches (*Taeniopygia guttata*), exploratory individuals have been described as having an adaptive advantage over less exploratory individuals, as their breeding performance is higher (McCowan et al. 2015; McCowan et al. 2014). Assuming that this adaptive advantage also applies to Gouldian finches (as suggested by Brazill-Boast et al. 2013), black-headed birds seem to lose this advantage first with increasing temperature while red-headed birds appear to lose it only under very high temperatures. Moreover, this model suggests the existence of a temperature range between 27 and 32°C for which birds of both morphs are equally exploratory and competitive (Fig. 7). This raises a question about the long-term effect of thermal conditions on individual interactions in a social species (as it is the case of the Gouldian finch), and the behavioural balance between morphs in the future. Examining the exploratory dynamics between morphs of Gouldian finches along a broader range of temperatures would therefore be necessary.



### Combined effects of heatwave intensity and morph

I have just described the traits for which the response was purely genetically-driven (i.e. differences between morphs), or environmentally-driven (i.e. differences between sham- and thermally-challenging conditions), but the main aim of this thesis was to examine the traits for which the response variations to different environmental conditions are genetically-driven (i.e. morph-specific response to thermally-challenging conditions). Thermal conditions differently affected black- and red-headed Gouldian finches in four ways: (1) body mass, (2) adult and offspring oxidative damage, (3) spermatozoa length, and (4) the onset of breeding (Fig. 8).



While the sperm length was not affected by thermal conditions in black-headed- males, the spermatozoa of red-headed males having experienced thermocritical conditions were shorter than those of red-headed males having experienced thermoneutral conditions. This suggests either higher plasticity and/or greater temperature-dependence of spermatogenesis in red-headed males. Shorter spermatozoa may reflect shorter spermatogenesis duration (Durairajanayagam et al., 2015; Ramm and Schärer, 2014), which supports the ideas of accelerated life-history strategies in red-headed birds following stressful conditions, and faster

pace-of-life of the red-headed morph. Moreover, previous studies already showed higher sperm variation of red-headed male Gouldian finches across different competitive conditions compared to black-headed males (Immler et al., 2010). Together with the results of this study, it suggests that, in contrast to black-headed males, red-headed males might control their breeding performance by altering sperm quality depending on environmental conditions.

Only black-headed females significantly reduced body mass, increased oxidative damage, but did not vary antioxidant capacity during the heatwaves, whereas red-headed individuals also decreased body mass, but increased oxidative damage at the end of the heatwave (see discussion of Study 2). Such higher effects of thermocritical heatwaves on the maintenance of black-headed females may be mainly explained by the activation of thermoregulatory processes in black-headed females, whereas these thermoregulatory processes were less activated in black-headed males and red-headed individuals. Even though they did not increase their antioxidant capacity during the heatwaves, black-headed females showed higher antioxidant capacity before an energetically-demanding condition (moult or breeding, Study 2). Then, it dropped to values similar to those of the other groups during this demanding condition, which suggests that black-headed females may anticipate antioxidant requirements by increasing antioxidant defences before energetically-demanding events, as they may be limited to do so during these events. However, such anticipatory reaction may be impossible in response to unpredicted environmental changes, as seen here in response to heatwave events. Despite being one of the first studies measuring oxidative parameters longitudinally, the main limitation of this study is that the investigation of the physiological response was very restricted, as it is more diverse than considered here. For instance, hormones such as corticosterone or prolactin may affect trade-offs between self-maintenance and reproduction (Angelier and Chastel, 2009; Angelier et al., 2016; Miller et al., 2009) and mediate individual response capacity to heatwaves (Wingfield et al., 1990; Wingfield et al., 2017). Because red-headed individuals seem to have a higher metabolic rate (Study 1) and stronger glucocorticoid responses to food manipulation (Pryke et al., 2012), I would predict stronger variation in corticosterone and prolactin levels with increasing temperatures in red-headed compared to black-headed birds. Moreover, preliminary data revealed that black-headed birds significantly increased their consumption of food rich in antioxidants under high temperatures, which may allow them to behaviourally counteract this elevation in oxidative damage (Study S2). However, we cannot predict with certainty whether black-headed Gouldian finches are actively able to take advantage of food properties against high oxidative damage, as several conditions have to be fulfilled, such as the

costs and benefits related to the use of exogenous in relation to endogenous antioxidants (see Table 1 in Beaulieu and Schaefer, 2013). However, these conditions have not been analysed in this study. Thus, further analysis are needed to have a complete picture of the oxidative status and the hormonal response between individuals under different environmental conditions.

Irrespective of the mechanisms, these higher thermally-induced effects on self-maintenance in black-headed females suggest that birds of both sexes, but interestingly also of both morphs, differ in their thermal tolerance, explaining why the physiological traits mainly differ (more so than behavioural traits) in response to temperature variations. Thus, my results suggest that black-headed birds (especially females) reach thermal limits at lower temperatures than red-headed birds (Fig. 3). Consequently, differences in thermoregulation and self-maintenance between morphs in response to an extreme heatwave event might also explain why black-headed females advanced their onset of breeding in comparison to red-headed females after the exposure to heatwaves reaching thermocritical temperatures (Study 3). This higher investment in reproduction after an energetically-challenging event might be counter-intuitive and is in contrast with the response of other tropical species (Table 3). However, in line with the 'terminal investment hypothesis' (Velando et al., 2006; Williams, 1966), previous exposure to highly stressful events may indicate that individuals have only limited time to breed before the next occurrence of stressful event, and that they should therefore increase reproductive investment (Marrot et al., 2017; Marrot et al., 2018). These results suggest that black-headed Gouldian finches respond more rapidly to a change in their breeding environment, and that birds may adjust their reproductive investment on the effects of pre-breeding environmental conditions based on their maintenance. However, to thoroughly test this hypothesis, it would be necessary to assess the costs of reproduction on future breeding outcome and survival.

**Table 3** Compilation table of the effects of high-temperature events on phenotypic traits in Gouldian finches and other tropical, closely-related species

	<u>Study</u>	Du Plessis et al., 2012; Funghi et al., 2019	Pattinson and Smit, 2017	Şekerciöğlü et al., 2012	Vinagre et al, 2014	Andrews et al., 2018	Hurley et al., 2018
	<u>Species</u>	Southern pied babbler; Zebra finch	Rufous-eared warbler	tropical birds	Rock gobi	Zebra finch	Zebra finch
	<u>Experimental approach</u>	observations on wild populations; experimenta approach in wild populations	observations on wild populations	Review		experimental approach and observations in the wild	simulated heatwaves
	<u>Phenotypic trait</u>						
<b>Behavioural traits</b>	Foraging	-	-				
	Aggression						
	Docility						
	Neophilia/neophobia						
	Food choice						
<b>Physiological traits</b>	Body mass	-		-			
	Body size					-	
	Oxidative status				-		
	Telomere dynamics						
<b>Reproductive traits</b>	Sperm morphometry						-
	Egg volume						
	Parental care						
<b>Breeding performance</b>	Clutch initiation						
	Hatching success						
	Clutch size						
	Fledging success						
	Brood size						
	Overall productivity						
	Chicks' sex-ratio						
<b>Breeding outcome</b>	Chick's body mass						
	Chick's growth						
	Chick's oxidative status						
<b>Survival</b>	Survival						

	<u>Study</u>	Zhang et al., 2018	Gardner et al., 2017; McKechnie and Wolf, 2010	Moreno and Pape Møller, 2011,	This thesis
	<u>Species</u>	desert agama	desert birds; red-winged fairy-wren ( <i>Malurus elegans</i> ) and white-browed scrubwrens ( <i>Sericornis frontalis</i> )		Gouldian finch
	<u>Experimental approach</u>	simulated heatwave	Review; observations in the wild	Review	immediate effects of simulated heatwaves
	<u>Phenotypic trait</u>				M:/; F:-
<b>Behavioural traits</b>	Foraging				/
	Aggression				/
	Docility				/
<b>Physiological traits</b>	Neophilia/neophobia				/
	Food choice				R:/; B: +
	Body mass				R:/; B: -
	Body size				/
<b>Reproductive traits</b>	Oxidative status				R:/; B: -
	Telomere dynamics	-			/
	Sperm morphometry				R:-; B: /
	Egg volume				R:+; B: /
<b>Breeding performance</b>	Parental care				+
	Clutch initiation			-	R:/; B: +
	Hatching success			-	/
	Clutch size				/
<b>Breeding outcome</b>	Fledging success			-	/
	Brood size				/
	Overall productivity			-	/
	Chicks' sex-ratio				-
	Chick's body mass				/
<b>Survival</b>	Chick's growth				/
	Chick's oxidative status				-
	Survival		-	-	/

## Conclusion and Perspectives

Intuitively, one would predict the better competitor to be more advantaged under challenging conditions. In the Gouldian finch, the red-headed morph seems to have the highest competitive advantages (e.g. socially dominant, higher mating opportunities; Brazill-Boast et al., 2013; Pryke and Griffith, 2006; Pryke et al., 2007). However, despite differences in sperm variation between morphs in response to environmental variation, the reproductive outcome of red-headed pairs did not differ from that of black-headed pairs. Both morphs increased their investment into reproduction after exposure to a thermocritical heatwave event, but the offspring produced by pairs previously experiencing thermocritical heatwaves showed transiently higher oxidative damage and altered behaviour at fledging (Study 3) compared to offspring of pairs after exposure to a heatwave event within thermoneutrality. However, how such differences affect the phenotypic response capacity and survival chances of the offspring to environmental change remain to be examined. The examination of transgenerational effects would provide a first insight into the life-long consequences of heatwave exposure. Based on my results, I cannot conclude which morph-specific strategy may be more advantageous when coping with heat events, as the breeding outcome after the heatwaves was similar in both morphs. Thus, both morphs may be considered as ‘losers’ in the face of extreme temperature change.

Moreover, compared to other tropical, closely-related monomorphic species, the Gouldian finch does not seem to have more advantages, as the negative effects of high temperature already highlighted in previous studies are reflected in both morphs (Table 3). However, the reproductive outcome in terms of reproductive investment and offspring condition did not differ between morphs. Thus, the higher phenotypic and genetic variation available for selection to act upon may not be significant enough to allow evolutionary rescue in the Gouldian finch. Moreover, theoretical and empirical work predict that a population that undergoes evolutionary rescue is characterised by a population decline (as maladaptive individuals are not able to survive and breed) followed by a population recovery (as adaptive individuals survive and manage to breed afterwards, Bell and Gonzalez, 2009; Holt and Gomulkiewicz, 2004). Given the evidence associating telomere length with individual survival probability (Bauch et al., 2014; Salomons et al., 2009; Zhang et al., 2018), differences between individuals in telomere attrition after heatwave events may also indicate lifespan differences between morphs and which morph may be more sensitive to decline. In this study, telomeres of red- and black-headed birds did not shorten throughout the 5-month study despite self-maintenance differences

between morphs during the heatwaves. Therefore, the results of this study suggest that no morph seems more disadvantaged than the other morph in terms of survival following exposure to heat events. If high phenotypic variability associated with polymorphism does not confer them higher advantages, as this study suggests, Gouldian finches may be highly vulnerable to environmental changes, as they show already lower capacity of evaporative cooling at high temperatures compared to other small tropical seed-eaters (Burton and Weathers, 2003). Whether high phenotypic variability between individuals, as typically observed in polymorphic species, may represent an evolutionary rescue under rapid environmental change remains to be examined. These conclusions should however be taken with caution, as I do not exclude the possibility of a significant impact of intra- and inter-specific interactions in the context of environmental change, especially in a highly social bird such as the Gouldian finch (King et al., 2015; Evans and Fidler, 2005). For instance, a recent study suggests that Gouldian finches use long-tailed finches (*Phoephila acuticauda*) as an indicator of safety in risky situations (O'Reilly et al., 2019). Thus, the simple presence of one phenotype (i.e. presence of another morph) or a competitor (i.e. presence of another species, long-tailed finch; Brazill-Boast et al., 2011; O'Reilly et al., 2019) may impact the responses observed here. Consequently, if inter-morph interactions are important enough to compensate for the decrease of a given morph, then inter-morph variability may allow for evolutionary rescue. The degree of the impact of such social effects on the population dynamics of Gouldian finches under environmental change will therefore depend on the Gouldian finch ecological flexibility. Another major consequence of current climate change is a shortage of food abundance and quality available for animals. Similarly, previous studies already highlighted that red-headed individuals are more sensitive to food manipulation than black-headed birds (higher changes in body condition and immune function, delayed breeding, smaller broods, lower-quality offspring due to reduced parental provisioning rate; Pryke et al., 2012). Thus, other factors relating to environmental change not considered in this study can be as important as high-temperature events, and could lead to different processes and consequences in the context of coping with these environmental challenges. Further studies are highly needed to answer these remaining questions.

This study was restricted to the examination of phenotypic responses at the individual level (Fig. 6). Knowing the sensitivity and the response differences among individuals is a first step to understand inter-individual coping variability to climate change. However, the consequences of climate change go beyond the individual level. The next step would be to use my results at the individual level to extrapolate the effects at the population level. Physiological markers

across individuals have been used in recent ecological studies to indicate individual chances of surviving and reproducing, and *in fine* to predict animal population health (Beaulieu et al., 2013; Wikelski and Cooke, 2006). In this context, markers of oxidative status, i.e. the balance between oxidative damage and antioxidant defences, may be used as indicators of demographic status. Indeed, a shift towards higher oxidative damage can lead to decreased fertility and survival probability (Bize et al., 2008; Helfenstein et al., 2010; Monaghan et al., 2009). In this study, antioxidant capacity did not vary between the thermal conditions, but hydroperoxide levels, one marker of oxidative damage, significantly increased in black-headed females only under thermocritical heatwaves. In this case, I would predict a higher disadvantage of the black-headed morph compared to the red-headed morph in the face of heatwaves.

Finally, studies investigating the implications of individual responses on social structure and population persistence by assessing fitness differences between morphs, as well as the consequences of such variations on inter-individual and inter-specific interactions, would give a thorough view of the consequences of heatwaves on population dynamics. Variation in morph frequency within populations, due to lifespan or survival differences after extreme temperature events, may increase intra-morph competition and limit mating possibilities, affecting individual fitness. For example, skewed morph ratios in populations of common lungwort (*Pulmonaria officinalis*) have shown lower female breeding success and a loss in genetic diversity (Brys et al., 2008; Meeus et al., 2012). Even though Gouldian finches breed assortatively, the ecological and evolutionary consequences of morph rate variation may be comparable. Examining the consequences of extreme thermal events on population dynamics across several polymorphic *versus* monomorphic species would finally allow us to accurately conclude whether polymorphism confers better advantages for evolutionary rescue compared to monomorphism.

To conclude, the results of this thesis are expected to give a first insight into morph-specific responses to thermocritical heatwaves and whether morph-specific phenotypes explain such response differences. While the behavioural responses overall did not differ between morphs or according to temperature intensity, some parameters of self-maintenance and reproduction measured in this study differed in response to morph and temperature intensity. However, no morph appears to have more advantages than the other in the face of intense heatwaves. Both morphs showed some negative behavioural and physiological responses, both

invested similarly in reproduction following intense heatwaves (in terms of productivity), and the offspring of parents of both morphs having experienced such thermocritical heatwaves were similarly affected. Based on these results, both morphs of the Gouldian finch may ‘lose’ in the face of extreme heat events, and high intraspecific variability does not seem to be significant enough to allow for evolutionary rescue in the polymorphic Gouldian finch. However, in order to thoroughly understand the evolution of polymorphism and the benefits of high intraspecific variability in the light of climate change, further studies examining the lifelong consequences (e.g. transgenerational effects, effects on lifetime reproductive success) on individuals, as well as the consequences on population dynamics are highly necessary.

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# PART III

## Publication list

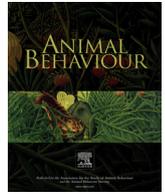
# Immediate effects of heatwaves

## **Study 1: Red does not always outperform black: morph-specific behavioural variation in response to environmental changes**

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# Red does not always outperform black: morph-specific behavioural variation in response to environmental changes

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Variable selection pressures on individuals contribute to the occurrence of distinct phenotypes within species. Rapidly changing environmental conditions due to climate change are likely to alter these selection pressures and hence the current balance between phenotypes within species. In birds, colour polymorphism has been related to alternative behavioural strategies, potentially underlying different responses to extreme environmental conditions between colour morphs. Here, we examined how red- and black-headed Gouldian finches, *Erythrura gouldiae*, responded to experimentally induced heatwaves of different intensities by measuring their feeding behaviour, their response to novelty and their behavioural reactivity. As red-headed birds have been described as physiologically more plastic than black-headed birds, we expected them to alter their behaviour more strongly in response to heatwaves. Even though black-headed males fed overall less than other birds (thereby suggesting lower energy requirements), differences in feeding behaviour between morphs remained unaffected by thermal conditions. However, birds' response to novelty varied between morphs under variable thermal conditions. Indeed, before thermal treatment, black-headed birds, irrespective of their sex, spent less time near a novel object than red-headed birds, thereby suggesting lower novelty interest. However, because only red-headed birds reduced the time spent close to a novel object during heatwaves, differences in novelty interest between morphs disappeared under these thermal conditions. These results suggest that behavioural plasticity in response to thermal conditions differ between colour morphs of Gouldian finches, thereby showing that behavioural differences between morphs are not static. How such behavioural adjustments within species affect the balance between colour morphs in natural populations facing extreme environmental conditions remains to be determined.

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Extreme climatic events have become more frequent and severe, thereby representing an increasing threat for humans and wildlife (Parmesan, 2006; Reside, Vanderwal, Garnett, & Kutt, 2015; Wingfield, 2013). For instance, heatwaves have led to unprecedented mortality of animals particularly in tropical regions (McKechnie & Wolf, 2009; Saunders, Mawson, & Dawson, 2011; Welbergen, Klose, Markus, & Eby, 2008), presumably because of the narrow thermal tolerance of tropical species (Khaliq, Hof, Prininger, Böhning-Gaese, & Pfenninger, 2014; Şekerciöglu, Primack, & Wormworth, 2012). To survive these extreme events, such as heatwaves, animals have two adaptive options: staying and adjusting their behaviour to the new environmental conditions or dispersing to more favourable conditions (Parmesan, Root, & Willig,

2000; Pulido & Berthold, 2004). In the first case, animals may adjust not only their physiology (e.g. increased evaporative cooling under hot conditions) but also their behaviour (e.g. reduced activity and search for shaded refuges; Wolf, 2000). Despite these adjustments, prolonged exposure to elevated temperatures may still negatively affect the fitness of animals by impinging upon foraging or breeding activities (Cunningham, Martin, & Hockey, 2015; Du Plessis, Martin, Hockey, Cunningham, & Ridley, 2012).

Within species, individuals respond differently to environmental changes, leading to variable reaction norms (Dingemanse, Kazem, Réale, & Wright, 2010). Variable selection pressures on individuals using different strategies contribute to such intraspecific behavioural variability. In polymorphic species for instance, behavioural strategies, such as reproductive, agonistic, antipredator or exploratory strategies, typically differ between morphs (Brazill-Boast, Griffith, & Pryke, 2013; Brodie, 1992; Dijkstra, Hemelrijk, Seehausen, & Groothuis, 2009; Peleg, Charter, Leshem, Izhaki, & Roulin, 2014; Van Rhijn, 1973; Williams, King, & Mettke-

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Hofmann, 2012). Different strategies are likely to confer adaptive advantages to each morph depending on the conditions it experiences. Thus, balancing selection, in addition to disruptive and frequency-dependent selection, is one hypothesis explaining the persistence and maintenance of intraspecific polymorphism (Roulin, 2004). However, because the current rate of climate change acts as a new selective agent within polymorphic species (Roulin, 2014), the costs and benefits associated with the use of alternative strategies is likely to vary in the future. If these costs and benefits remain similar, then strategy differences between morphs should remain constant. In contrast, if these costs or benefits become higher in one morph than in the other, then strategy differences between morphs should decrease, increase or disappear. These potential scenarios highlight the need to examine behavioural adjustments in polymorphic species under varying conditions and not only under stable conditions (Réale, Reader, Sol, McDougall, & Dingemanse, 2007).

Here, we experimentally examined the impact of heatwaves of different intensities on behavioural traits in Gouldian finches, *Erythrura gouldiae*, of the two main colour morphs: red- and black-headed birds. Given the effects of high-temperature exposure on the expression of behavioural traits (Beever et al., 2017; Wong & Candolin, 2015; Zhao & Feng, 2015), we first expected all birds to adjust their overall behaviour in response to thermal conditions (i.e. before versus during the heatwaves) and this behavioural response was expected to be stronger under a thermocritical heatwave (i.e. a heatwave characterized by a temperature above thermoneutrality, for which birds need to increase energy expenditure to thermoregulate) than under a thermoneutral one (i.e. a heatwave characterized by a temperature within thermoneutrality, for which energy expenditure for thermoregulation is minimal). We also expected red-headed birds to be more affected than black-headed birds, as they have been described as showing a higher physiological response when facing environmental changes (e.g. change in their social environment), possibly due to higher energy requirements (Mettke-Hofmann, 2012; Pryke et al., 2007, 2012; Williams et al., 2012).

## METHODS

### Experimental Design

A total of 112 young adult Gouldian finches (approximately 1 year old, information gathered from closed leg rings and respective breeders) of the two main colour morphs (56 red-headed and 56 black-headed birds) were obtained from 12 local private breeders. We ensured that all birds were of the wild-type and were reared by parents. The experiment was conducted in two sessions (each including 28 red-headed and 28 black-headed birds). Each bird was randomly paired with another bird from a different breeder, but of the same morph and of the opposite sex. Pairs were housed in individual cages (99.6 × 48.0 cm and 52.0 cm high) placed in two climate chambers (2.8 × 4.2 m and 2.5 m high; seven red-headed and seven black-headed pairs in each climate chamber). We ensured birds from different breeders and morphs were equally distributed in the two climate chambers. Climate chambers were initially set at 32 ± 1 °C, with a humidity of 50 ± 5%, a full-spectrum light source with a 12:12 h (light:dark) cycle (0700–1900 hours) and an airflow of 700 m<sup>3</sup>/h (Beaulieu, 2016; Evans & Fidler, 2005). These thermal conditions reflect the conditions that these tropical birds can currently encounter in some regions of their natural habitat in Northern Australia during the hottest months of the year, but also presumably more frequently in the next few decades because of climate warming (CSIRO and Bureau of Meteorology, 2015; Evans & Fidler, 2005). Cages were equipped with two

perches, shell grit and cuttlefish bones. Birds had access to water and mixed seeds for tropical finches (Deli Nature 40–Exoten Basis, Beyers, Belgium) ad libitum and to water for bathing every day for 1 h to improve welfare (Krause & Ruploh, 2016). Birds could acclimate to these conditions for at least 2 weeks before starting behavioural measurements.

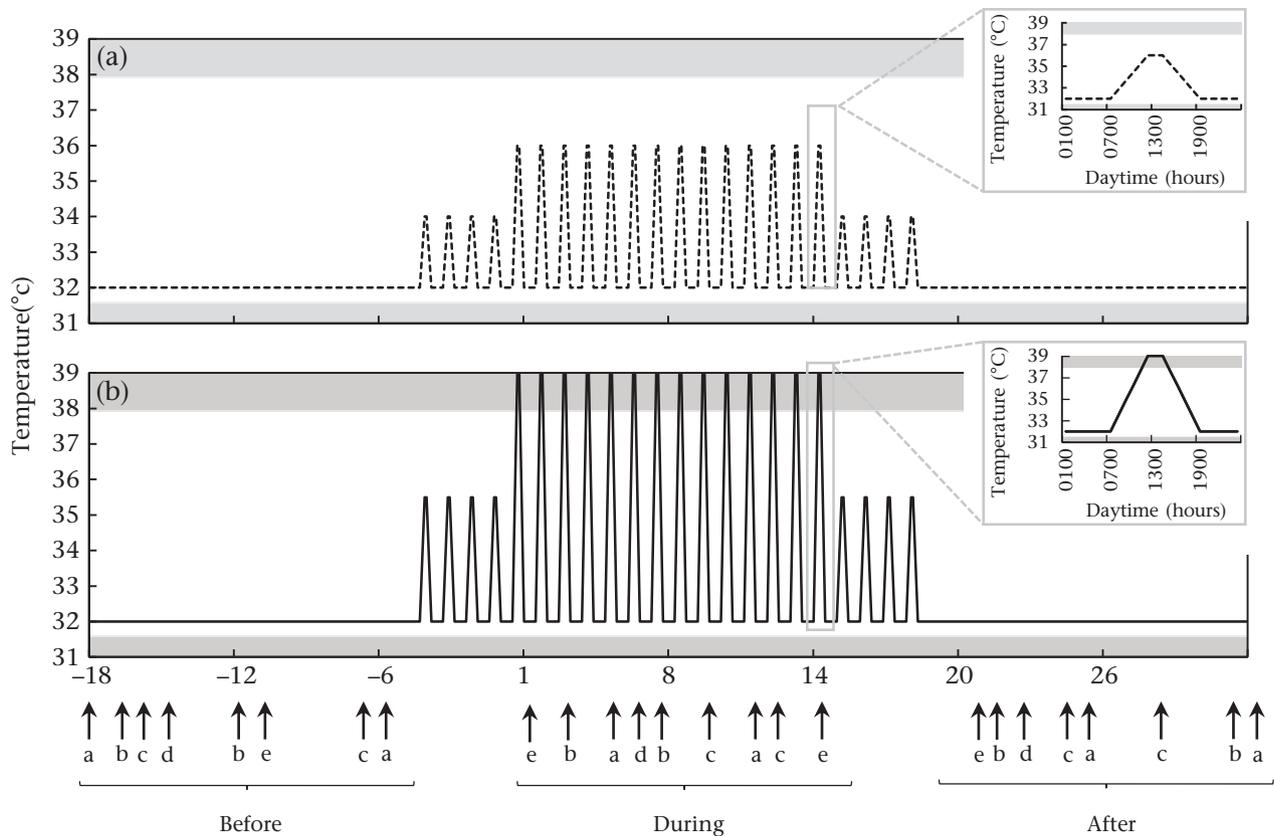
In our experiment, temperatures were chosen to mimic heatwaves occurring within and beyond the thermoneutral zone of Gouldian finches (i.e. the range of ambient temperatures for which birds produce minimal metabolic heat: 31.8–38.0 °C; Burton & Weathers, 2003; Fig. 1). Thus, initial temperature was set at 32 °C (i.e. lower critical temperature of Gouldian finches) for 14 days, followed by an intermediate temperature increase (maximal temperature of 34 and 35.5 °C in thermoneutral and thermocritical heatwaves, respectively) for 4 days to expose birds progressively to the high temperatures of 14-day-long heatwaves they experienced afterwards (maximal temperature of 36 and 39 °C in thermoneutral and thermocritical heatwaves, respectively). After the heatwaves, birds were exposed to an intermediate temperature (as before the heatwave), before experiencing 32 °C again (Fig. 1). During the intermediate phases and the heatwaves, birds experienced maximal temperatures only for 2 h per day from 1200 to 1400 hours (plateau temperature; Fig. 1), with a progressive increase before and decrease after the plateau, and stable conditions at night. The length of our heatwaves (14 days) corresponds to the duration of long heatwaves as projected for the future in Australia (Frich et al., 2002; Perkins, Alexander, & Nairn, 2012). Moreover, the intensity of these heatwaves corresponds to the temperature birds experience during current, and will experience during future, heatwaves in their natural habitat, where average temperature is predicted to increase by 3–4 °C by 2070 (CSIRO and Bureau of Meteorology, 2015). We exchanged temperature conditions between the two sessions in both climate chambers to control for a potential effect of the climate chamber per se.

### General Approach for Behavioural Measurements

As interindividual differences in behavioural traits may be driven by interindividual differences in energetic requirements (Careau, Thomas, Humphries, & Réale, 2008; Crino, Buchanan, Trompf, Mainwaring, & Griffith, 2017), the diurnal feeding behaviour of birds of both morphs was examined first. Then, to test for behavioural differences between colour morphs in response to high temperatures, we conducted behavioural tests reflecting (1) the response of individuals to novelty (object exploration and avoidance behaviour) and (2) their behavioural reactivity (capture time, aggressive defence, docility) across different ambient temperatures. Most behavioural traits were measured longitudinally: twice before, twice during and twice after the heatwaves (except capture time which was measured once before, twice during and once after the heatwave, and diurnal feeding behaviour which was measured once before and once during the heatwave; Fig. 1). Each bird, therefore, acted as its own control, thereby reducing potential error variation due to individual heterogeneity. All the tests were conducted in the middle of the day (corresponding to the plateau hours during the heatwaves), when Gouldian finches feed less (Beaulieu & Schaefer, 2014), and when the influence of feeding on their general behaviour was expected to be minimal.

### Feeding Behaviour

At the end of the day preceding behavioural monitoring, feeding glasses (9.5 × 9.5 cm and 8.0 cm high) filled with staple seeds were placed in the middle of the cages. The feeding behaviour of birds was then monitored with digital cameras (Colour-Miniature-



**Figure 1.** Chronogram of the experiment conducted under (a) thermoneutral and (b) thermocritical conditions. Behavioural traits (a = aggressiveness and docility; b = neophilia; c = neophobia; d = diurnal feeding behaviour; e = capture time) were measured before, during and after the heatwaves. Insets represent temperature variation over 24 h during the thermoneutral and the thermocritical heatwaves. The grey area corresponds to temperatures outside the thermoneutral zone of Gouldian finches (Burton & Weathers, 2003).

Camera RS Components, Corby, U.K.) placed on the ceiling of each cage, and connected to a digital video recorder (Vi-P14, Video-switch, U.K.) and a monitor outside the climate chambers. Video recording automatically started before the light was turned on, and stopped after the light was turned off. Videos were subsequently manually encoded with VLC media player 2.2.2 (Free Software Foundation, Inc., Boston, MA, U.S.A.). A feeding event was defined as a series of pecks, starting with a first peck and finishing when individuals stopped eating for at least 5 s (Beaulieu & Schaefer, 2014). In total, 32 251 feeding events were observed during 384 h of video. For further analyses, we summed the duration of all feeding events for each bird, for every hour of the day (diurnal feeding pattern) and for each day (total feeding duration).

#### Response to Novelty

Object exploration was recorded with the same digital cameras as those used to monitor feeding behaviour. In each recording trial, the behaviour of all birds was simultaneously recorded with no experimenter being present in the chambers.

To test object neophilia (i.e. object attraction), the novel object (e.g. plastic star, shiny metal bell, glass vial) was placed on the end of a perch, positioned at the centre of the cage in a neutral location, away from food and water (Williams et al., 2012). The latency to approach the novel object, as well as the time spent close to it (i.e. within one bird length marked as a black line on the perch) was recorded for 1 h. Neophilia duration and latency were repeatable across time (intraindividual correlation: duration:  $r = 0.20$ , 95% confidence interval, CI: 0.01, 0.36,  $N = 112$ ,  $P = 0.02$ ; latency:

$r = 0.65$ , 95% CI: 0.18, 0.85,  $N = 23$ ,  $P = 0.01$ ), and were therefore summed (duration) or averaged (latency) across trials for analysis.

To test object neophobia (i.e. hesitation to feed in the presence of a novel object), novel objects (e.g. coloured duct tape, little wooden clothes pin, aluminium foil) were placed on the whole edge of the feeder filled with staple seeds (Williams et al., 2012). The feeder was the same as the feeder used to monitor feeding behaviour. The latency to enter the feeder, as well as the time spent in it, were measured for 1 h. Birds were food deprived for 1 h before behavioural monitoring to minimize any confounding effects related to variable physiological status between birds. Duration was repeatable across time (intraindividual correlation:  $r = 0.63$ , 95% CI: 0.55, 0.70,  $N = 112$ ,  $P < 0.001$ ) and was therefore summed for analysis. However, latency was not repeatable across time ( $r = -0.04$ , 95% CI:  $-1.18$ , 0.51,  $N = 30$ ,  $P = 0.54$ ) and was excluded for further analyses, as it did not reliably reflect a consistent behaviour within individuals. The neophobic effect triggered by the novel object per se was examined by calculating the difference in time spent feeding between the neophobia test (when the novel object was present on the feeder) and feeding monitoring (when the novel object was absent from the feeder) for the same hour of the day.

To avoid object habituation, we used a different novel object in consecutive recording trials, and the same object was used only after more than a month after its first exposure (as described in Mettke-Hofmann & Greenberg, 2001). In total, 11 distinct objects were used for the neophilia and neophobia tests, each object being the same for all birds in each trial to ensure that behavioural differences between birds were not due to the use of intrinsic

differences between objects. In total, 1559 approach events were observed during 96 h of video in both tests.

### Behavioural Reactivity

We measured docility, aggressive defence reaction and capture time to estimate the reactivity of birds in response to a threat (e.g. Careau et al., 2008; Møller, Christiansen, & Mousseau, 2011; Møller & Ibáñez-Álamo, 2012; Noguera, Metcalfe, Surai, & Monaghan, 2015; Réale et al., 2007). Docility and aggressive reactions were measured by the same experimenter (R.F.) throughout the two experimental sessions. Docility was assessed by measuring how often birds moved to escape when held in the left hand for 10 s. Docility scores ranged from zero, when the bird did not move at all, to six when it moved six times during the measurement time. This measure was used as a proxy for the propensity of birds to escape when threatened by a predator (i.e. the experimenter; Møller et al., 2011; Møller & Ibáñez-Álamo, 2012; Noguera et al., 2015). Aggressive defence reaction was subsequently assessed by holding the bird in the left hand, and by approaching the right index finger in front of the beak six times for 10 s (Møller & Ibáñez-Álamo, 2012; Noguera et al., 2015). The number of times the birds pecked the finger of the experimenter was noted (score range 0–6) and used as an index of their propensity to aggressively react to a potential predator. We also assessed capture time by scoring the time needed to catch both birds (one after the other) within each cage. Likewise, the time needed to catch a bird indirectly reflected the ability of birds to escape a potential threat (Careau et al., 2008; Réale et al., 2007).

Behavioural reactivity was highly repeatable across time (intraindividual correlation: capture time:  $r = 0.65$ , 95% CI: 0.49, 0.76; aggressiveness:  $r = 0.84$ , 95% CI: 0.80, 0.87; docility:  $r = 0.72$ , 95% CI: 0.66, 0.78;  $N_{\text{capture time}} = N_{\text{aggressiveness}} = N_{\text{docility}} = 112$ , all  $P < 0.001$ ), and were therefore, each separately, averaged (capture time) or summed (aggressiveness and docility) across trials for analyses.

### Statistical Methods

To reduce related behavioural variables, we first ran a principal component analysis (PCA) on the correlation matrix, including five variables that were repeatable and for which we had a sufficient sample size (neophilia duration, neophobia duration, docility, aggressiveness and capture time). Based on the factor loading matrix after varimax rotation (Table 1), 69% of the variance was explained by the first three components of the PCA, with the first component reflecting the response to novelty (PC1; neophilia and neophobia durations), the second component reflecting the response to threat (PC2; aggressiveness, capture time) and the third component reflecting docility. The standardized scores were further used to estimate the behavioural response of red- and black-headed Gouldian finches to high temperatures. As few birds

**Table 1**  
Factor loading matrix for each principal component in the PCA

Principal component	PC1	PC2	PC3
Neophilia duration	<b>0.76</b>	0.03	0.17
Neophobia duration	<b>0.77</b>	−0.03	−0.12
Docility	0.06	0.003	<b>0.93</b>
Aggressiveness	−0.16	<b>0.75</b>	0.28
Capture time	0.16	<b>0.78</b>	−0.26
Eigenvalue	1.22	1.17	1.04
% Variance	24.32	23.45	20.83

The behavioural traits reflected by each principal component are given in bold.

approached the novel object during the heatwaves, temporal variation in latency could not be measured. The latency to approach the novel object, as well as neophobic effects in terms of latency ( $\Delta$  latency = latency with object – latency without object), were therefore only compared between morphs and sexes before the heatwaves. Neophobic effects were still examined before the heatwave and across thermal conditions in terms of proportion of birds approaching the novel object ( $\Delta$  proportion = proportion with object – proportion without object) and in terms of time they spent close to the novel object ( $\Delta$  duration = duration with object – duration without object).

We first wanted to examine how behavioural differences between colour morphs before the heatwaves (i.e. at 32 °C) in our study compared with the results of previous studies examining similar behavioural traits in Gouldian finches (King, Williams, & Mettke-Hofmann, 2015; Mettke-Hofmann, 2012; Williams et al., 2012). Towards this end, we conducted either linear mixed models (LMM; neophilia latency,  $\Delta$  latency) or generalized linear mixed models (GLMM; with gamma (feeding behaviour, PC1, PC2, PC3,  $\Delta$  duration) or binomial ( $\Delta$  proportion) distribution) with behavioural traits before the heatwaves as dependent variables, with morph (red- and black-headed birds) and sex (males and females) as independent factors, and cage and session as random factors. We further examined the diurnal feeding pattern of birds throughout the day using a GLMM with a negative binomial distribution with morph and sex as independent factors, daytime (hour of the day) and daytime<sup>2</sup> (to test for potential quadratic effects) as repeated factors, and individuals nested within their cage and session as random factors.

Then, to examine how these traits varied with thermal conditions, we conducted a GLMM with gamma (feeding behaviour, PC1, PC2, PC3,  $\Delta$  duration) or binomial ( $\Delta$  proportion) distribution, with colour morph, sex, heatwave treatment (thermoneutral and thermocritical) and time (before, during and after the heatwaves) as independent factors. We controlled for the nonindependence of the repeated measures from the same individuals across time, as well as the nonindependence of males and females in the same cage by including time as a repeated factor, and individual identity nested within the cage and session as random factors. We also controlled for the potential effects of the different objects per se on the behavioural response of birds to novelty by including the objects as a random factor in the models. In addition, to examine the effects of heatwaves on the diurnal feeding pattern of birds, GLMMs with negative binomial distribution were used with time (before and during the heatwaves), daytime (hour of the day) and daytime<sup>2</sup> (to test potential quadratic effects) as independent factors, with time and daytime as repeated factors, and individual and session as random factors. In this case, analyses were conducted separately for each sex and each heatwave intensity to examine how each individual modified its own feeding behaviour across the day because of changes in thermal conditions.

Statistical models initially included all interactions, but final models were chosen in a backwards-deletion procedure using the corrected Akaike's information criterion (AICc). Post hoc comparisons were performed using Bonferroni correction. Residuals were tested and visually inspected for normality and heteroscedasticity. All the analyses were performed using SPSS (v. 22, 2013; SPSS Inc., Chicago, IL, U.S.A.). All results are given as means  $\pm$  SE.

### Ethical Note

Animal experimentation, handling and husbandry complied with the EU animal welfare regulation and were approved by the ethical committee of Mecklenburg-Western Pomerania (LALLF: Permit No. 7221.3-2-041/15 and approval No. ZooBea3936/3/15). As

no regulations for captive Gouldian finches per se exist, we followed most of the regulations for zebra finches, *Taeniopygia guttata*, commonly used as a biological model in ecological studies (Beaulieu, 2016). The intensity of heatwaves (within thermoneutrality or 1 °C over thermoneutrality), as well as their duration (2 h per day for 14 days), represented a moderate physiological challenge that Gouldian finches may experience in their natural habitat, and with which they are likely to have to cope more frequently in the future due to climate change (CSIRO and Bureau of Meteorology, 2015; Evans & Fidler, 2005). For their welfare, the birds included in our study could bathe during heatwaves to decrease heat stress (Krause & Ruploh, 2016). All birds were checked daily for potential health problems or signs of discomfort due to thermal treatments, but no abnormality or mortality was observed during our study. The sample size chosen of 14 individuals under each experimental condition (two thermal conditions, two sexes, two colour morphs) was needed for a suitable statistical power (0.8). After the study, birds were returned to local breeders and to zoological parks.

## RESULTS

### Morph Differences before the Heatwaves

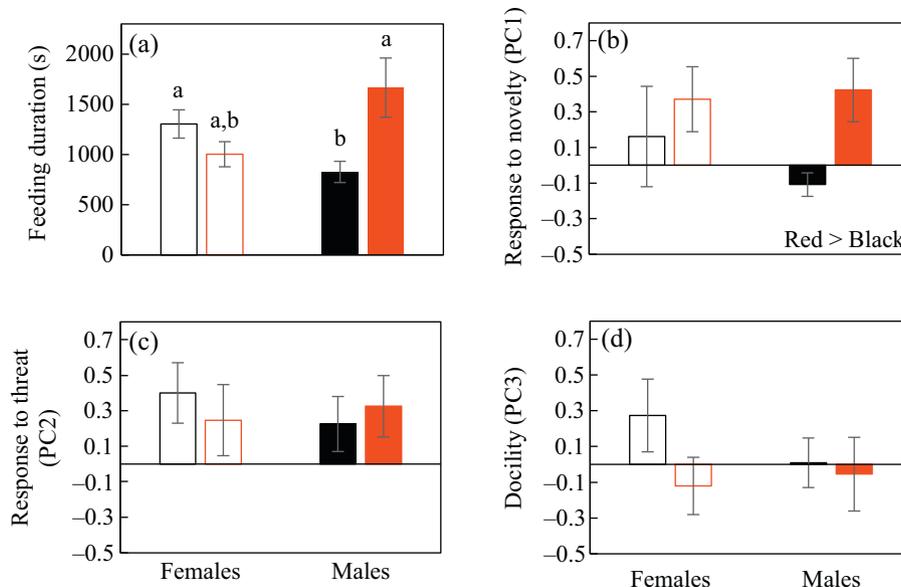
Over the whole day, the feeding behaviour of birds depended both on their colour morph and sex. Indeed, a significant interaction between sex and morph indicated that black-headed males spent approximately 40% less time feeding than black-headed females (post hoc test:  $P = 0.032$ ) and half as long as red-headed males (post hoc test:  $P = 0.037$ ). Red- and black-headed females fed similarly long (post hoc test:  $P = 0.61$ ; Fig. 2a), and females of both morphs did not differ significantly from red-headed males (all post hoc tests:  $P > 0.21$ ). Despite these differences between morphs and sexes, the feeding behaviour of birds followed the same temporal pattern across the day, with all birds feeding predominantly in the morning and in the evening (daytime<sup>2</sup>: all  $P < 0.001$ ; Table A1, Fig. 3).

Even though birds of both morphs did not differ in terms of latency to approach the novel object (black-headed birds:

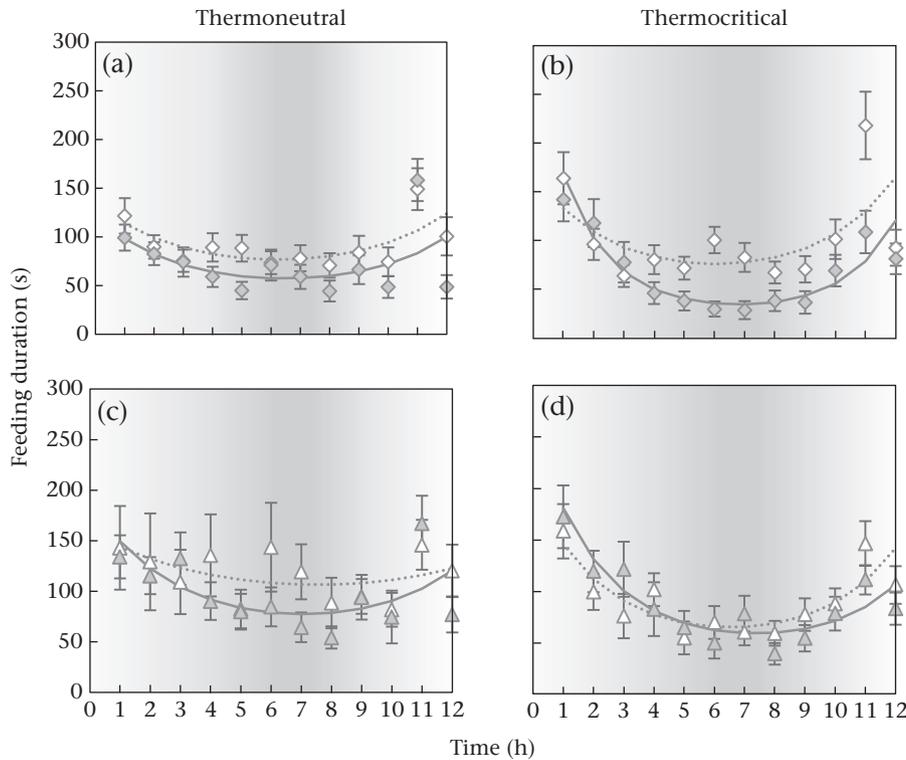
$1728 \pm 248$  s; red-headed birds:  $1458 \pm 154$  s; Table A1), red-headed individuals spent more time near a novel object than black-headed individuals, irrespective of their sex (Table 2, Fig. 2b). Moreover, neophobic effects were weaker in red-headed than in black-headed birds, as the proportion of red-headed birds feeding in the feeder did not vary significantly after adding the novel object in the feeder (from 0.95 without a novel object to 0.57 with a novel object), while the proportion of black-headed birds decreased significantly (from 0.93 to 0.34; Table A1). However, the time birds spent inside the feeder was not affected by the presence of the novel object irrespective of their morphs (Table A1). Similarly, red- and black-headed birds did not differ significantly in their response to threat or in terms of docility (Table 2, Fig. 2c and d).

### Temperature-induced Behavioural Variation in Both Morphs

Similar to initial differences before the heatwaves, we found that the feeding behaviour of birds depended on their morph and sex with black-headed males feeding the least (Table 3). Moreover, females were more susceptible to the heatwaves than males, as in contrast to males their overall feeding behaviour was strongly reduced during heatwaves, irrespective of their morph and heatwave intensity (feeding duration in females before and during the heatwaves:  $1154 \pm 95$  and  $834 \pm 79$  s, respectively, post hoc test:  $P < 0.001$ ; feeding duration in males before and during the heatwaves:  $1247 \pm 165$  and  $1113 \pm 113$  s, respectively, post hoc test:  $P = 0.56$ ; Fig. 4a). Additionally, the analyses of the two plateau hours in the middle of the day, when temperature was maximal and stable, revealed that the feeding behaviour of females was more affected at that time by thermocritical conditions, as it was reduced by approximately 60% (feeding duration in females before and during the thermocritical heatwave:  $184 \pm 25$  and  $59 \pm 14$  s, respectively; post hoc test:  $P < 0.001$ ; Table A2, Fig. 3b), while it was not significantly affected by thermoneutral conditions (feeding duration in females before and during the thermoneutral heatwave:  $151 \pm 22$  and  $130 \pm 21$  s, respectively; post hoc test:  $P = 0.49$ ; Fig. 3a). Accordingly, only the feeding behaviour of females showed a steeper curve across the day during than before the thermocritical heatwave (time \* daytime<sup>2</sup>:  $\chi^2_1 = 17.73$ ,  $P < 0.001$ ; Table A2,



**Figure 2.** (a) Initial feeding duration, (b) estimates of the response to novelty, (c) estimates of the response to threat and (d) estimates of docility in red- (red bars) and black-headed (black bars) females (open bars) and males (solid bars). Bars with different letters differ significantly.



**Figure 3.** Intraindividual feeding duration during the whole day (mean  $\pm$  SE) before (open symbols) and during (filled symbols) the heatwave. (a) Females in the thermoneutral condition, (b) females in the thermocritical condition, (c) males in the thermoneutral condition and (d) males in the thermocritical condition. The lines represent means estimated by our models before (dashed lines) and during (solid lines) the heatwaves. Time is given as the number of hours after the light was turned on in the morning.

Fig. 3). In contrast, the feeding behaviour of males did not vary because of the heatwaves during the hottest hours of the day (feeding duration in males before and during the heatwaves:  $131 \pm 23$  and  $128 \pm 30$  s, respectively; post hoc test:  $P = 0.16$ , Fig. 3c and d) and varied across the day similarly before and during the heatwaves (Table A2, Fig. 3).

Red-headed birds spent less time close to a novel object during the heatwaves, and spent as much time close to a novel object after as before the heatwaves, irrespective of their sex and the intensity of the heatwave (post hoc tests:  $P_{\text{before-during}} < 0.001$ ;  $P_{\text{during-after}} < 0.001$ ;  $P_{\text{before-after}} > 0.99$ ; Table 3, Fig. 4b). In contrast, black-headed birds only tended to spend less time close to a novel object during the heatwaves (post hoc tests:  $P_{\text{before-during}} = 0.07$ ;  $P_{\text{during-after}} = 0.15$ ;  $P_{\text{before-after}} > 0.99$ ; Table 3, Fig. 4b). Moreover, the object triggered stronger neophobic effects during the thermoneutral than during the thermocritical heatwave, irrespective of morphs. Indeed, a higher proportion of individuals did not approach the feeder because of the novel object during than before the thermoneutral heatwave (0.43 and 0.77, respectively; post hoc test:  $P < 0.001$ ) while this proportion did not differ significantly

during and before the thermocritical heatwave (0.61 and 0.54 respectively; post hoc test:  $P > 0.99$ ; Table A2, Fig. A1a, b). Finally, neophobic effects on the time spent inside the feeder did not differ between morphs and did not vary across thermal conditions (Table A2).

Overall, birds responded to threat less strongly during than before or after the heatwaves, irrespective of their intensity (post hoc tests:  $P_{\text{before-during}} < 0.001$ ;  $P_{\text{during-after}} < 0.001$ ;  $P_{\text{before-after}} > 0.99$ ; Table 3, Fig. 4c). However, docility estimates did not vary during the heatwaves, but individuals, irrespective of sex, morph and thermal conditions, became more docile after the heatwaves (post hoc tests:  $P_{\text{before-during}} = 0.11$ ;  $P_{\text{during-after}} < 0.001$ ;  $P_{\text{before-after}} = 0.007$ ; Table 3; Fig. 4d), presumably indicating habituation effects.

## DISCUSSION

Before heatwaves, Gouldian finches of different colour morphs showed differences in feeding behaviour and in their response to novelty, with black-headed males feeding the least and black-headed birds spending less time near a novel object than red-

**Table 2**  
Results of statistical tests examining the effects of sex and morph on behavioural traits before the heatwaves.<sup>a</sup>

Response variable	Diurnal feeding duration		Response to novelty (PC1)		Response to threat (PC2)		Docility estimate (PC3)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sex	0.04	0.85	0.56	0.46	0.05	0.82	0.32	0.57
Morph	2.60	0.11	<b>4.04</b>	<b>0.047</b>	0.07	0.79	1.71	0.19
Sex*Morph	<b>12.50</b>	<b>0.001</b>	0.96	0.33	0.81	0.37	0.9	0.35
Test used	GLMM with gamma distribution–log link		GLMM with gamma distribution–log link		GLMM with gamma distribution–log link		GLMM with gamma distribution–log link	

Significant results are given in bold. Analyses were made on the results of a PCA (response to novelty, response to threat and docility). GLMM: generalized linear mixed model.

<sup>a</sup> Temperature at 32 °C.

**Table 3**Results of statistical tests examining the effects of sex and morph on behavioural traits across time and thermal conditions.<sup>a</sup>

Response variable	Diurnal feeding duration		Response to novelty (PC1)		Response to threat (PC2)		Docility estimate (PC3)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sex	1.01	0.32	0.82	0.37	0.75	0.39	0.21	0.65
Morph	1.23	0.27	<b>4.44</b>	<b>0.036</b>	0.90	0.350	1.55	0.22
Time	<b>10.55</b>	<b>0.001</b>	0.60	0.55	<b>61.64</b>	<b>&lt; 0.001</b>	<b>13.58</b>	<b>&lt; 0.001</b>
Treatment	0.00	0.97	0.03	0.87	0.22	0.64	0.36	0.55
Time*Sex	<b>5.90</b>	<b>0.016</b>	1.31	0.27	1.69	0.19	0.23	0.80
Time*Morph	1.32	0.25	<b>3.36</b>	<b>0.036</b>	0.36	0.70	0.45	0.64
Time*Treatment	0.37	0.55	0.73	0.48	0.99	0.37	1.32	0.27
Treatment*Morph	0.02	0.90	1.55	0.21	1.00	0.32	0.85	0.36
Treatment*Sex			1.14	0.29	3.33	0.07	0.49	0.49
Sex*Morph	<b>10.00</b>	<b>0.002</b>	0.18	0.67	3.39	0.07	0.86	0.36
Time*Treatment*Morph	1.14	0.29	1.21	0.30	2.72	0.07	0.87	0.42
Time*Treatment*Sex			0.99	0.37	0.51	0.60	0.66	0.52
Time*Sex*Morph			0.80	0.45	1.06	0.35	0.01	0.99
Treatment*Sex*Morph			0.57	0.45	0.47	0.49	0.05	0.82
Time*Treatment*Sex*Morph			0.03	0.98	1.63	0.20	0.68	0.51
Test used	GLMM with repeated measures—gamma distribution log link		GLMM with repeated measures—gamma distribution log link		GLMM with repeated measures—gamma distribution log link		GLMM with repeated measures—gamma distribution log link	

Significant results are given in bold. Analyses were made on the results of a PCA (response to novelty, response to threat and docility). GLMM: generalized linear mixed model.

<sup>a</sup> Temperatures at 32 °C before the heatwaves, 36 °C or 39 °C during the heatwaves and 32 °C after the heatwaves.

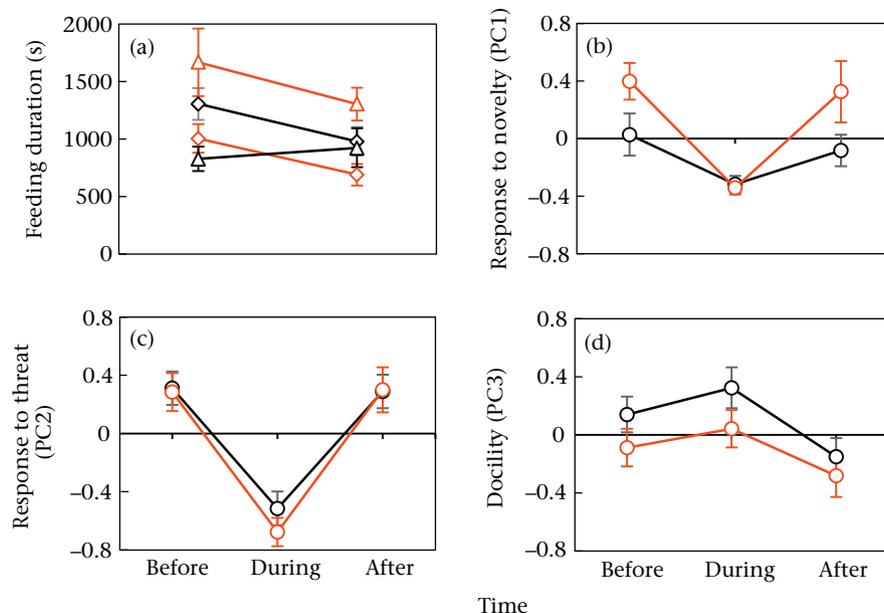
headed birds. However, no differences in behavioural reactivity could be highlighted between morphs. During heatwaves, feeding behaviour and response to threat were similarly reduced in both morphs. However, the response to novelty between thermal conditions was morph specific, with red-headed birds being more affected by heatwaves than black-headed birds.

#### Initial Behavioural Differences Between Morphs

We found that black-headed males spent half as long feeding as red-headed males. This suggests that black-headed males have lower energy requirements, presumably because they also have lower testosterone levels than red-headed birds (Pryke et al., 2012). However, other factors may explain these lower energy requirements, as black-headed males also fed less than black-headed females in our study. Irrespective of the underlying mechanisms, lower energy requirements in black-headed males

were expected to be reflected by a steeper U-shaped feeding behaviour across the day, as feeding requirements should be rapidly met in the morning and in the evening (Morton, 1967). However, the feeding behaviour of black-headed males followed the same U-shape pattern across the day as in other birds and as already observed in this species (Beaulieu & Schaefer, 2014 in black-headed individuals only). Interestingly, Gouldian finches in the study by Beaulieu and Schaefer (2014) spent four times as long feeding as in the current study. Such differences are probably related to the different thermal conditions used in the studies (25 versus 32 °C), with conditions below thermoneutrality being associated with higher energy requirements (Burton & Weathers, 2003). This suggests that overall energy requirements in our study were low (as expected for captive birds exposed to thermoneutral conditions).

In our study, red-headed birds spent more time near a novel object and experienced lower neophobic effects than black-headed



**Figure 4.** (a) Feeding duration, (b) estimates of the response to novelty, (c) estimates of the response to threat and (d) estimates of docility variation before, during and after the heatwaves in red- (red lines) and black-headed (black lines) individuals (females:  $\diamond$ ; males:  $\Delta$ ; both sexes:  $\circ$ ).

birds, suggesting that they are bolder than black-headed birds. These results are in sharp contrast with the results of previous studies showing that red-headed Gouldian finches were less bold than black-headed birds (King et al., 2015; Mettke-Hofmann, 2012; Williams et al., 2012). As birds in our study were not studied in the same social environment as in previous studies (birds in the presence of a sexual partner of the same morph in our study versus individual birds or birds in the presence of another bird of the same sex and of the opposite morph in previous studies), the social exposure experienced by Gouldian finches may differently affect their response to novelty with red-headed birds becoming more exploratory in contact with red-headed birds (as in our study) but less exploratory when alone or in contact with black-headed birds (previous studies). Accordingly, in the neophilia test, the latency of red-headed birds to approach a novel object was much lower in our study (where birds were in contact with a red-headed partner) than in Williams et al.'s (2012) study (where birds were individually tested; approximately 1500 versus 2300 s, respectively), while the latency of black-headed birds to approach a novel object was exactly the same in both studies (approximately 1500 s). Another possible reason why the results of our study differ from those of previous studies may come from different housing conditions (birds kept at a temperature close to the lower critical temperature in our study versus birds kept at lower temperatures in previous studies) which may alter the physiology and behaviour of birds (Beaulieu, 2016). Irrespective of the reasons for discrepancies between studies, these differences interestingly suggest that the behaviour of red- and black-headed Gouldian finches do not differ consistently but that behavioural differences between morphs vary depending presumably on social and environmental conditions (see also Fig. A2 and A3 for the correlation analysis between behavioural traits within each morph, sex and thermal condition).

#### *Temperature-induced Behavioural Variation in Both Morphs*

Owing to the activation of thermoregulatory processes, Gouldian finches were expected to present a higher energetic debt after being exposed to the thermocritical than to the thermoneutral heatwave (Burton & Weathers, 2003). This hypothesis is reinforced by the fact that Gouldian finches appeared unable to feed more during the thermocritical heatwave despite the need to compensate for these higher energy requirements. A possible explanation for this lack of feeding adjustments is that birds were unable to increase their activity to feed more, and instead favoured heat-dissipating behaviours (e.g. panting, wing spreading; Whitfield, Smit, McKechnie, & Wolf, 2015). Moreover, only females fed less in the middle of the day during the thermocritical heatwave than before the heatwave, suggesting that they were more affected than males by thermocritical conditions (potentially because of different thermoneutral ranges between males and females).

The overall decreased activity of birds during heatwaves was confirmed by their simultaneous reduced response to novelty and to threat. Moreover, a stronger activity decrease under thermocritical conditions may explain why neophobic effects were weaker under such conditions than under thermoneutral conditions. Interestingly, response to novelty varied more strongly in red-headed than in black-headed birds. These differential effects between morphs are unlikely to be directly mediated by thermoregulatory differences due to pigmentation per se, as colour differences between morphs occur only over a minor surface of their body (approximately 4%). This is in contrast to other polymorphic species (e.g. snakes, damselflies, butterflies, birds) where larger differences in colour may confer different thermoregulatory advantages and differently affect the overall behaviour of animals of different morphs (Forsman, 2000; Watt, 1969). In Gouldian finches, where pigmentation differences

between morphs are minimal, differential temperature effects on the behaviour of red- and black-headed birds must therefore be indirect. For instance, behavioural changes may be mediated by stronger changes in neurotransmitter (e.g. serotonin) production at the central level in red-headed birds exposed to variable conditions.

We found that temperatures affected neophobic effects only for the proportion of red-headed birds visiting the novel object but not for the time they spent close to the novel object. This suggests that thermal conditions acted on the decision of birds to approach a novel object (i.e. their interest in the novel object) but not on their capacity to tolerate the proximity of a novel object. This hypothesis is reinforced by the fact that, across studies, the proportion of birds visiting the novel object in a neophilia test (i.e. the proportion of birds actively deciding to approach the novel object) decreases with increasing temperatures (24 °C: 0.75; 32 °C: 0.54; 36 °C: 0.20; 39 °C: 0.20; Williams et al., 2012, this study). Overall, this suggests that the exploratory propensity of Gouldian finches, and especially red-headed birds, is directly and negatively related to the thermal conditions they experience, as it decreases at high temperatures.

#### *Conclusion*

There has been little agreement on the advantage of intraspecific variation in behaviour for species adaptation to environmental change (Bolton et al., 2015, 2016; Forsman, 2016; Forsman, Ahnesjö, Caesar, & Karlsson, 2008). Here, we found that the effects of thermal conditions on the behavioural balance between colour morphs in Gouldian finches were inconsistent, depending on behavioural traits, and were mostly visible on their response to novelty. Whether this altered behavioural balance between morphs in response to changing conditions affects differences in reproductive performance or survival, leading to reduced fitness of one morph relative to the other, remains to be investigated. If so, such changes will affect not only morph proportions but also the overall interaction dynamics within populations, species and habitats.

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**Appendix**

**Table A1**  
Results of statistical tests examining the effects of sex, morph (and daytime) on behavioural traits before the heatwaves<sup>1</sup>

Response variable	Plateau feeding duration		Feeding duration across the day				Object neophilia latency		Δ Object neophobia duration		Δ Object neophobia proportion		Δ Object neophobia latency	
			Females		Males		F	P	F	P	F	P	F	P
Explanatory variables	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Sex	0.38	0.54					2.80	0.11	0.03	0.87	0.12	0.73	0.001	0.98
Morph	0.31	0.58			<b>5.32</b>	<b>0.021</b>	2.79	0.11	0.31	0.58	<b>5.11</b>	<b>0.026</b>	2.84	0.10
Daytime			<b>16.15</b>	<b>&lt; 0.001</b>	<b>24.48</b>	<b>&lt; 0.001</b>								
Daytime <sup>2</sup>			<b>12.46</b>	<b>&lt; 0.001</b>	<b>31.93</b>	<b>&lt; 0.001</b>								
Sex*Morph	2.87	0.09					17.76	0.08	2.55	0.11	0.04	0.85	0.02	0.90
Morph*Daytime			0.02	0.90	0.01	0.91								
Morph*Daytime <sup>2</sup>			0.02	0.89	0.41	0.52								
Test used	GLMM with gamma distribution–log link		GLMM with repeated measures–negative binomial distribution log link		GLMM with repeated measures–negative binomial distribution log link		LMM		GLMM with gamma distribution–log link		GLMM with binomial distribution–logit link		LMM	

Significant results are given in bold. GLMM: generalized linear mixed model; LMM: linear mixed model.

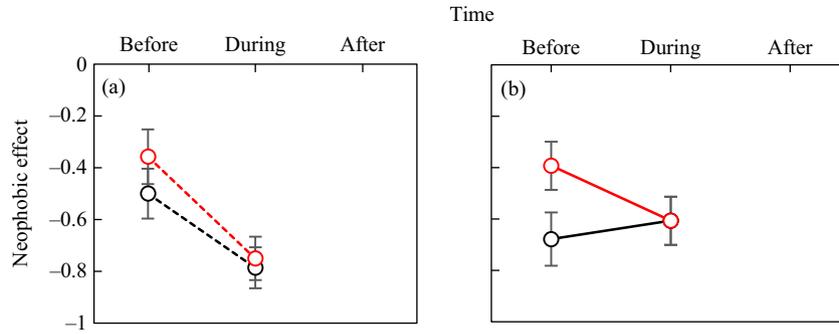
<sup>1</sup> Temperature at 32 °C.

**Table A2**  
Results of statistical tests examining the effects of sex and morph on behavioural traits across time and thermal conditions<sup>1</sup>

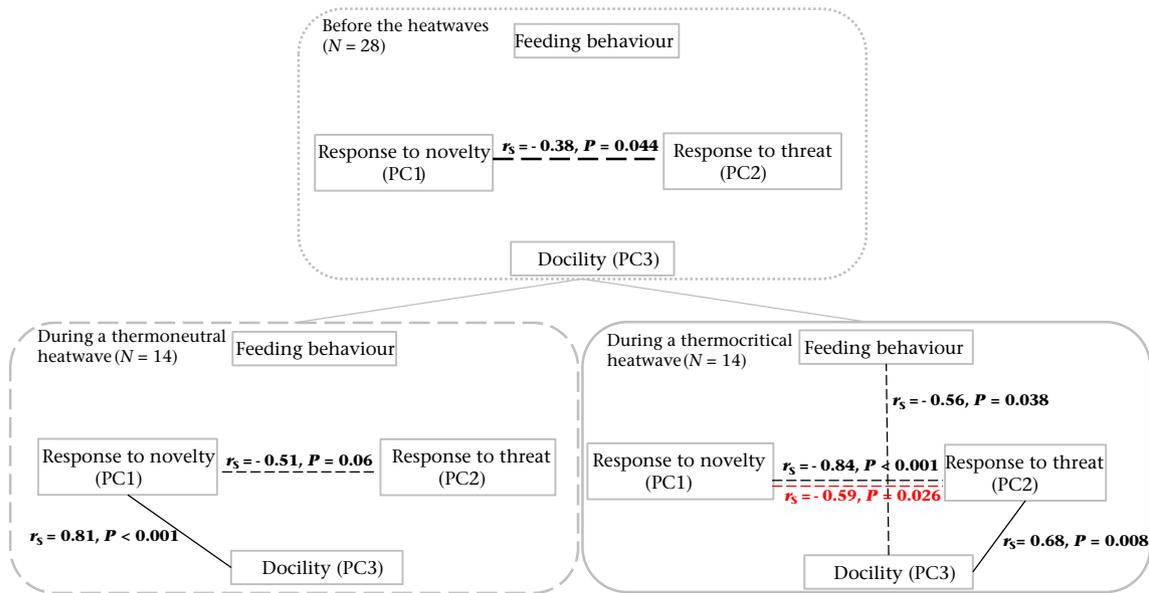
Response variable	Plateau feeding duration		Feeding duration across the day								Δ Object neophobia duration		Δ Object neophobia proportion	
			Females in TC		Females in TN		Males in TC		Males in TN		F	P	F	P
Explanatory variables	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Sex	0.94	0.33									0.79	0.38	0.18	0.68
Morph	1.32	0.25									0.12	0.73	3.19	0.08
Time	<b>18.59</b>	<b>&lt; 0.001</b>	<b>9.39</b>	<b>0.002</b>	0.01	0.92	3.54	0.06	1.51	0.22	0.002	0.97	<b>7.08</b>	<b>0.008</b>
Daytime			<b>87.17</b>	<b>&lt; 0.001</b>	3.80	0.05	<b>126.32</b>	<b>&lt; 0.001</b>	<b>12.28</b>	<b>&lt; 0.001</b>				
Daytime <sup>2</sup>			<b>80.99</b>	<b>&lt; 0.001</b>	1.93	0.17	<b>114.54</b>	<b>&lt; 0.001</b>	<b>8.53</b>	<b>0.004</b>				
Treatment	<b>4.36</b>	<b>0.038</b>									0.03	0.87	0.40	0.53
Time*Morph	0.05	0.82									0.40	0.53	1.54	0.22
Time*Treatment	3.85	0.05									0.06	0.81	<b>5.08</b>	<b>0.025</b>
Treatment*Morph	0.98	0.32									0.001	0.99	0.40	0.53
Treatment*Sex	0.05	0.82									0.05	0.83	0.19	0.66
Sex*Morph	<b>7.96</b>	<b>0.005</b>									3.31	0.07	2.27	0.13
Time*Sex	<b>5.52</b>	<b>0.020</b>									0.63	0.43	0.02	0.90
Time*Daytime			<b>19.96</b>	<b>&lt; 0.001</b>	0.43	0.51	3.34	0.07	0.48	0.49				
Time*Daytime <sup>2</sup>			<b>13.04</b>	<b>&lt; 0.001</b>	0.26	0.61	2.71	0.10	0.01	0.91				
Time*Treatment	0.63	0.43									0.06	0.82	1.38	0.24
Time*Treatment*Morph														
Time*Treatment*Sex	<b>16.95</b>	<b>&lt; 0.001</b>									<b>7.72</b>	<b>0.006</b>	0.01	0.91
Treatment*Sex*Morph											0.21	0.65	1.92	0.17
Treatment*Sex*Morph											0.07	0.79	2.30	0.13
Test used	GLMM <sup>2</sup> with repeated measures–gamma distribution log link		GLMM <sup>2</sup> with repeated measures–negative binomial distribution log link		GLMM <sup>2</sup> with repeated measures–negative binomial distribution log link		GLMM <sup>2</sup> with repeated measures–negative binomial distribution log link		GLMM <sup>2</sup> with repeated measures–negative binomial distribution log link		GLMM <sup>2</sup> with repeated measures–gamma distribution log link		GLMM <sup>2</sup> with repeated measures–binomial distribution logit link	

Significant results are given in bold. TC: thermocritical conditions; TN: thermoneutral conditions. GLMM: generalized linear mixed model.

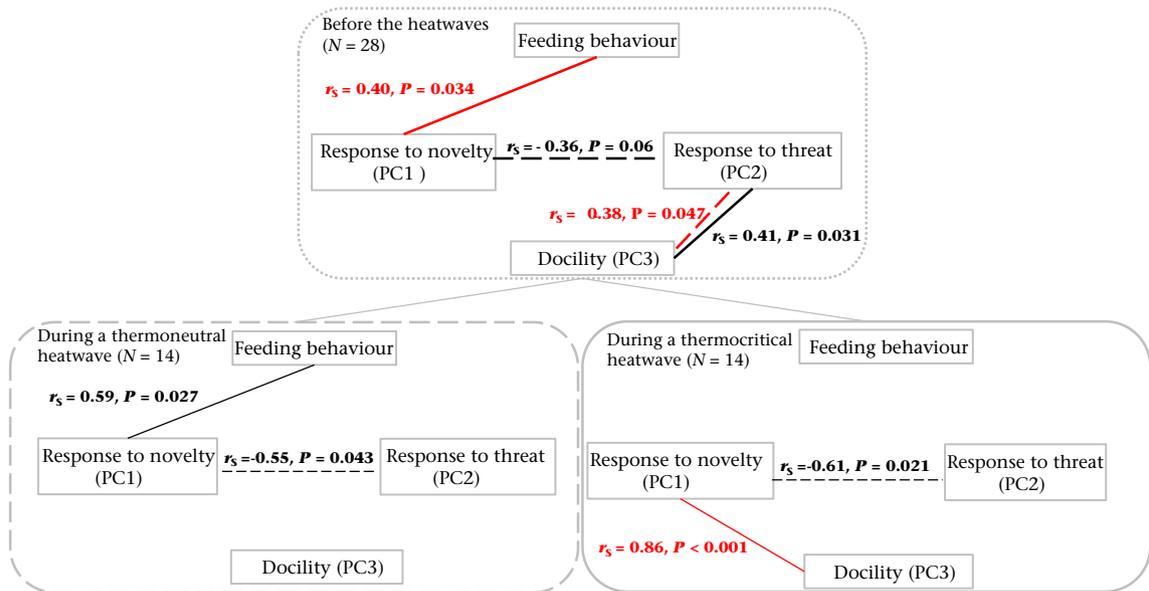
<sup>1</sup> Temperatures at 32 °C before the heatwaves, 36 °C or 39 °C during the heatwaves and 32 °C after the heatwaves.



**Figure A1.** Difference in the proportion of red- (red lines) and black-headed (black lines) individuals feeding in a feeder with and without novel objects (neophobic effects) before, during and after the (a) thermoneutral and (b) thermocritical heatwaves.



**Figure A2.** Trait correlation variation in females between treatments and morphs (red lines: red-headed; black lines: black-headed) before and during the heatwaves. Solid lines: positive correlations; dashed lines: negative correlations.



**Figure A3.** Trait correlation variation in males between treatments and morphs (red lines: red-headed; black lines: black-headed) before and during the heatwaves. Solid lines: positive correlations; dashed lines: negative correlations.



## **Study 2: Morph- and sex-specific effects of challenging conditions on maintenance parameters in the Gouldian finch**

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## RESEARCH ARTICLE

# Morph- and sex-specific effects of challenging conditions on maintenance parameters in the Gouldian finch

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## ABSTRACT

Intraspecific discrete polymorphism is associated with the use of alternative life-history strategies, reflected by distinct reproductive or copying strategies in individuals of different morphs. Yet, morph-specific costs and benefits related to different life-history strategies remain unclear. Here, we examined in the polymorphic Gouldian finch (*Erythrura gouldiae*) whether markers of somatic maintenance (body mass, oxidative status and telomere length) differed between red- and black-headed birds under energetically demanding conditions (during heatwaves of different intensity, and during moult or breeding following heatwaves). During heatwaves, red-headed birds showed a homogeneous response, as males and females initially tended to gain mass and had reduced plasma hydroperoxide levels (a marker of oxidative damage) irrespective of heatwave intensity. In contrast, black-headed birds showed a stronger and more heterogeneous response, as black-headed males gained mass at the beginning of the thermoneutral heatwave and showed stable oxidative status, whereas black-headed females lost mass and tended to show higher hydroperoxide levels at the end of the thermocritical heatwave. Following heatwaves, we found morph-specific oxidative costs owing to moult or reproduction, with oxidative markers varying only in black-headed birds. Again, oxidative markers varied differently in black-headed males and females, as plasma antioxidant capacity decreased in moulting or breeding females, whereas males showed higher hydroperoxide levels with larger broods. For the first time, our study highlights that within polymorphic species, some individuals appear more vulnerable than others when coping with energetically demanding conditions. In the context of climate change, such differential effects may ultimately alter the currently observed balance between morphs and sexes within natural populations.

**KEY WORDS:** Body mass variation, Endogenous traits, Inter-morph variability, Moulting-breeding strategy, Oxidative status, Telomere shortening

## INTRODUCTION

The regulation of self-maintenance (i.e. the ability of functioning and surviving) parameters is usually assumed to be constant and generalisable across individuals within the same species. For instance, immune efficiency, moulting costs and homeostasis (e.g. redox balance) are typically defined intraspecifically (Valladares

et al., 2014). Physiological thermal tolerance is also typically defined intraspecifically although thermoneutral ranges may not necessarily be identical between individuals. Indeed, because of extrinsic (e.g. early-life experience, social environment) or intrinsic factors (e.g. genetic, maternal effects), intraspecific phenotypic variability may result in a variety of maintenance strategies within the same species (Kempnaers et al., 2008; Schwartz and Bronikowski, 2013). To reduce such interindividual physiological differences, animals may differently alter their behaviour. For example, individuals unable to adjust their physiology to extreme temperatures may still be able to keep maintenance parameters stable by reducing their overall physical activity (Beaulieu et al., 2015a; Wolf, 2000).

In polymorphic species, morphological traits typically covary with life-history traits, as reflected by distinct reproductive, copying or foraging strategies in each morph (Roulin, 2004). Such phenotypic differences between morphs may result in different maintenance costs under stressful conditions. For instance, in the polymorphic Australian painted dragon (*Ctenophorus pictus*) (Rollings et al., 2017), morph-specific reproductive strategies are associated with different maintenance costs in terms of telomere dynamics. One mechanism for the coexistence of distinct phenotypes within polymorphic species is the use of alternative life-history strategies, with the highest fitness advantages and minimal maintenance costs for each morph under different conditions (e.g. ecological conditions or morph frequency). Such intraspecific differences in maintenance investment owing to the use of different life-history strategies may be exacerbated under extreme environmental conditions if some individuals of a given morph are more affected than others by such conditions (Dingemanse et al., 2010; Schwartz and Bronikowski, 2013). Intraspecific phenotypic variability in polymorphic species therefore appears inseparable from environmental variability. However, the examination of morph differences in life-history strategies and their maintenance costs under variable environmental conditions remains mostly overlooked.

Here, we tested whether challenging conditions affected differently the somatic maintenance (body mass, oxidative status and telomere length) of Gouldian finches (*Erythrura gouldiae*) of the two main colour morphs (red- and black-headed birds). More specifically, birds were first exposed to either a thermocritical heatwave (temperature above thermoneutrality, associated with increased energetic needs for thermoregulation) or a thermoneutral heatwave (temperature within thermoneutrality, associated with minimal energetic needs for thermoregulation). Because of the higher energetic requirements expected under thermocritical conditions, we first expected maintenance parameters to be more strongly affected by thermocritical than by thermoneutral conditions (i.e. accentuated body mass loss, lower antioxidant defences, higher oxidative damage and faster telomere shortening under thermocritical than under thermoneutral conditions). We then examined whether such maintenance differences between thermal treatments led to

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subsequent differences in moult and breeding initiation between both morphs, as the exposure to challenging conditions, such as thermocritical conditions, may have postponed effects on life-history decisions (Harrison et al., 2010). Birds were therefore expected to be more likely to refrain from moulting or breeding after a thermocritical heatwave than after a thermoneutral heatwave, as these activities are themselves energetically demanding and may force animals into an allostatic overload (i.e. energy requirements exceed energy supply) that would accentuate the deterioration of maintenance parameters (Lindström et al., 1993; Monaghan and Nager, 1997; Murphy and King, 1992). Because red-headed Gouldian finches have been described as being more aggressive and dominant, and feeding more (at least males) than black-headed birds (Fragueira and Beaulieu, 2019; Pryke et al., 2012; Williams et al., 2012), the effects of thermocritical conditions on maintenance parameters were first expected to be stronger in red-headed birds, with presumably higher energy requirements than in black-headed birds. However, because red-headed birds decrease their activity more strongly during heatwaves than black-headed birds (Fragueira and Beaulieu, 2019), they may be able to minimise the effects of thermocritical conditions on maintenance parameters. In contrast, the maintenance of black-headed birds, altering their behaviour less strongly during heatwaves, may be physiologically more affected by thermocritical conditions, which in turn may reduce their capacity to moult or breed.

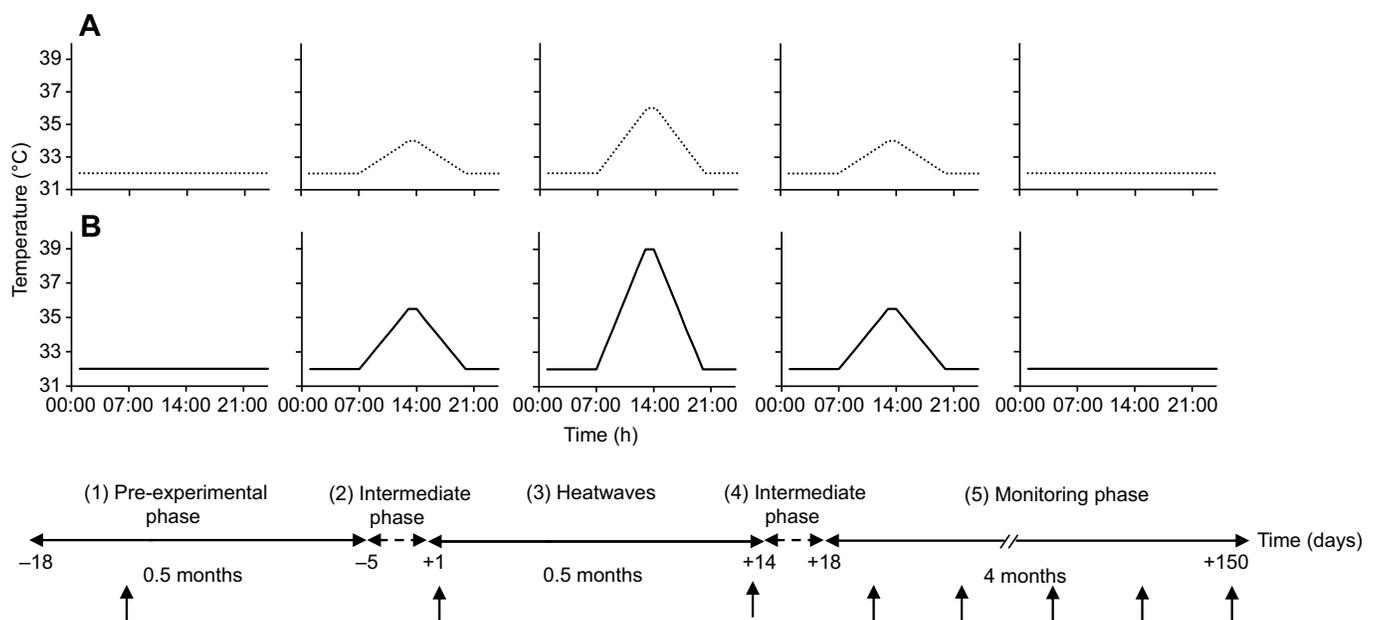
## MATERIALS AND METHODS

### Experimental design

A total of 112 wild-type, young adult Gouldian finches [*Erythrura gouldiae* (Gould 1844); approximately 1 year old] were obtained from local breeders. Across our study, birds had access to water, shell grit, cuttlefish bones and mixed seeds (Deli Nature 40 - Exoten Basis, Beyers, Belgium) *ad libitum*, and were given a bath daily. Monomorphic breeding pairs were initially randomly assigned into experimental cages (99.6×48.0×52.0 cm) in two different climate

chambers (2.8×4.2×2.5 m; 7 red-headed and 7 black-headed pairs in each climate chamber in two sessions) set at 32±1°C (i.e. within the thermoneutral range of Gouldian finches: 31.8–38.0°C; Burton and Weathers, 2003), with 50±5% humidity and a 12 h:12 h light:dark cycle. Birds were allowed to habituate to these conditions for at least 2 weeks before measurements. Then, all birds were exposed to the same experimental event sequence (Fig. 1): (1) a pre-experimental phase at 32°C for 14 days (day –18 to day –5), followed by (2) an intermediate phase at 34°C (thermoneutral intermediate treatment) or 35.5°C (thermocritical intermediate treatment) for 4 days (day –4 to day –1), (3) a heatwave at 36°C (thermoneutral heatwave) or 39°C (thermocritical heatwave) for 14 days (day 1 to day 14), (4) an intermediate phase similar to the intermediate phase before the heatwave (34°C or 35.5°C; day 15 to day 18) and (5) a monitoring phase at 32°C for 4 months when birds could moult or breed (day 19 to day 150). During both intermediate phases (phases 2 and 4) and during the heatwaves (phase 3), birds experienced maximal temperatures only for 2 h each day from 12:00 to 14:00 h, with a progressive temperature increase before and temperature decrease after these 2 h of exposure (Fig. 1). We progressively exposed birds to temperature changes during intermediate phases so they would have time to adjust their behaviour and physiology to the new conditions. During the heatwaves, maximal temperatures were either within or beyond thermoneutrality, whereas temperature was always within this range otherwise. The 14-day-long heatwaves that we induced were designed to reflect long heatwaves that tropical birds, such as Gouldian finches, will likely experience in the future in Australia (IPCC, 2015).

After this experimental phase, each cage was supplemented with a nest box, nest material, seed sprouts and egg food (Witte Molen Eggfood, Witte Molen, Netherlands), and the light:dark cycle was changed to 13 h:11 h light:dark. Each bird had then the possibility to breed or moult. To monitor moult (i.e. start and end of moult) and breeding (i.e. clutch initiation, clutch size and brood size) status, each cage and nest was checked daily. We defined individual



**Fig. 1. Chronogram of the experiment conducted under the different thermal conditions.** (A) Thermoneutral (dashed lines) and (B) thermocritical (solid lines). Arrows along the time axis represent when Gouldian finches were weighed and sampled. Each graph represents temperature variation over 24 h before the heatwaves under pre-experimental conditions (1), during the intermediate phases (2 and 4), during the heatwaves (3) and during the monitoring phase (5). This experiment was repeated twice in two different sessions conducted in two consecutive years.

moulting periods from the time birds started losing body and/or wing feathers until complete feather replacement, and breeding periods from the first egg being laid until chick fledging. For birds that both moulted and bred consecutively, we only considered the first event following heatwaves in the analyses. Moreover, three males for which moult and breeding overlapped were excluded from our analyses, as the respective effects could not be distinguished.

### Maintenance measurements

Body mass, markers of oxidative status and telomere length were measured longitudinally to examine how birds regulated their maintenance across time. Temporal variation in body mass reflects how birds regulate their energy balance (Rozman et al., 2003) whereas temporal variation in oxidative status may reflect how they prioritise self-maintenance over other functions, such as reproduction (Beaulieu et al., 2015b). For instance, antioxidant defences decrease with brood size in parent zebra finches (*Taeniopygia guttata*; Alonso-Alvarez et al., 2006; Wiersma et al., 2004), thereby indicating that they sacrifice their maintenance for their current reproduction (note that this pattern is not necessarily seen in all species; Stier et al., 2012). Low antioxidant defences may lead to an imbalance between pro-oxidants [e.g. reactive oxygen species (ROS)] and antioxidant defences, and to increased levels of oxidative damage on key biological molecules, such as lipids, proteins and DNA sequences, including telomeres (i.e. non-coding sequences at the end of eukaryotic chromosomes stabilizing the genome) (Finkel and Holbrook, 2000). Accordingly, high ROS levels have been described *in vitro* as accelerating telomere shortening (Von Zglinicki, 2002), which in turn may lead to accelerated senescence and reduced survival probability (Bize et al., 2009). Variation in markers of oxidative status and telomere dynamics can therefore be used to examine how animals prioritise their maintenance (Young, 2018).

Blood was collected from each bird across the experimental period (once before the heatwaves, twice during the heatwaves, then monthly across the 4 months of monitoring; Fig. 1) at a fixed time (13:00–16:00 h). Immediately after capture, blood (ca. 80  $\mu$ l) was taken from the brachial vein, and then the birds were weighed ( $\pm 0.1$  g). Blood was centrifuged for 10 min at 4000 g, and plasma and erythrocytes were separated and stored at  $-80^{\circ}\text{C}$  until analysis.

To examine plasma oxidative status, we measured one marker of antioxidant defence (total plasma antioxidant capacity using the OXY-adsorbent test; Diacron International, Grosseto, Italy) and one marker of oxidative damage (plasma hydroperoxide levels using the d-ROM test; Diacron International). Both markers of oxidative status have previously been measured in the plasma of Gouldian finches and other bird species (Beaulieu et al., 2014; Beaulieu and Schaefer, 2014; for measurement principles, see Costantini, 2016). In the assays, samples were balanced across plates for morph, sex and treatment. Intra- and inter-plate coefficients of variation for the OXY and ROM were 15% ( $n=48$  replicates) and 7% ( $n=42$  replicates), and 16% ( $n=42$  replicates) and 9% ( $n=42$  replicates), respectively, which fall within the range reported in Costantini (2011).

Telomere length was measured in erythrocytes before the heatwaves and at the end of the monitoring period using the terminal restriction fragment (TRF) procedure as previously described in birds (Salomons et al., 2009) but adjusted for Gouldian finches. This procedure consists in extracting DNA from ca. 5  $\mu$ l of red blood cells using the CHEF Mammalian Genomic DNA Plug kit (Bio-Rad Laboratories). The extracted DNA in agarose plugs was then digested overnight at  $37^{\circ}\text{C}$  with a mixture of the restriction enzymes *Hinf*I (15 U), *Hind*III (30 U) and *Msp*I (30 U) in NEB2 buffer. The restricted DNA and the  $^{32}\text{P}$

end-labelled size standards [(a) 1 kb DNA ladder, New England Biolabs; Molecular Weight Marker XV, Roche Diagnostics, Basel, Switzerland, range 2.4–48.5 kb; (b) Ultra-long DNA ladder, PFG Marker I, NEB Midrange] were separated through a 0.8% agarose gel by pulsed-field gel electrophoresis at  $14^{\circ}\text{C}$  for 22 h with  $4.8\text{ V cm}^{-1}$ . In a second step, gels were dried (gel dryer model 538, Bio-Rad Laboratories) and hybridised overnight at  $37^{\circ}\text{C}$  with a  $^{32}\text{P}$ -end-labelled oligonucleotide [(5'-CCCTAA-3')] bound to the single-stranded overhang of telomeres. Finally, gels were exposed overnight to a phosphor screen (PerkinElmer, Waltham, MA, USA), and the radioactive signal, obtained from the phosphor image (Cyclone<sup>TM</sup> Storage Phosphor System, PerkinElmer), could be further analysed through densitometry using ImageJ 1.50i. The lower limit of the measurement was set at 2.4 kb, corresponding to the smallest observable band of the 1 kb DNA ladder and the upper limit at 195 kb, corresponding to the highest observable band of the Ultra-long DNA ladder. The background, set between 200 and 250 kb, was subtracted from the optical density measurements. The inter-gel coefficient of variation was 10.5%. All raw data generated in the study are available as Dataset 1.

### Statistics

First, we analysed variation in body mass and oxidative status during the heatwaves using general (plasma antioxidant capacity) and generalised (body mass, plasma hydroperoxide levels; gamma-distributed) linear mixed models with thermal treatment (thermoneutral, thermocritical), sex (male, female), morph (black- and red-headed) and time (day  $-10$ , day  $+1$ , day  $+14$ ) as independent factors, and individual birds nested in their cage as a random factor.

For birds that started moult or breeding, we analysed whether the thermal treatment affected moult/breeding strategy using a generalised linear mixed model with a binary function with moult–breeding status as a dependent factor (moult: 0, breeding: 1), thermal treatment (thermoneutral, thermocritical), sex (male, female) and morph (black-, red-headed) as independent factors, and cage as a random factor. Birds were considered breeding when at least one egg was laid.

To examine whether maintenance parameters were differently affected by moult and breeding in both morphs, we used general (antioxidant capacity, log telomere length) and generalised (body mass, plasma hydroperoxide levels; gamma-distributed) linear mixed models with morph, sex, thermal treatment, time (before, during moulting or breeding) and moult/breeding strategy (not moulting/not breeding; moult; breeding) as independent factors, and birds nested in their cage as a random factor. In these analyses, we only considered birds with fledglings (i.e. having completed reproduction) as breeding birds, in order for all breeding individuals to be comparable between each other in terms of breeding advancement and to cover the whole reproduction period. We also examined whether brood size had an impact on the temporal dynamics of the measured physiological traits [i.e. difference ( $\Delta$ ) between before and during breeding] using general ( $\Delta$ antioxidant capacity) and generalised ( $\Delta$ body mass,  $\Delta$ hydroperoxide levels and  $\Delta$ telomere length corrected for regression to the mean) mixed models with sex, morph and brood size as independent factors, and cage as a random factor.

Final models were selected using the corrected Akaike's information criterion (AICc) in a backwards-deletion procedure. When the factor 'morph' was involved in a significant three- or four-way interaction, we repeated our models for each morph separately to clarify how each morph responded across conditions. For all other interactions, *post hoc* comparisons were performed using Benjamini–Hochberg corrections. All analyses were performed

using SPSS (v. 22). For clarity, results (given as means±s.e.m.) are presented depending on the statistical outcomes (i.e. depending on significant factors or interactions between factors).

### Ethics

The animal care and experimentation complied with the EU animal experimentation regulations approved by the ethical committee of Mecklenburg-Western Pomerania (LALLF: permit no. 7221.3-2-041/15; ZooBea3936/3/15).

## RESULTS

### Maintenance status during heatwaves (day –10 to day +14)

During the heatwaves, body mass varied depending on morph, sex and heatwave intensity (Table 1). After repeating our model within each morph, we found that red-headed birds tended to gain mass at the beginning of the heatwaves and irrespective of their intensity (time:  $F_{2,155}=13.84$ ,  $P<0.001$ ; *post hoc* test:  $P_{-10-1d}=0.07$ ; Table S1, Fig. 2A), but they lost this gain afterwards ( $P_{1-14d}<0.001$ ). Similarly, black-headed males gained mass at the beginning of the heatwave but only during the thermoneutral heatwave (thermal treatment×time×sex:  $P=0.034$ ; *post hoc* test:  $P_{-10-1d}=0.049$ ; Table S1). In contrast, the body mass of black-headed males during the thermocritical heatwave and the body mass of females during the thermoneutral heatwave remained constant across time (all  $P>0.17$ ). Black-headed females during the thermocritical heatwave also maintained their body mass constant at the beginning of the thermocritical heatwave (*post hoc* test:  $P_{-10-1d}=0.29$ ), but their body mass significantly decreased afterwards (*post hoc* test:  $P_{1-14d}=0.03$ ,  $P_{-10-14d}=0.045$ ; Table S1, Fig. 2B).

Antioxidant capacity remained unchanged across heatwaves, irrespective of morph and sex (Table 1, Fig. 2C,D). However, similar to body mass, plasma hydroperoxide levels tended to vary differently across time depending on morph, sex and heatwave intensity (Table 1). After repeating our model within each morph, we found that plasma hydroperoxide levels varied across time in red-headed birds (time:  $F_{2,155}=3.40$ ,  $P=0.036$ ; Table S1), as they slightly decreased at the beginning of the heatwaves ( $P_{-10-1d}=0.048$ ) and returned to initial levels afterwards ( $P_{1-14d}=0.57$ ,  $P_{-10-14d}=0.09$ ;

Fig. 2E). Plasma hydroperoxide levels also appeared to vary across time in black-headed birds, but depending on sex and heatwave intensity (thermal treatment×sex×time:  $F_{2,156}=3.17$ ,  $P=0.045$ ; Table S1). Indeed, hydroperoxide levels tended to increase in black-headed females at the end of the thermocritical heatwave (*post hoc* test:  $P_{-10-14d}=0.07$ ,  $P_{1-14d}=0.10$ ) whereas they did not vary in other black-headed birds (all other *post hoc* tests:  $P>0.10$ ; Fig. 2F).

### Effects of moult and breeding on maintenance status (day +21 to day +150)

In the monitoring period, 95 birds out of the 112 birds included in our study (85%) moulted or bred (i.e. produced at least one egg). Among these birds, the proportion of birds moulting (53%) or reproducing first (47%) did not differ between black- and red-headed birds (Table 1). Irrespective of morph and thermal treatment, maintenance parameters did not significantly differ between pre-moulting and pre-breeding birds (body mass:  $F_{1,80}=1.91$ ,  $P=0.17$ ; antioxidant capacity:  $F_{1,46}=0.38$ ,  $P=0.54$ ; hydroperoxide levels:  $F_{1,78}=0.88$ ,  $P=0.35$ ), even though telomeres tended to be longer in pre-moulting than in pre-breeding birds ( $F_{1,48}=3.47$ ,  $P=0.07$ ; Table S2).

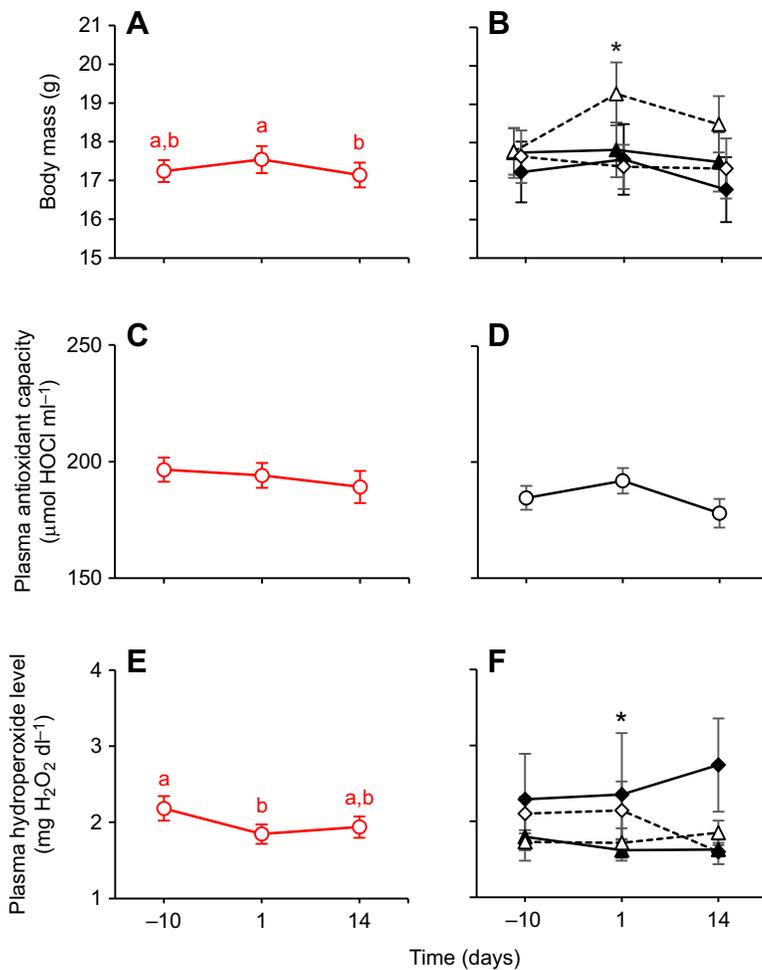
Body mass varied differently through time in moulting and breeding birds (Table 2). Indeed, moulting birds kept their body mass stable ( $P_{\text{before-during moult}}=0.55$ ) and comparable to that of non-moulting/non-breeding birds ( $P=0.63$ ) whereas breeding birds exhibited a significant mass loss ( $P_{\text{before-during breeding}}=0.015$ ; Fig. 3A). This body mass loss (i.e. difference between before and during breeding) was independent of colour morph and brood size (all  $P>0.09$ ; Table S3).

Even though antioxidant capacity did not differ between moulting and breeding individuals (Fig. 3C), a significant interaction between sex, morph and time suggested different antioxidant dynamics in birds depending on their sex and morph (Table 2). Although no sex differences were observed in red-headed birds across time after repeating our model within each morph (sex×time:  $F_{1,39}=1.35$ ,  $P=0.25$ ; Table S4, Fig. 4A), antioxidant capacity varied differently in black-headed females and males (sex×time:  $F_{1,38}=6.14$ ,  $P=0.018$ ; Table S4). Multiple comparison analyses revealed that antioxidant capacity was higher in black-

**Table 1. Summary table of statistical tests examining the effects of thermal treatment (TT), time (–10, +1, +14 days before/after the start of the heatwaves), sex and colour morph on Gouldian finch body mass, plasma antioxidant capacity and plasma hydroperoxide levels during the heatwaves**

Test used	GLMM – gamma log link with repeated measures			LMM – repeated measures			GLMM – gamma log link with repeated measures			GLMM – binary logit link		
	Body mass			Antioxidant capacity			Hydroperoxide level			Proportion of moulting and breeding birds		
Response variable	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
Explanatory variables												
TT	1, 311	0.11	0.74	1, 104	1.98	0.16	1, 310	0.88	0.35	1, 87	0.25	0.62
Time	2, 311	9.85	<0.001	2, 207	1.94	0.15	2, 310	2.62	0.08			
Morph	1, 311	0.65	0.42	1, 104	2.25	0.14	1, 310	0.07	0.80	1, 87	0.08	0.78
Sex	1, 311	0.07	0.79	1, 104	1.53	0.22	1, 310	0.002	0.96	1, 87	0.08	0.78
TT×Time	2, 311	2.11	0.12	2, 207	1.04	0.36	2, 310	0.25	0.78			
TT×Morph	1, 311	0.86	0.35	1, 104	0.06	0.80	1, 310	1.62	0.20	1, 87	0.25	0.62
TT×Sex	1, 311	0.07	0.79	1, 104	0.09	0.77	1, 310	0.14	0.71	1, 87	0.005	0.94
Time×Morph	2, 311	0.13	0.88	2, 207	0.58	0.56	2, 310	0.45	0.64			
Time×Sex	2, 311	0.54	0.59	2, 207	0.01	0.99	2, 310	1.17	0.31			
Sex×Morph	1, 311	2.03	0.16	1, 104	0.16	0.69	1, 310	3.15	0.08	1, 87	0.08	0.78
TT×Time×Morph	2, 311	0.50	0.61	2, 207	1.10	0.34	2, 310	3.04	0.049			
TT×Sex×Morph	1, 311	0.57	0.45	1, 104	1.53	0.22	1, 310	0.28	0.60			
TT×Time×Sex	2, 311	0.71	0.50	2, 207	2.28	0.11	2, 310	2.39	0.09	1, 87	0.02	0.89
Sex×Morph×Time	2, 311	3.30	0.038	2, 207	2.31	0.10	2, 310	0.25	0.78			
TT×Time×Morph×Sex	2, 311	5.87	0.003	2, 207	1.01	0.37	2, 310	2.67	0.07			

GLMM, generalized linear mixed model; LMM, linear mixed model. Italicized values indicate  $P<0.05$ .



**Fig. 2. Maintenance status during the heatwaves. Body mass, plasma antioxidant capacity and plasma hydroperoxide levels across time in red-headed Gouldian finches (A,C,E), irrespective of sex and thermal treatment (○;  $N=56$ ), and in black-headed Gouldian finches (B,D,F) for each sex (females, ◇,  $N=28$ ; males, △,  $N=28$ ) and thermal treatment (thermoneutral, dashed black lines; thermocritical, solid black lines). Asterisks indicate significant interactions between time, sex and thermal treatment in black-headed birds. Different letters indicate significant differences between time points in red-headed birds.**

headed females than in black-headed males (*post hoc* test:  $P=0.019$ ; Fig. 4B) before moulting or breeding. Moreover, antioxidant values decreased with time only in moulting or breeding black-headed females (*post hoc* tests:  $P_{\text{black-females}}=0.019$ ,  $P_{\text{red-females}}=0.23$ ,  $P_{\text{black-males}}=0.73$ ,  $P_{\text{red-males}}=0.38$ ), resulting in no remaining sexual differences in black-headed birds during moult or breeding (*post hoc* test:  $P=0.76$ ; Fig. 4B). Even though plasma hydroperoxide levels showed the same temporal pattern as body mass, multiple comparison analyses revealed no differences between groups (all  $P>0.24$ ; Fig. 3D).

Morph differences in the regulation of oxidative status were also visible when examining temporal variation in oxidative markers (i.e. difference between before and during breeding) in relation to brood size. Indeed,  $\Delta$ antioxidant capacity and  $\Delta$ hydroperoxide levels tended to differ between sexes, morphs and brood sizes (sex $\times$ morph $\times$ brood size:  $P=0.06$ ; Table S3), and Spearman correlations conducted for each sex within each morph revealed that  $\Delta$ antioxidant capacity positively correlated with brood size only in red-headed females ( $r_s=0.593$ ,  $P=0.05$ ,  $n=10$ ), whereas  $\Delta$ oxidative damage positively correlated with brood size only in black-headed males ( $r_s=0.702$ ,  $P=0.035$ ,  $n=9$ ; Fig. S1). All other correlations between temporal variation in oxidative markers and brood size were not significant (all  $P>0.27$ ).

Telomere length showed a significant morph $\times$ sex interaction (Table 2), with red-headed males exhibiting shorter telomeres than red-headed females ( $39.3\pm 1.07$  and  $43.8\pm 1.13$  kbp, respectively; *post hoc* test:  $P=0.012$ ) and black-headed males ( $42.4\pm 0.91$  kbp;

*post hoc* test:  $P=0.021$ ). In contrast to males, telomere length tended to be shorter in black-headed females ( $39.9\pm 0.65$  kbp) than in red-headed females (*post hoc* test:  $P=0.07$ ). However, telomere length did not vary across time, nor between moulting or breeding birds, irrespective of the thermal conditions they previously experienced (Table 2, Fig. 3B).

## DISCUSSION

Whether individuals of different morphs are differently affected by challenging conditions and differently engage in costly activities is largely unknown. In this study, we monitored longitudinally markers of somatic maintenance in red- and black-headed Gouldian finches across a sequence of different events: first, a thermally challenging experimental period, and second, a subsequent monitoring period when birds could freely moult or breed. Overall, we found that Gouldian finches of different morphs responded differently to energetically demanding conditions (heatwaves, moult and reproduction), with red-headed males and females showing a homogeneous response, whereas the response of black-headed males and females diverged.

### Maintenance status during heatwaves

We found that red-headed males and females tended to gain body mass at the beginning of the thermoneutral and thermocritical heatwaves. The food provided to birds, their low physical activity in breeding cages, and the absence of competition with other birds probably explain this mass gain across time. However, red-headed

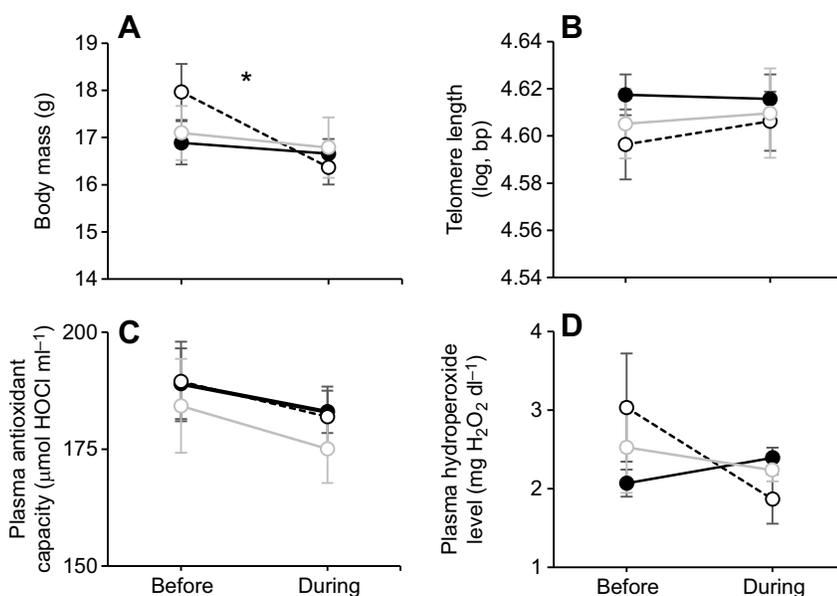
**Table 2. Summary table of statistical tests examining the effects of thermal treatment, time (before and during moult or breeding), moult/breeding status (i.e. moult/breeding strategy: moult or reproduction), sex and morph on body mass, plasma antioxidant capacity, plasma hydroperoxide levels and telomere length**

Test used	GLMM with repeated measures – gamma log link			LMM – repeated measures			GLMM with repeated measures – gamma log link			LMM with repeated measures – log transformed		
	Body mass			Plasma antioxidant capacity			Plasma hydroperoxide level			Telomere length		
Response variable	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
TT	1, 147	0.16	0.69	1, 85	0.03	0.86	1, 149	2.43	0.12	1, 98	0.001	0.97
Time	1, 147	<b>14.38</b>	<b>&lt;0.001</b>	1, 83	1.36	0.25	1, 149	0.62	0.43	1, 77	0.05	0.82
Strategy	2, 147	0.38	0.68	2, 84	0.03	0.97	2, 149	1.73	0.18	2, 92	0.86	0.43
Morph	1, 147	0.77	0.38	1, 85	0.03	0.86	1, 149	<b>5.09</b>	<b>0.025</b>	1, 99	0.04	0.85
Sex	1, 147	0.03	0.87	1, 86	0.57	0.45	1, 149	3.71	0.06	1, 92	1.04	0.31
TT×Time	1, 147	0.66	0.43	1, 80	2.19	0.14	1, 149	1.72	0.19	1, 80	1.02	0.32
TT×Strategy	2, 147	0.32	0.73	2, 85	0.15	0.86	2, 149	2.35	0.10	2, 96	0.18	0.84
TT×Morph	1, 147	2.84	0.09	1, 83	0.09	0.76	1, 149	0.01	0.94	1, 92	1.49	0.23
Time×Strategy	2, 147	<b>7.25</b>	<b>0.001</b>	2, 82	0.01	0.99	2, 149	<b>3.21</b>	<b>0.043</b>	2, 77	0.11	0.90
Time×Morph	1, 147	0.05	0.83	1, 80	2.72	0.10	1, 149	0.02	0.88	1, 80	0.001	0.98
Strategy×Morph	2, 147	0.15	0.86	2, 85	1.00	0.37	2, 149	<b>3.18</b>	<b>0.045</b>	2, 96	1.62	0.20
TT×Sex	1, 147	0.20	0.66	1, 82	0.87	0.36	1, 149	0.15	0.70	1, 92	1.17	0.28
Sex×Strategy	2, 147	0.04	0.96	2, 86	0.04	0.96	2, 149	0.28	0.76	2, 92	0.15	0.86
Sex×Morph	1, 147	3.85	0.05	1, 83	3.51	0.07	1, 149	0.13	0.72	<b>1, 91</b>	<b>9.04</b>	<b>0.003</b>
TT×Time×Strategy				2, 81	0.17	0.85	2, 149	1.09	0.34	2, 79	0.31	0.74
TT×Time×Morph				1, 81	3.24	0.08	1, 149	0.12	0.73	1, 77	1.64	0.20
TT×Strategy×Morph				2, 84	0.29	0.75	2, 149	0.30	0.74			
Time×Strategy×Morph				2, 80	0.004	>0.99	2, 149	2.16	0.12	2, 79	1.08	0.35
TT×Sex×Strategy				2, 83	0.07	0.93	2, 149	0.64	0.53			
TT×Sex×Morph				1, 84	0.23	0.63	1, 149	0.14	0.71			
TT×Sex×Time				1, 80	1.61	0.21	1, 149	1.62	0.21	1, 78	0.35	0.55
Sex×Strategy×Morph	2, 147	0.10	0.91	2, 83	0.60	0.55	2, 149	0.43	0.65			
Sex×Morph×Time	1, 147	0.20	0.66	<b>1, 80</b>	<b>9.70</b>	<b>0.003</b>	1, 149	0.43	0.52	1, 77	0.37	0.54
Sex×Time×Strategy				2, 79	1.47	0.24	2, 149	0.53	0.59	2, 75	0.19	0.83

Italicized values indicate  $P < 0.05$ .

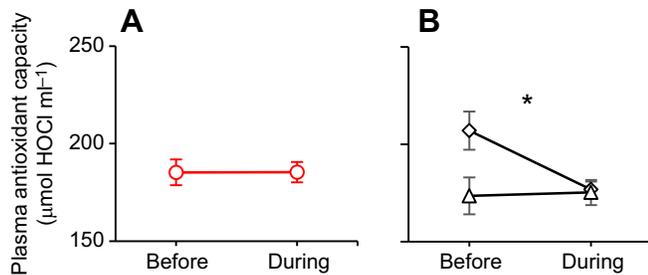
birds still lost mass at the end of the heatwaves, suggesting that initial adjustments were only temporary and not sufficient to compensate for the effects of high temperature on their maintenance in the long term. Similarly, black-headed males under thermoneutral conditions also gained mass at the beginning of the heatwave. The fact that black-headed males experiencing thermocritical conditions were unable to do so suggests that either their energy requirements were higher because of the activation of thermoregulatory processes

(Wolf, 2000), or they maintain fat reserves at a low level to reduce insulation (Clark, 1979). Similarly, black-headed females losing body mass at the end of the thermocritical heatwave, may have used these strategies. Irrespective of the mechanisms underlying mass changes in Gouldian finches exposed to variable thermal conditions, the fact that these changes differ between red- and black-headed males and females suggests that birds of both morphs and sexes may show different thermal tolerances. This may be due



**Fig. 3. Maintenance status during moult/reproduction.**

(A) Body mass, (B) telomere length, (C) plasma antioxidant capacity and (D) plasma hydroperoxide levels before and during moult (solid line;  $N=51$ ), breeding (dashed line;  $N=33$ ) and in non-moulting/non-breeding individuals (grey line;  $N=17$ ) irrespective of sex, thermal treatment and colour morph. The asterisk indicates a significant interaction between time and strategy.



**Fig. 4. Plasma antioxidant capacity before and during moult or breeding.** (A) Red-headed males and females together (○;  $N=56$ ), and (B) black-headed females (◇;  $N=28$ ) and males (△;  $N=28$ ) separately. The asterisk indicates a significant interaction between time and sex.

to the fact that red-headed birds reduce their activity during heatwaves (Fragueira and Beaulieu, 2019), which may allow them to save on costly thermoregulatory mechanisms at least at the beginning of heatwaves, thus keeping their total energy expenditure constant. In contrast, black-headed birds negligibly reducing their activity (Fragueira and Beaulieu, 2019) may have to use such costly physiological strategies immediately at the beginning of thermocritical heatwaves (males) or during heatwaves irrespective of their intensity (females).

Intriguingly, we found that red-headed birds showed reduced levels of plasma hydroperoxides at the beginning of the heatwaves. This is in sharp contrast with previous studies showing higher oxidative damage in birds exposed to hot conditions (Altan et al., 2003; Lin et al., 2008), and highlights the difficulty in predicting the effects of thermal conditions on oxidative markers. Low levels of hydroperoxides in red-headed birds may be explained by the fact that birds were chronically exposed to high temperatures (as opposed to an acute exposure), which allowed them to activate uncoupling proteins. However, this hypothesis seems unlikely, as activating uncoupling proteins generating heat would increase even more the risk of hyperthermia for birds. Low levels of hydroperoxides in red-headed birds may rather be due to the overall reduction of their physical activity during heatwaves (Fragueira and Beaulieu, 2019) to minimize the effects of heat exposure on their overall energy expenditure and ROS production (Beaulieu et al., 2015a). Conversely, the absence of behavioural adjustments in black-headed birds may explain why black-headed females tended to show higher levels of hydroperoxides in the long term under thermocritical conditions.

Overall, our results suggest that the maintenance of Gouldian finches was more affected by thermal conditions in black-headed birds than red-headed birds, presumably because of a lack of behavioural adjustments in black-headed birds. These results are in contrast to the hypothesis that potentially higher energy requirements in dominant red-headed birds should result in higher maintenance costs under thermally challenging conditions. For instance, red-headed males feeding more than black-headed males (thereby suggesting higher energy requirements; Fragueira and Beaulieu, 2019) were not more affected by thermal conditions. However, the maintenance of black-headed females feeding more than black-headed males (Fragueira and Beaulieu, 2019) appears more affected than that of males. Higher energetic requirements in black-headed females may therefore explain differential maintenance costs between black-headed males and females. Consequently, the hypothesis that higher intrinsic requirements should result in higher maintenance costs under thermocritical conditions appears to hold true between black-headed males and females, but not between red- and black-headed birds.

### Effects of moult and breeding on maintenance status

Although we exposed birds to conditions promoting reproduction by providing nest material and supplementary food, only less than half of them started breeding over the 4-month period following heatwave exposure. This low proportion was not due to the severity of the thermal conditions that birds previously experienced, as it was similar in birds previously experiencing the thermoneutral or the thermocritical heatwave. The low proportion of breeders may rather be due to the fact that the monitoring period was too short or that partners were not fully compatible despite belonging to the same colour morphs (morphs usually mate assortatively in this species; Pryke and Griffith, 2007). In contrast to our prediction, the intensity of the heatwave that birds previously experienced did not affect their propensity to preferentially start moulting or breeding. Moreover, this result was independent of their colour morph, thereby suggesting that black- and red-headed birds follow similar rules to initiate either moult or breeding. The fact that birds moulting first tended to show longer telomeres supports the hypothesis that telomere dynamics and feather regeneration are inter-related, possibly because of the common effects of telomerase on cell proliferation (Reichert et al., 2014). Overall, our results suggest that birds of both morphs recovered from the heatwaves before moulting or breeding, and that the initiation of moult and breeding is an individually based rather than a morph-specific strategy.

Body mass and plasma hydroperoxide levels showed the same temporal pattern, with pre-breeding birds showing higher values than pre-moulting and non-moulting/non-breeding birds, but converging towards them while breeding. This suggests that reproduction per se affected more strongly the maintenance of birds than moulting irrespective of their morph. However, the antioxidant capacity of birds still showed morph-specific temporal variation, with initially elevated antioxidant defences decreasing in moulting or breeding black-headed females, while remaining stable in red-headed females and in males of both morphs. This suggests that black-headed females may anticipate antioxidant requirements by increasing antioxidant defences before moulting or breeding, as they may be limited to do so during these energetically demanding events. Accordingly, black-headed females did not increase antioxidant capacity with brood size whereas red-headed females did so. In contrast to red-headed females, black-headed males showed higher levels of hydroperoxides with increasing brood size. Overall, these results suggest that the oxidative balance of moulting or breeding black-headed birds is more variable than that of red-headed birds, and that black-headed males and females alter different components of this balance while breeding.

Telomeres did not shorten over the course of our 5-month study irrespective of the morph or thermal treatment of birds. This is surprising given that telomeres typically shorten rapidly in short-lived species (Tricola et al., 2018), such as Gouldian finches (maximal longevity: 6 years, AnAge; Tacutu et al., 2012). The absence of telomere shortening in our study may be due to the fact that telomeres were measured in adults (as opposed to growing individuals; Salomons et al., 2009) and that Gouldian finches exhibit very long telomeres (mean telomere length of 40 kb in our study versus 25 kb in zebra finches or 47 kb in great tits, which also exhibit ultralong telomere length; Atema et al., 2019), which may mask the attrition of shorter telomeres. Despite this limitation, we still found that red-headed males exhibited the shortest telomeres among all birds, suggesting that they hatch with shorter telomeres, that their growth has stronger effects on telomere attrition, and/or that their telomeres shorten more rapidly over periods of time longer than that considered here. The same hypotheses might apply to black-headed females tending to show shorter telomeres than red-headed females.

## Conclusions

In polymorphic species, colour polymorphism has been found to be related to distinct life-history strategies. Accordingly, our results show that somatic maintenance in Gouldian finches of different morphs was differently affected by energetically demanding conditions, with red-headed males and females showing a slight but homogeneous physiological response, whereas black-headed males and females showed a stronger but heterogeneous response. How differences in maintenance regulation between morphs and sexes contribute to distinct fitness differences in this species remains to be examined. Notably, it would be interesting to examine the ultimate consequences of a homogeneous physiological response between breeding partners relative to a heterogeneous response. In the context of climate change, such an approach would allow us to determine whether differences in maintenance regulation between morphs and sexes make some individuals within polymorphic species more vulnerable than others to novel conditions, which in turn may strongly affect population dynamics under natural conditions.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: R.F., S.V., M.B.; Methodology: R.F., S.V., M.B.; Validation: S.V., M.B.; Formal analysis: R.F.; Investigation: R.F.; Resources: S.V., M.B.; Data curation: R.F.; Writing - original draft: R.F.; Writing - review & editing: R.F., S.V., M.B.; Supervision: S.V., M.B.; Project administration: M.B.; Funding acquisition: M.B.

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## Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.196030.supplemental>

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# Postponed effects of heatwaves

## **Study 3: Reduced offspring quality despite increased parental investment after heatwave exposure in a polymorphic bird**

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**Reduced offspring quality despite increased parental investment after heatwave exposure in a polymorphic bird.**

## 1 **Abstract**

2 Within species, responses to extreme climatic events may differ between individuals using  
3 distinct life-history strategies, thereby potentially resulting in climate change ‘winners’ and  
4 ‘losers’. Such dichotomy may occur in polymorphic species where individuals of distinct  
5 morphs differ in life-history strategies. Here, we examined the reproductive performance of  
6 Gouldian finches (*Erythrura gouldiae*) of the two main colour morphs (red- and black-headed  
7 birds) after exposure to a simulated heatwave reaching thermocritical temperatures, as well as  
8 potential carry-over effects in their offspring. Because of high investment into self-maintenance  
9 under challenging conditions, we expected lower reproductive performance after heatwave  
10 exposure. In contrast to our prediction, birds invested more into reproduction (larger eggs,  
11 higher chick provisioning rate) relative to sham birds only experiencing temperature change  
12 within their thermoneutral range. However, offspring paid the cost of the pre-exposure of their  
13 parents to thermocritical conditions, as their condition was reduced and their behaviour was  
14 altered. Despite differences in reproductive and developmental traits between red- and black-  
15 headed birds and morph-specific effects of thermal conditions on some reproductive traits  
16 (laying latency and sperm length), the overall reproductive performance of both morphs did not  
17 differ. These results suggest that red- and black-headed Gouldian finches, and especially their  
18 offspring, are both climate change ‘losers’, and that polymorphism does not represent an  
19 evolutionary rescue in this species. Whether both morphs are indeed climate change losers will,  
20 however, depend on their capacity to recover relative to the recurrence of extreme climatic  
21 events.

22

23 Keywords: carry-over effects, extreme climatic event, Gouldian finch,  
24 maintenance/reproduction, oxidative status, postponed effects

## 25 **1. Introduction**

26 More severe, more frequent, and longer extreme climatic events are predicted for the next  
27 decades (Easterling et al. 2000, IPCC 2015). Among these extreme climatic events, sweltering  
28 heatwaves (i.e. prolonged periods of temperature exceeding the mean temperature expected at  
29 that time) have already caused sudden mass mortality among human and animal populations  
30 (Argaud et al. 2007, Welbergen et al. 2008, McKechnie and Wolf 2009) and have therefore  
31 been described as ‘silent killers’ (Luber and McGeehin 2008). Just recently, almost a third of  
32 the population of spectacled flying foxes (*Pteropus conspicillatus*) died over two days of  
33 extreme heat in northern Australia (Akst, 2019). These detrimental effects on population size  
34 may be further accelerated if the reproductive capacity of the animals surviving a heatwave is  
35 subsequently reduced. Indeed, animals exposed to high temperatures are expected to allocate  
36 resources to self-maintenance, which may, in turn, compromise their subsequent capacity to  
37 reproduce (Stearns 1989). However, even though the immediate effects of high temperatures  
38 have received much attention in ecological studies, their postponed effects in surviving  
39 individuals have been mostly neglected.

40 Within species, not all individuals may be equally able to cope with extreme climatic events,  
41 thereby resulting in ‘winners’ and ‘losers’ (Somero 2010). For instance, the mortality of young  
42 and female flying foxes (*Pteropus sp.*) following heat exposure is higher than that of adult males  
43 (Welbergen et al. 2008). Such interindividual differences in sensitivity to extreme climatic  
44 events may be explained by different life-history strategies, with individuals investing more  
45 strongly into self-maintenance having better chances to survive. The relationship between life-  
46 history strategies and the ability to survive and reproduce following an extreme climatic event  
47 may be highly visible in polymorphic species, if individuals of different morphs use distinct  
48 life-history strategies. Indeed, differences in the Darwinian fitness of each morph under variable  
49 environmental conditions underlies the very maintenance of different morphs within species

50 (Roulin 2004, Emaresi et al. 2014). In the case of frequently-occurring heatwaves, the ‘winning’  
51 morph would be the one with the lowest mortality during exposure and/or the highest ability to  
52 reproduce afterwards, and should therefore be selected under harsher conditions.  
53 Polymorphisms may therefore be considered as a potential evolutionary rescue for the whole  
54 species when coping with changing environmental conditions (Forsman and Wennersten 2015).  
55 Here, we examined the postponed effects of extreme heatwaves on reproduction, as well as  
56 potential carry-over effects in their offspring, by comparing the reproductive performance of  
57 breeding Gouldian finches (*Erythrura gouldiae*) of the two main colour morphs (red- and black-  
58 headed) after a heatwave above thermoneutrality (i.e. thermocritical conditions) relative to  
59 temperature change within thermoneutrality range (i.e. sham-controlled conditions). To have a  
60 thorough view of the effects of thermal conditions on reproduction, we examined both parental  
61 reproductive performance and offspring traits. Because of higher energetic demands under  
62 thermocritical than under thermoneutral conditions (Burton and Weathers 2003), we first  
63 expected the former to induce detrimental effects on reproduction. This should be reflected by  
64 reduced reproductive performance (e.g. delayed breeding onset, smaller eggs and spermatozoa  
65 and reduced parental provisioning, fewer fledglings) and/or reduced offspring quality (e.g.  
66 lower body mass, reduced antioxidant defences, more oxidative damage, higher behavioural  
67 reactivity). Parents may also modulate reproductive investment following heatwave exposure  
68 by altering the sex-ratio of their offspring, if one sex is more costly to produce than the other  
69 (Pryke et al. 2011; Sheldon, 1999). We previously found that traits associated with self-  
70 maintenance deteriorate in adult black-headed female Gouldian finches after a thermocritical  
71 heatwave (lower body mass, higher oxidative damage; (Fragueira et al. 2019). As reduced body  
72 condition and higher oxidative damage may negatively affect breeding performance (Wiersma  
73 et al. 2004, Costantini et al. 2010), we expected the overall reproductive performance of black-  
74 headed pairs to be more strongly affected relative to that of red-headed pairs. Thus, we predicted

75 that the black-headed morph would be the climate change ‘loser’ in this polymorphic species  
76 under extreme environmental conditions.

77

## 78 **2. Methods**

### 79 ***2.1. Experimental design***

80 During two consecutive years, a total of 112 wild-type, young adult Gouldian finches (approx.  
81 one-year-old) were obtained from local breeders. All birds were reared in captivity, have not  
82 been hand-fed and were all naïve, meaning that they have not bred before. Pairs were formed  
83 from birds of the same colour morph and from different breeders, and assigned to experimental  
84 cages (99.6 x 48.0 x 52.0 cm) in two different climate chambers (2.8 x 4.2 x 2.5 m; 7 red-headed  
85 and 7 black-headed pairs in each climate chamber each year) set at  $32 \pm 1^\circ\text{C}$  (i.e. within the  
86 thermoneutral range of Gouldian finches:  $31.8\text{--}38.0^\circ\text{C}$ ; Evans & Fidler, 2005),  $50 \pm 5\%$   
87 humidity, and with a 12:12 L:D cycle. Across our study, birds had access to water, shell grit,  
88 cuttlefish bones and mixed seeds (Deli Nature 40 - Exoten Basis, Beyers, Belgium) *ad libitum*,  
89 and were given a bath daily for an hour. After at least two weeks under such conditions, birds  
90 were exposed to experimental changes in thermal conditions: either thermally-challenging  
91 conditions, with temperatures above thermoneutrality (i.e. thermocritical heatwave) or sham-  
92 controlled conditions, under which birds experienced temperature changes within  
93 thermoneutrality (i.e. thermoneutral exposure) for two weeks, with a temperature maximum  
94 ( $39^\circ\text{C}$  for thermocritical and  $36^\circ\text{C}$  for thermoneutral conditions) for two hours in the middle of  
95 the day (see Fragueira and Beaulieu 2019 for more information). The 14-day-long heatwave  
96 was designed to reflect long heatwaves that tropical birds, such as Gouldian finches, will likely  
97 experience in the future in Australia (IPCC 2015). After these 14 days, birds could freely breed  
98 for four months. The temperature was set again at  $32^\circ\text{C}$ , each cage was supplemented with a  
99 nest box, nest material, germ sprouts and egg food, and the L:D cycle was changed to 13:11

100 L:D (Evans & Fidler, 2005). All birds survived the heatwave, and the proportion of birds  
101 initiating breeding was independent of colour morph and thermal treatment.

102

## 103 **2.2. Parental reproduction**

### 104 a. General approach

105 Nest boxes were inspected every morning to monitor phenology (the onset of breeding,  
106 incubation, hatching, and fledging) and reproductive outcome (clutch size, hatching success,  
107 brood size at fledging). For each pair, we considered all breeding attempts following thermal  
108 treatment (pairs varied between zero and three clutches, except for one couple, who initiated  
109 five clutches in total, but where only the first three clutches were considered).

110

### 111 b. Spermatozoa length

112 During the first month following thermal treatment, males were captured to collect sperm  
113 (approx. 1-3  $\mu$ L). Males for which we had not enough measurable sperm cells and were not  
114 incubating were recaptured once a week (recaptures varied from zero to three times). To collect  
115 sperm, we massaged males' cloaca and collected ejaculates in glass capillaries (Wolfson 1952).  
116 One droplet was immediately smeared on a glass slide with 5  $\mu$ L of a fixator (5%  
117 formaldehyde). From each slide, we took pictures of 10 intact sperm cells with a Leica DFC  
118 7000T microscope (Leica Microsystems GmbH, Germany) at 40 $\times$  magnification and phase  
119 contrast 2 in order to calculate both the ejaculate mean and intra-individual correlation (ICC)  
120 in sperm morphology (Kleven et al. 2008). Total spermatozoa length from each sperm cell was  
121 measured using ImageJ 1.50i programme. These measurements were highly repeatable within  
122 each male (ICC:  $r = 0.76$ , CI 95%: 0.57, 0.89;  $P < 0.001$ ).

123

### 124 c. Egg volume

125 Eggs ( $N = 108$ ) were collected across the monitoring period. To avoid excessive disturbance  
126 during incubation (which in turn may have affected hatching success), we refrained from  
127 collecting eggs that were incubated and hatched, but only collected eggs that were abandoned  
128 by breeding birds. For further analyses, we only considered eggs coming from entire clutches  
129 to reduce the potential effects of laying order on egg volume (Sockman et al. 2006). Note,  
130 however, that these data are only representative of unhatched eggs. To calculate egg volume,  
131 the length and width of each unhatched egg were measured with a digital calliper ( $\pm 0.01$  mm;  
132 volume =  $0.51 * (\text{length} * \text{width}^2)$  (Hoyt 1979). Egg volume was highly repeatable for each  
133 female (intra-individual correlation:  $r = 0.68$ , CI 95%: 0.34, 0.86;  $P < 0.001$ ;  $N = 21$  females).

134

#### 135 d. Parental provisioning

136 Parental provisioning was recorded along chick growth (at days 6, 11 and 16 after hatching)  
137 throughout the whole day with a miniature camera (RS company, Corby, UK) placed on the  
138 ceiling of the cage and directed towards the entrance of the birds' nest. This allowed us to  
139 calculate how often parents entered the nest, which we used as a proxy of parental provisioning.  
140 Only the first brood produced by a pair was considered for these analyses. In total, 806 h of  
141 video recordings from 19 provisioning pairs (11 red-headed pairs and 8 black-headed pairs)  
142 were analysed manually using VLC Player (Version 2.2.6), and a total of 6089 provisioning  
143 events were observed.

144

### 145 **2.3. Offspring condition**

#### 146 a. Sex determination and general approach

147 The sex of offspring was determined by observing breast colouration after birds acquired their  
148 adult plumage (males have a darker purple breast than females). All other offspring traits (body  
149 size, mass and behaviour) were measured after collecting blood at fledging, one month after

150 fledging, and two months after fledging on the same day time (13.00-16.00). We did not  
151 measure any offspring trait before fledging to avoid any side-effects on nestling growth or nest  
152 abandonment.

153

#### 154 b. Body size and mass

155 To assess body size, beak width and length, tarsus length, and wing length (from the basis of  
156 the alula to the tip of the longest primary remige) were measured using a calliper (beak width  
157 and length, tarsus length;  $\pm 0.01$  mm) or a ruler (wing;  $\pm 1$  mm). Chicks were also weighed on  
158 a digital scale ( $\pm 0.1$  g).

159

#### 160 c. Behaviour

161 The birds' handling responses were measured in a standardised way by the same experimenter,  
162 as previously done in zebra finches (*Taeniopygia guttata*; Noguera, Metcalfe, Surai, &  
163 Monaghan, 2015) and Gouldian finches (Fragueira and Beaulieu 2019). Briefly, docility was  
164 measured by counting how often birds struggled when held in hand for 10 s, aggressiveness  
165 was assessed by counting biting frequency when approaching the right index finger in front of  
166 the beak six times every 2 s, and breathing rate was measured by counting chest movements for  
167 10 s (Møller and Ibáñez-Álamo 2012, Noguera et al. 2015). Docility and aggressiveness were  
168 used as behavioural responses to stressful conditions (Noguera et al. 2015). Measuring  
169 breathing rate allowed us to characterize non-invasively the reactivity of the autonomic nervous  
170 system to stressors (Carere and van Oers 2004). Birds in poorer conditions were expected to be  
171 less docile, to be more aggressive, and to breathe more rapidly than birds under  
172 thermoneutrality.

173

#### 174 d. Oxidative status

175 Immediately after capture, blood (approx. 80  $\mu$ L) was collected from the brachial vein with a  
176 heparinized capillary, centrifuged (10 min., 4000 G) to isolate plasma, and frozen at -80°C for  
177 further analyses. To examine the chicks' plasma oxidative status, we measured one marker of  
178 antioxidant defences (total antioxidant capacity using the OXY-adsorbent test; Diacron  
179 International, Grosseto, Italy) and one marker of oxidative damage (hydroperoxide  
180 concentration using the d-ROM test; Diacron International, Grosseto, Italy), already used to  
181 measure the oxidative status of adult Gouldian finches and other bird species (Beaulieu &  
182 Schaefer, 2014; Fragueira et al. 2019; for measurement principles, see Costantini, 2016). In the  
183 assays, samples were balanced across plates with respect to clutch, parental morph, and thermal  
184 treatment. Intra- and inter-plate coefficients of variation for the OXY and ROM were 13% ( $N$   
185 = 5 replicates) and 3% ( $N$  = 5 replicates), and 17% ( $N$  = 4 replicates) and 6% ( $N$  = 5 replicates),  
186 respectively.

187

#### 188 **2.4. Statistics**

189 First, we analysed whether thermal treatments differently affected reproductive traits of red-  
190 and black-headed pairs using generalized linear models (spermatozoa length, onset of breeding,  
191 total number of fledglings) with thermal treatment (thermoneutral, thermocritical) and morph  
192 (red- and black-headed) as fixed factors. When data were available for several consecutive  
193 breeding attempts, we also conducted general linear (egg volume, incubation duration, chick-  
194 rearing duration) or generalized (clutch size, hatching success, brood size, sex-ratio (i.e. male  
195 proportion)) mixed models with thermal treatment (thermoneutral, thermocritical), morph (red-  
196 and black-headed) as fixed factors, breeding attempt (breeding attempt 1 to 3) as repeated  
197 factor, and pair ID as random factor. To analyse parental provisioning rate, a generalized linear  
198 mixed model with repeated measures was used with thermal treatment (thermoneutral,  
199 thermocritical), morph (red- and black-headed) and sex as fixed factors, chicks' age (day 6, 11

200 or 16) as repeated measures, brood size as covariate, and parent ID nested within the cage as  
201 random factors.

202 We estimated chicks' body size using a principal-component analysis (PCA) including  
203 four variables (beak width, beak length, tarsus length, and wing length), resulting in one PC  
204 with an eigenvalue of 2.42 and explaining 61% of the variance. To examine whether parental  
205 morph and the thermal treatment previously experienced caused carry-over effects in their  
206 offspring, we analysed variation in chicks' condition and behavioural traits after fledging using  
207 general (body mass, body size, antioxidant capacity) and generalized (docility, breathing rate,  
208 aggressiveness, hydroperoxide level) linear mixed models with thermal treatment  
209 (thermoneutral, thermocritical), parental morph (red- and black-headed), and time (at fledging,  
210 one month after fledging, two months after fledging) as independent factors, and fledgling ID  
211 nested within brood, as well as the breeding attempt from which fledglings were produced as  
212 random factors.

213 For all significant effects, post-hoc comparisons were controlled for false-discovery rates  
214 according to Benjamini & Hochberg (1995). All analyses were performed using SPSS (v. 22;  
215 Chicago, IL). Results are given as means  $\pm$  s.e.

216

## 217 **3. Results**

### 218 ***3.1. Parental reproduction***

219 Spermatozoa length of red- and black-headed males was differently affected by thermal  
220 conditions, with red-headed males producing 5% smaller spermatozoa after a thermocritical  
221 heatwave (post-hoc test:  $P = 0.015$ ) while spermatozoa length remained unaffected by thermal  
222 conditions in black-headed males ( $P = 0.24$ ; Table 1; Fig. 1A). Conversely, black-headed pairs  
223 started breeding more than a month earlier after exposure to thermocritical conditions than after

224 exposure to thermoneutral conditions (post-hoc test:  $P = 0.03$ ), while the onset of breeding was  
225 not affected by thermal conditions in red-headed pairs ( $P = 0.66$ ; Table 1; Fig 1B).

226 The effects of thermal treatment and morph on egg volume depended on breeding  
227 attempt (Table 2). Indeed, only during the first breeding attempt following thermal treatment,  
228 females of both morphs produced 6% larger eggs after a thermocritical heatwave relative to  
229 females only experiencing thermoneutral conditions, and red-headed females produced 7%  
230 larger eggs than black-headed females (Table 2; Fig. 1C). These trends disappeared in  
231 subsequent breeding attempts (Fig. S1A-B). Irrespective of morph and thermal treatment,  
232 clutch size was highest for the second breeding attempt ( $4 \pm 1$  eggs,  $5 \pm 1$  eggs and  $4 \pm 1$  eggs  
233 for the first, second and third breeding attempt, respectively). Incubation duration ( $14 \pm 1$  days),  
234 hatching success ( $29 \pm 4\%$ ), chick-rearing duration ( $22 \pm 1$  days), brood size ( $0.9 \pm 0.2$  mean  
235 chicks per brood), and the total number of fledglings produced per pair ( $1.1 \pm 0.3$  mean total  
236 fledglings produced per pair) did not differ between morphs and thermal treatments (Tables 1,  
237 2).

238 Even though males provisioned more often hatchlings than females ( $30 \pm 4$  and  $25 \pm 5$   
239 visits, respectively), both parents previously exposed to a thermocritical heatwave provisioned  
240 their offspring more than three times as frequently as parents previously exposed to  
241 thermoneutral conditions, irrespective of their morph (Table 1, Fig. 1D). Within broods, sex-  
242 ratio was also significantly affected by treatment (Table 2), with fewer males being produced  
243 following exposure to thermocritical conditions than to thermoneutral conditions ( $35 \pm 11$  and  
244  $67 \pm 11\%$ , respectively; Fig. 1E). However, the total number of fledglings produced did not  
245 differ between thermal treatments or morphs (Table 1; Fig. 1F).

246

### 247 **3.2. Offspring traits**

248 Chicks gained mass and size after fledging (Table 3), and this gain was more pronounced in  
249 red-headed than in black-headed fledglings (Fig. 2A). At fledging, offspring from red-headed  
250 parents were lighter than offspring from black-headed parents (post-hoc test:  $P = 0.001$ ), but  
251 this difference faded afterwards (all  $P > 0.99$ ). Additionally, offspring from red-headed parents  
252 continued to grow after one month after fledging (post-hoc tests:  $P < 0.001$ ) while offspring  
253 from black-headed parents had reached maximal size 30 days after fledging ( $P = 0.28$ ; Fig. 2B).  
254 Despite an additional significant morph by treatment interaction (Table 3), we could not detect  
255 significant differences in body size between offspring of different morphs and from parents  
256 previously experiencing different thermal conditions (post-hoc tests: all  $P > 0.07$ ).

257 Irrespective of the colour morph of their parents, offspring from parents having previously  
258 experienced a thermocritical heatwave showed 40% lower antioxidant capacity than those from  
259 parents having experienced a thermoneutral heatwave (Table 3; Fig. 2C). In contrast,  
260 hydroperoxide levels depended on both parental morph and thermal treatment as well as on the  
261 age of offspring (Table 3). Overall, hydroperoxide levels increased (or tended to increase) with  
262 time in all groups (post-hoc tests: all  $P < 0.06$ ), except in offspring produced by red-headed  
263 parents previously exposed to thermocritical conditions ( $P = 0.56$ ; Fig. 2D). Moreover,  
264 fledglings produced by black-headed parents were strongly affected by the thermal conditions  
265 that their parents previously experienced, as offspring produced by black-headed parents  
266 exposed to thermocritical conditions showed levels of hydroperoxide 80% higher than those  
267 experienced by offspring produced by black-headed parents exposed to thermocritical  
268 conditions (post-hoc tests:  $P < 0.001$ ). Such a difference in hydroperoxide levels was not  
269 observed in offspring produced by red-headed pairs ( $P = 0.22$ ; Fig. 2D).

270 Docility did not significantly differ between morphs, thermal treatments or offspring age  
271 ( $0.28 \pm 0.06$  mean struggle movements; Table 3; Fig. S2). However, offspring from parents

272 having experienced thermoneutral conditions became more aggressive with time after fledging  
273 (post-hoc tests:  $P = 0.027$  between days 30 and 60), whereas offspring from parents having  
274 experienced thermocritical conditions remained as aggressive after fledging as they were at  
275 fledging (post-hoc tests: all  $P > 0.99$ ; Table 3; Fig. 2E). Furthermore, 30-day old offspring from  
276 parents previously experiencing thermocritical conditions breathed three times as slowly  
277 offspring from parents previously experiencing thermoneutral conditions (post-hoc test:  $P =$   
278 0.005; Fig. 2F).

279

## 280 **4. Discussion**

281 The challenges of current climate change for animals is not only to survive extreme climatic  
282 events but also to be able to successfully reproduce afterwards. Here, we found that breeding  
283 Gouldian finches exposed to a simulated heatwave beyond their thermal tolerance subsequently  
284 increased their reproductive investment, as they advanced breeding, produced larger eggs and  
285 provisioned their offspring more frequently. However, offspring condition was altered. These  
286 effects differed only marginally between both colour morphs.

287

### 288 ***4.1. Effects of thermal conditions on reproduction***

289 After experiencing a thermocritical heatwave, female Gouldian finches produced larger eggs  
290 and parents provisioned their offspring more frequently. Overall, these results suggest that  
291 Gouldian finches increased their reproductive investment after exposure to thermocritical  
292 conditions. This is in contrast with our hypothesis that challenging conditions should result in  
293 the reallocation of resources into self-maintenance, which should then be traded-off against  
294 subsequent reproduction (Wingfield et al. 2017). Several non-exclusive hypotheses may  
295 explain these apparently counter-intuitive results. First, in tropical species, such as Gouldian  
296 finches, the contrast between unfavourable and subsequent favourable environmental

297 conditions appears to be the main trigger for reproduction in the wild (Evans and Fidler, 2005).  
298 Besides a change in food abundance, a change in ambient temperature might also represent such  
299 an environmental cue that trigger breeding in Gouldian finches. Our results suggest that the  
300 stronger the contrast between the unfavourable and favourable conditions, the higher the  
301 reproductive investment. Accelerating life-history strategies by increasing reproductive  
302 investment following exposure to stressful conditions may be adaptive if the intensity of the  
303 stressful conditions experienced by animals is indicative of the recurrence of these conditions  
304 (Meehl, 2000). If so, previous exposure to highly stressful conditions may indicate animals that  
305 they have only limited time to breed before the next occurrence of stressful conditions, and that  
306 they should therefore increase reproductive investment (Marrot et al. 2017, 2018). Finally, in  
307 line with the terminal investment hypothesis (Velando et al. 2006; Williams, 1966), Gouldian  
308 finches may adjust their reproductive investment based on the effects of pre-breeding  
309 environmental conditions on their own maintenance. To fully test this hypothesis, we would,  
310 however, need to assess the future costs of reproduction on subsequent reproduction and  
311 survival.

312         Similar to other bird species, smaller eggs are generally less likely to hatch in Gouldian  
313 finches (Pryke and Griffith, 2009). However, in our study, the larger eggs laid after a  
314 thermocritical heatwave appeared to have the same hatching probability than smaller eggs laid  
315 after exposure to thermoneutral conditions. However, these results have to be considered with  
316 caution, as only eggs from unhatched clutches were analysed. Moreover, despite higher  
317 provisioning rate following exposure to thermocritical conditions, brood size at fledging was  
318 independent of thermal treatment. This suggests that egg and chick quality might have differed  
319 between thermal treatments, and that egg and chick quality produced by birds previously  
320 experiencing thermocritical conditions was poorer. Indeed, female birds may be limited to  
321 transfer resources (i.e. lower antioxidants) to their eggs after experiencing stressful conditions

322 (Surai et al. 2001, Royle et al. 2001), which may explain the lower antioxidant capacity of  
323 fledglings produced by pairs previously experiencing thermocritical conditions. Finally, low  
324 nutrient levels during development have also been associated with slow breathing rate in finches  
325 (Noguera et al. 2015), which we also transiently observed in offspring from parents having  
326 experienced thermocritical conditions. Finally, the fact that fewer males were produced  
327 following exposure to thermocritical conditions suggests that they are more costly to produce  
328 and need higher nutrient levels to develop. Interestingly, the behavioural differences that we  
329 initially observed between offspring from parents having experienced thermocritical and  
330 thermoneutral conditions progressively disappeared after fledging. This suggests that offspring  
331 from parents having experienced thermocritical conditions managed at least partially to recover  
332 once independent. Whether they may entirely recover in the long term, for instance in terms of  
333 antioxidant capacity or longer-lasting negative carry-over effects in adulthood (Dufty et al.  
334 2002, Nettle et al. 2015), remains to be examined.

335

#### 336 ***4.2. Morph-specific effects of thermal conditions***

337 Previous studies have highlighted strong differences in life-history strategies between both  
338 colour morphs in Gouldian finches, with red-headed birds being generally more aggressive, less  
339 exploratory, having priority access to high-quality nests and producing more fledglings  
340 (Brazill-Boast et al. 2013; Mettke-Hofmann, 2012; Pryke and Griffith, 2009b; Williams et al.  
341 2012), but being unable to adjust parental provisioning to food variation, and being  
342 physiologically more sensitive to environmental variation than black-headed birds (Pryke et al.  
343 2007, 2012). Here, red-headed birds were not more productive (in terms of number of fledglings  
344 produced) than black-headed birds, likely due to the absence of competition. Irrespective of  
345 thermal conditions, we found that red-headed birds produced larger unhatched eggs but smaller  
346 fledglings. This either suggests that egg volume from unhatched eggs does not reflect egg

347 volume from hatched eggs, or that egg volume is not related to egg quality. Lower body mass  
348 at fledging forced offspring of red-headed parents to catch up afterwards in terms of growth.  
349 This compensatory growth may, however, have subsequent costs (Criscuolo et al. 2008), which  
350 may compromise survival (Geiger et al., 2012).

351 Thermal conditions differently affected black- and red-headed Gouldian finches in three  
352 ways: (1) spermatozoa length, (2) onset of breeding, and (3) offspring hydroperoxide levels.  
353 While the sperm length was not affected by thermal conditions in black-headed- males, the  
354 spermatozoa of red-headed males having experienced thermocritical conditions were smaller  
355 than those of red-headed males having experienced thermoneutral conditions. This suggests  
356 higher plasticity and/or greater condition-dependence of spermatogenesis in this colour morph.  
357 Accordingly, males of both morphs produce longer sperm mid-piece in response to increased  
358 sperm competition, but only red-headed males increase sperm flagellum length under lower  
359 competitive conditions (Immler et al. 2010). Together with our study, this suggests that, in  
360 contrast to black-headed males, red-headed males might control their breeding performance by  
361 altering sperm quality depending on environmental conditions. Interestingly, shorter  
362 spermatozoa may reflect shorter spermatogenesis duration (Ramm and Schärer 2014,  
363 Durairajanayagam et al. 2015), which supports the idea of accelerated life-history strategies  
364 following stressful conditions, but here only in red-headed birds.

365 We also observed an acceleration of life-history strategies in black-headed birds  
366 following exposure to thermocritical conditions, as they initiated breeding earlier. This suggests  
367 that black-headed individuals responded more rapidly to a change in their breeding  
368 environment. This may be because black-headed birds adjust their reproductive investment  
369 based on the effects of pre-breeding environmental conditions on their own maintenance.  
370 Indeed, only black-headed females were in poorer condition after exposure to the critical  
371 heatwave (Fragueira et al. 2019). However, a rapid onset of breeding may result in lower

372 parental investment (e.g. lower maternal nutrient transfer during egg production), which may  
373 explain the fact that offspring produced by black-headed parents previously experiencing  
374 thermocritical conditions showed not only lower antioxidant defences but also higher  
375 hydroperoxide levels than offspring produced by black-headed parents previously experiencing  
376 thermoneutral conditions. Such a difference was not observed in offspring produced by red-  
377 headed parents, which initiated reproduction independently of thermal conditions.

378

379 In conclusion, despite some differences in reproductive and developmental traits  
380 between black- and red-headed Gouldian finches, the exposure to a thermocritical heatwave  
381 affected the reproductive performance of both morphs similarly, mostly by increasing their  
382 reproductive investment and by decreasing, at least temporarily, the quality of their offspring.  
383 Therefore, no morph appears to be more disadvantaged than the other following an intense  
384 heatwave, and red- and black-headed Gouldian finches can both be considered as ‘climate stress  
385 losers’. Therefore, when coping with intense heatwaves, polymorphism does not appear to  
386 represent an evolutionary rescue for Gouldian finches. The benefits and costs of the rapid and  
387 strong reproductive investment that we observed after stressful conditions will, however,  
388 depend on the recurrence of such extreme climatic events. Further studies should focus on the  
389 resilience of animals following exposure to challenging conditions in view of the projected  
390 duration and frequency of these events projected for the future.

391 **Competing interests**

The authors declare no conflict of interest.

392 **Data accessibility**

393 The data related to this manuscript will be available from the Dryad Digital Repository.

394

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501

## Tables

Table 1: Summary table of statistical tests examining the effects of thermal treatment, morph, parent sex, offspring age (6, 11, 16 days after hatching) and brood size on the reproduction performance of Gouldian finches

Response variable Explanatory variables	Spermatozoa length			Onset of breeding			Parental provisioning rate			Total number of fledglings		
	df	F	P	df	$\chi^2$	P	df	F	P	df	$\chi^2$	P
Treatment	<b>1,214</b>	<b>26.41</b>	<b>&lt;0.001</b>	<b>1</b>	<b>12.25</b>	<b>&lt;0.001</b>	<b>1,97</b>	<b>5.63</b>	<b>0.020</b>	1	0.63	0.43
Morph	1,214	3.21	0.08	<b>1</b>	<b>4.60</b>	<b>0.032</b>	1,97	0.50	0.48	1	0.12	0.73
Sex							<b>1,97</b>	<b>5.05</b>	<b>0.027</b>			
Age							1,97	0.45	0.64			
Brood size							<b>1,97</b>	<b>11.23</b>	<b>0.001</b>			
Treatment*Morph	<b>1,214</b>	<b>6.78</b>	<b>0.010</b>	<b>1</b>	<b>7.36</b>	<b>0.007</b>	1,97	1.83	0.18	1	1.38	0.24
Treatment*Sex							1,97	0.39	0.53			
Treatment*Brood size							<b>1,97</b>	<b>5.97</b>	<b>0.016</b>			
Morph*Sex							1,97	1.64	0.20			
Morph*Age							1,97	0.30	0.74			
Morph*Brood size							1,97	0.51	0.48			
Age*Brood size							1,97	0.58	0.56			
Treatment*Morph*Sex							1,97	2.17	0.14			
Morph*Age*Brood size							1,97	1.76	0.18			
Test used	GLMM <sup>1</sup> - Gamma distribution			GLM <sup>2</sup> - Gamma distribution			GLMM <sup>1</sup> with repeated measures - Gamma distribution			GLM <sup>2</sup> - Negative binomial distribution		

Significant results are given in bold.

<sup>1</sup> GLMM : generalized linear mixed model

<sup>2</sup> GLM : generalized linear model

1 Table 2: Summary table of statistical tests examining the effects of thermal treatment, parental morph and breeding attempt on the reproduction  
 2 performance of Gouldian finches

Response variable Explanatory variables	Clutch size			Egg volume			Incubation duration			Hatching success			Chick-rearing duration			Brood size			Sex-ratio		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Treatment	1,53	0.13	0.72	1,9	2.66	0.14	1,25	0.05	0.83	1,53	0.82	0.37	1,16	0.27	0.61	1,53	0.001	0.97	<b>1,16</b>	<b>5.44</b>	<b>0.033</b>
Morph	1,53	0.001	0.97	1,8	1.18	0.31	1,25	0.07	0.80	1,53	1.12	0.30	1,16	0.10	0.76	1,53	0.00	0.97	1,16	0.34	0.57
Breeding attempt	<b>2,53</b>	<b>4.040</b>	<b>0.023</b>	2,69	1.64	0.20	3,25	1.08	0.38	2,55	2.11	0.13	3,16	1.66	0.22	2,55	0.30	0.74	3,16	0.22	0.88
Treatment*Morph	1,53	0.20	0.66	1,8	0.02	0.89	1,25	0.001	0.97	1,53	3.51	0.07	1,16	1.58	0.23	1,53	0.002	0.97	1,16	0.002	0.97
Treatment*Attempt	2,53	1.27	0.29	<b>2,96</b>	<b>4.95</b>	<b>0.009</b>	2,25	0.18	0.84	2,55	0.11	0.90	2,16	0.11	0.89	2,55	0.78	0.47	2,16	1.57	0.24
Morph*Attempt	2,53	0.38	0.69	<b>2,67</b>	<b>8.23</b>	<b>0.001</b>	2,25	0.68	0.52	2,55	2.79	0.07	2,16	1.15	0.34	2,55	0.06	0.94	2,16	1.84	0.19
Treatment*Morph*Attempt	2,53	0.93	0.40				2,25	0.06	0.94	2,55	0.65	0.53	2,16	0.01	0.94	2,55	0.83	0.44	1,16	2.79	0.11
Test used	GLMM <sup>1</sup> with repeated measures - Negative binomial distribution			LMM <sup>2</sup> with repeated measures			LMM <sup>2</sup> with repeated measures			GLMM <sup>1</sup> with repeated measures - Gamma distribution			LMM <sup>2</sup> with repeated measures			GLMM <sup>1</sup> with repeated measures - Negative binomial distribution			GLMM <sup>1</sup> with repeated measures - Gamma distribution		

3 Significant results are given in bold.

4 <sup>1</sup> GLMM : generalized linear mixed model

5 <sup>2</sup> LMM : linear mixed model

6

7 Table 3: Summary table of statistical tests examining the effects of parental morph, thermal treatment, and time after fledging on offspring traits

Response variable Explanatory variables	Body mass			Body size (PC1)			Antioxidant capacity			Hydroperoxide level			Docility			Aggressiveness			Breathing rate		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Parental morph	1, 25	2.85	0.10	1,37	0.83	0.37	1, 29	0.03	0.86	<b>1, 50</b>	<b>16.22</b>	<b>&lt; 0.001</b>	1, 111	< 0.001	> 0.99	1, 111	1.37	0.25	1, 111	0.17	0.69
Treatment	1, 36	0.02	0.90	1,37	0.650	0.43	<b>1, 30</b>	<b>8.45</b>	<b>0.007</b>	<b>1, 62</b>	<b>4.05</b>	<b>0.049</b>	1, 111	0.380	0.54	<b>1, 111</b>	<b>5.14</b>	<b>0.025</b>	1, 111	0.93	0.34
Time	<b>2, 72</b>	<b>64.94</b>	<b>&lt; 0.001</b>	<b>2,73</b>	<b>334.96</b>	<b>&lt; 0.001</b>	2, 67	0.75	0.48	<b>2, 99</b>	<b>4.95</b>	<b>0.009</b>	2, 111	0.260	0.77	2, 111	1.93	0.15	<b>2, 111</b>	<b>8.97</b>	<b>&lt; 0.001</b>
Morph* <i>Treatment</i>	1, 36	0.08	0.78	<b>1,37</b>	<b>6.46</b>	<b>0.015</b>	1, 31	0.24	0.63	<b>1, 61</b>	<b>7.91</b>	<b>0.007</b>	1, 111	0.001	0.97	1, 111	0.80	0.38	1, 111	2.84	0.10
Morph* <i>Time</i>	<b>2, 72</b>	<b>3.45</b>	<b>0.037</b>	<b>2,73</b>	<b>3.45</b>	<b>0.037</b>	2, 67	0.38	0.69	2, 99	2.55	0.08	2, 111	0.210	0.81	2, 111	0.36	0.70	2, 111	0.27	0.76
Treatment* <i>Time</i>	2, 72	2.74	0.07	2,73	1.77	0.18	2, 67	1.35	0.27	2, 99	0.31	0.73	2, 111	0.050	0.95	<b>2, 111</b>	<b>3.12</b>	<b>0.048</b>	<b>2, 111</b>	<b>8.92</b>	<b>&lt; 0.001</b>
Morph* <i>Treatment</i> * <i>Time</i>	2, 72	0.18	0.84	2,73	1.06	0.35	2, 67	0.87	0.43	<b>2, 99</b>	<b>3.30</b>	<b>0.041</b>	2, 111	0.130	0.88	2, 111	1.33	0.27	2, 111	2.25	0.11
Test used	LMM <sup>1</sup> with repeated measures			LMM <sup>1</sup> with repeated measures			LMM <sup>1</sup> with repeated measures			LMM <sup>1</sup> with repeated measures - log transformed			GLMM <sup>2</sup> with repeated measures - Poisson distribution			GLMM <sup>2</sup> with repeated measures - Poisson distribution			GLMM <sup>2</sup> with repeated measures - Poisson distribution		

8 Significant results are given in bold.

9 <sup>1</sup> LMM : linear mixed model

10 <sup>2</sup> GLMM: generalized linear mixed model

11

12 **Figure legends**

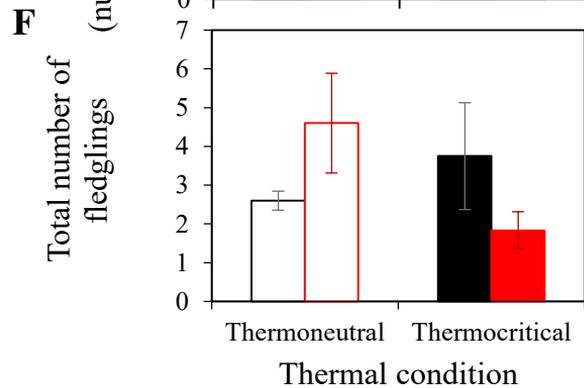
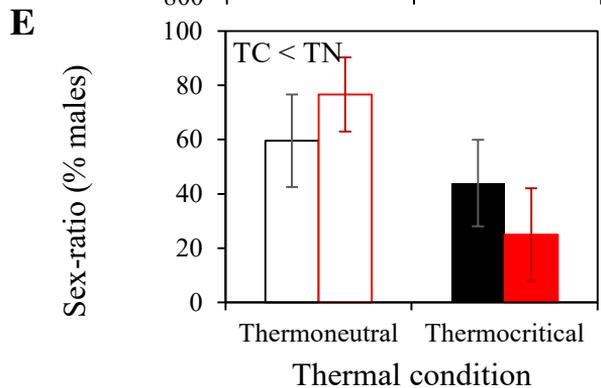
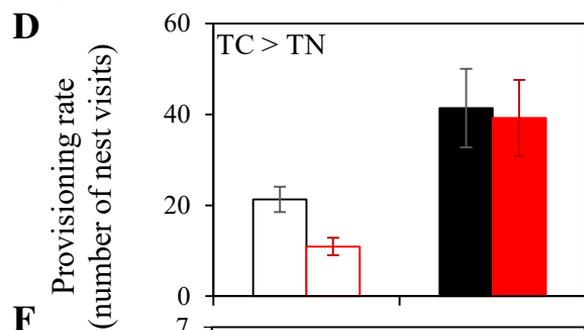
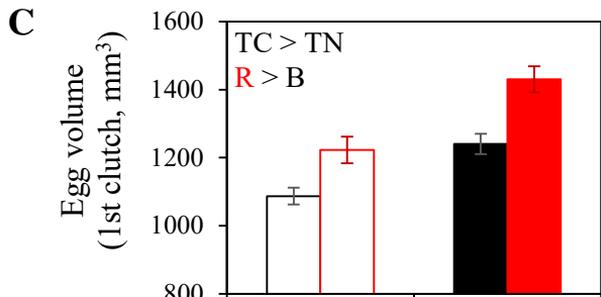
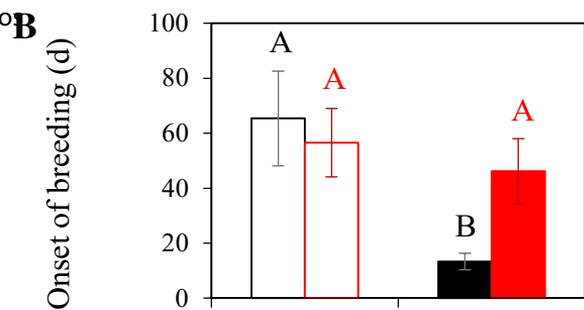
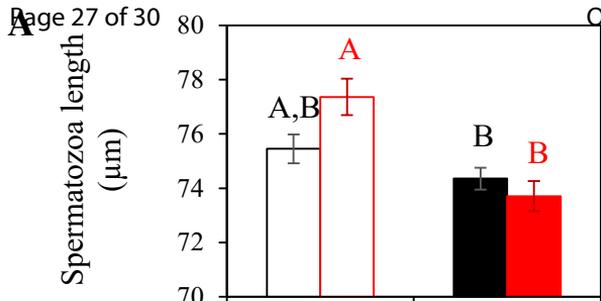
13 **Figure 1 Parental reproductive performance in relation to thermal conditions and morph.**

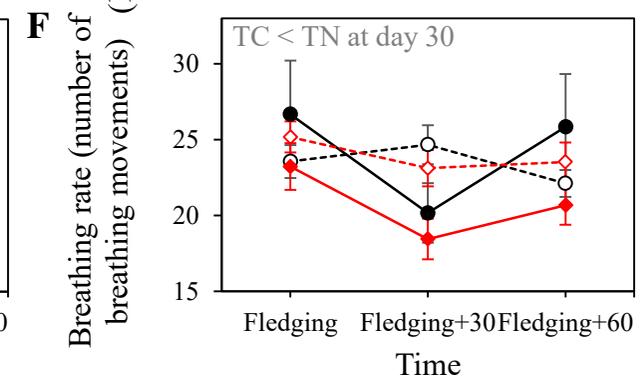
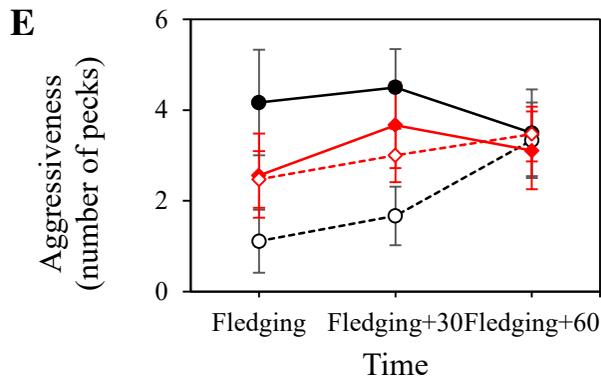
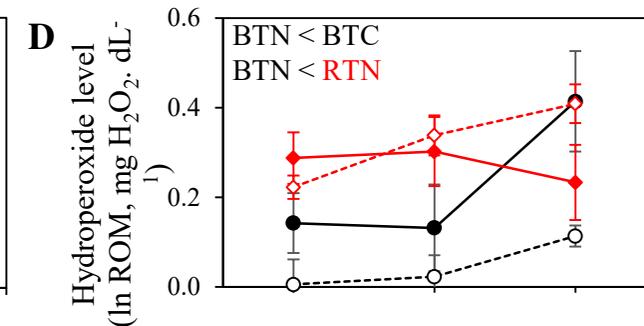
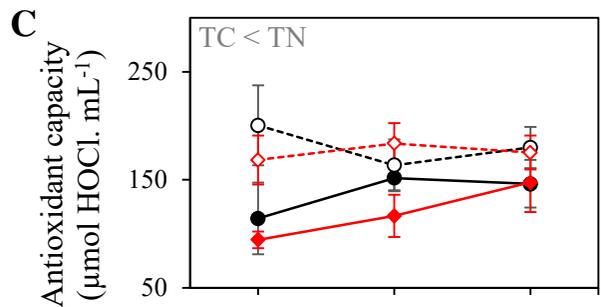
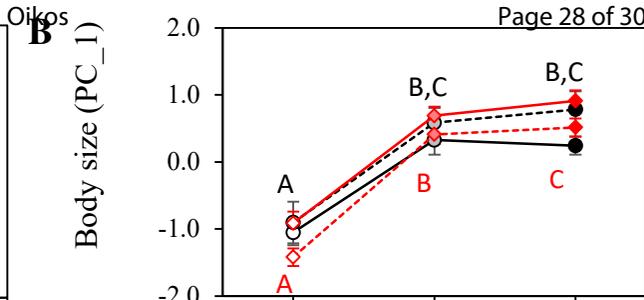
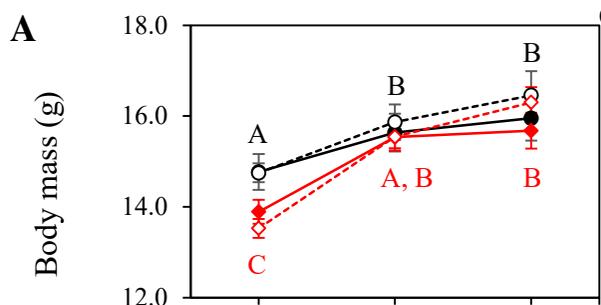
14 (A) Spermatozoa length, (B) onset of breeding, (C) egg volume, (D) mean provisioning rate  
15 after hatching, (E) chicks' sex-ratio and (F) total number of fledglings produced by black-(black  
16 bars; B) and red-headed (red bars; R) pairs after exposure to thermoneutral (open bars; TN) and  
17 thermocritical (solid bars; TC) conditions. Different letters represent significant differences.

18

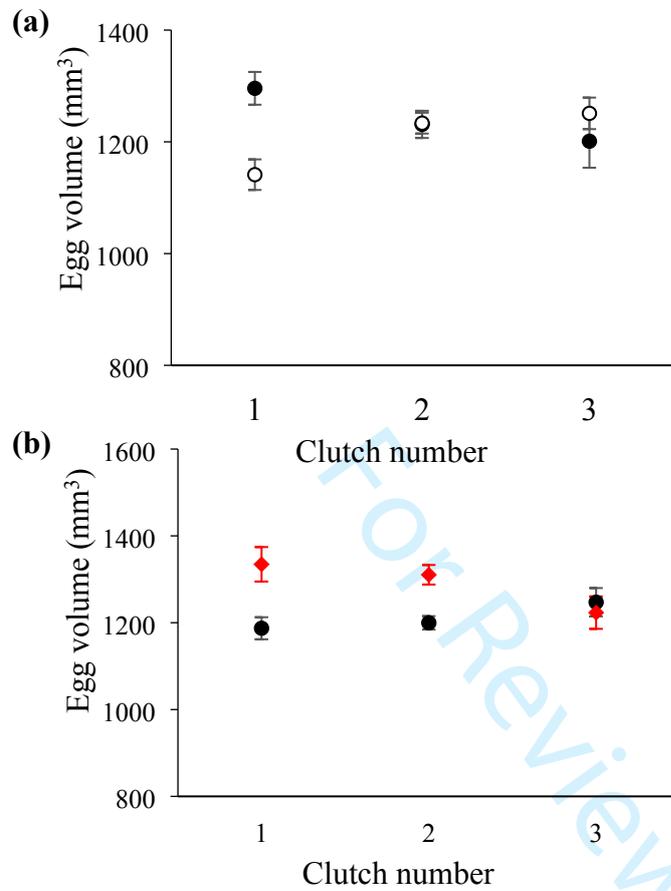
19 **Figure 2 Offspring traits in relation to parental morph, thermal treatment, and age after**

20 **fledging.** (A) Body mass, (B) body size, (C) plasma antioxidant capacity, (D) plasma  
21 hydroperoxide level, (E) aggressiveness, and (F) breathing rate 0, 30, and 60 days after fledging  
22 in red-headed ( $\diamond$ ; R) and black-headed ( $\circ$ ; B) parents previously experiencing thermoneutral  
23 (dashed lines; TN) or thermocritical (solid lines; TC) conditions. Different letters represent  
24 significant differences.

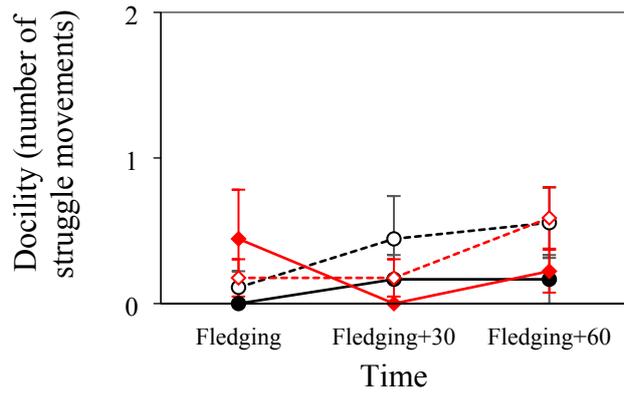




## Supplementary figures



**Figure S1** Egg volume in the different breeding attempts (a) between thermocritical (●) and thermoneutral (○) conditions, and (b) between red- (◇) and black-headed (○) individuals.



**Figure S2** Offspring's docility rate 0, 30, and 60 days after fledging in red-headed ( $\diamond$ ) and black-headed ( $\circ$ ) parents previously experiencing thermoneutral (dashed lines) or thermocritical (solid lines) conditions.



## Supplementary data

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### Box S1 Morph-specific courtship display in the Gouldian finch

Dominique Treschnak<sup>1</sup>, Rita Fragueira<sup>1</sup>, Michaël Beaulieu<sup>1,2</sup>, Monika Eberhard<sup>1</sup>

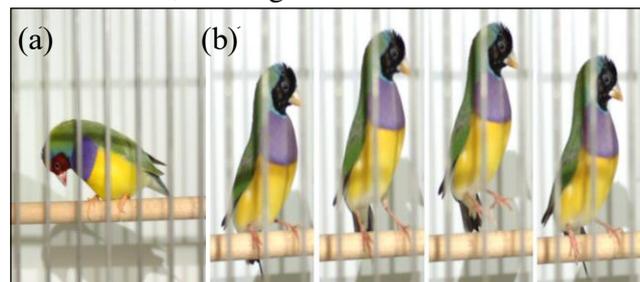
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#### Manuscript in preparation

Animals display a variety of courtship displays, including a variety of song repertoires, different courtship behaviours, like tapping, wing flapping, saults or head shaking, all of which honestly signal male quality (Candolin, 2003; Ota et al., 2015). Females use these cues to choose their breeding mate, accordingly. Complex multimodal courtship displays have therefore evolved in males through female choice, according to sexual selection. In the Gouldian finch, males and females show two main head colour morphs, red- and black-headed. The two morphs show alternative behaviours (Mettke-Hofmann, 2012; Pryke and Griffith, 2006; Williams et al., 2012), however only males have a ritualistic courtship behaviour. First, the male adopts an oblique posture, the feathers of the head are fluffed, and the tail directed towards the female. In a second step, he makes a series of rapid head movements (i.e. head shaking; Fig. S1) in front of the females, before starting a series of little jumps in an upright

posture, as he sings his courtship song (figure 5b; Evans and Fidler, 2003). The female may then fly away or agree to mate with him, making her choice based on this

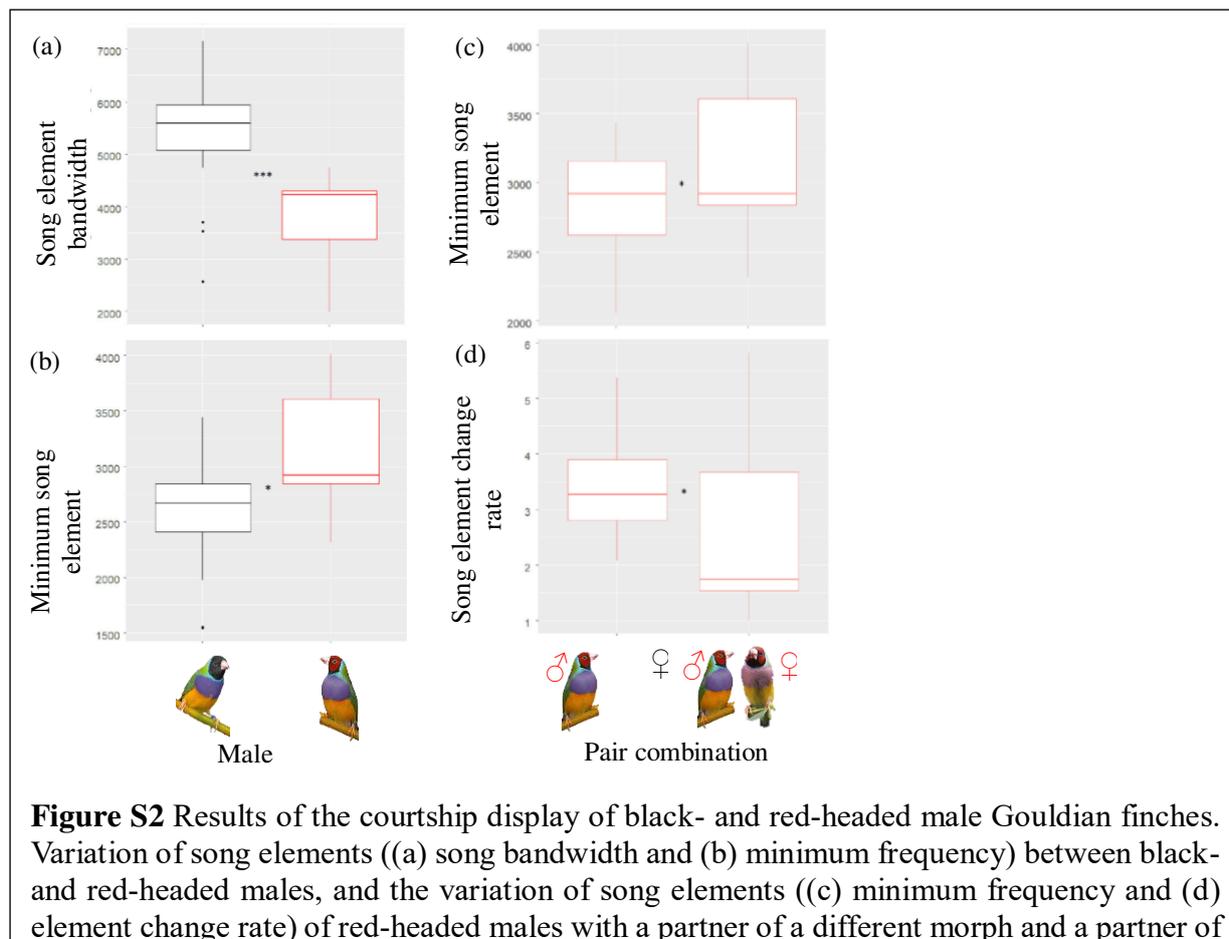


**Figure S1** Courtship display of Gouldian finches consists in several, ritualistic behaviours: (a) head shaking and (b) little jumps in an upright position, with head feathers fluffed, tail directing towards the female, and singing along this display. (Pictures taken by D. Treschnak.)

multimodal courtship behaviour. A previous study revealed that red-headed females preferentially mate with males of the similar morph, whereas black-headed females do not show morph-specific preference (Pryke and Griffith, 2007). Because red-headed males are chosen in both morphs, this raises the question whether the courtship display of red-headed males is more elaborated than

the one of black-headed males. To answer this question, we analysed the courtship displays of males with three same morph females, but also with different morph females, which were randomly chosen. The courtship displays were video-recorded with

shorter than the song bandwidth of black-headed males (Fig. S2). Furthermore, red-headed males sang at higher frequency and used less song elements when coupled with a red-headed female than when coupled with a black-headed female (Fig. S2). However,



a high speed camera (300 fps; Phantom Flex, Vision Research, USA), song elements were monitored with an audio-recorder (TASCAM DR-40, TEAC Europe GmbH, Germany).

We found that red- and black-headed males showed different song elements, with red-headed males singing with higher frequency, but with a song bandwidth 30%

red- and black-headed males did not show any difference in their courtship behaviour (e.g. frequency of head shaking, jumping frequency, jumping height).

These results give a first indication that the song elements of red- and black-headed morphs differ, and that males adjust their courtship song depending on the

partner's morph. Indeed, previous studies already highlighted the importance of male choice in the Gouldian finch, with males mating assortatively. Hence, further studies

should investigate the function of this multimodal courtship behaviour in relation to the maintenance of polymorphism.

## Box S2 Dietary antioxidants to counteract temperature-induced oxidative imbalance

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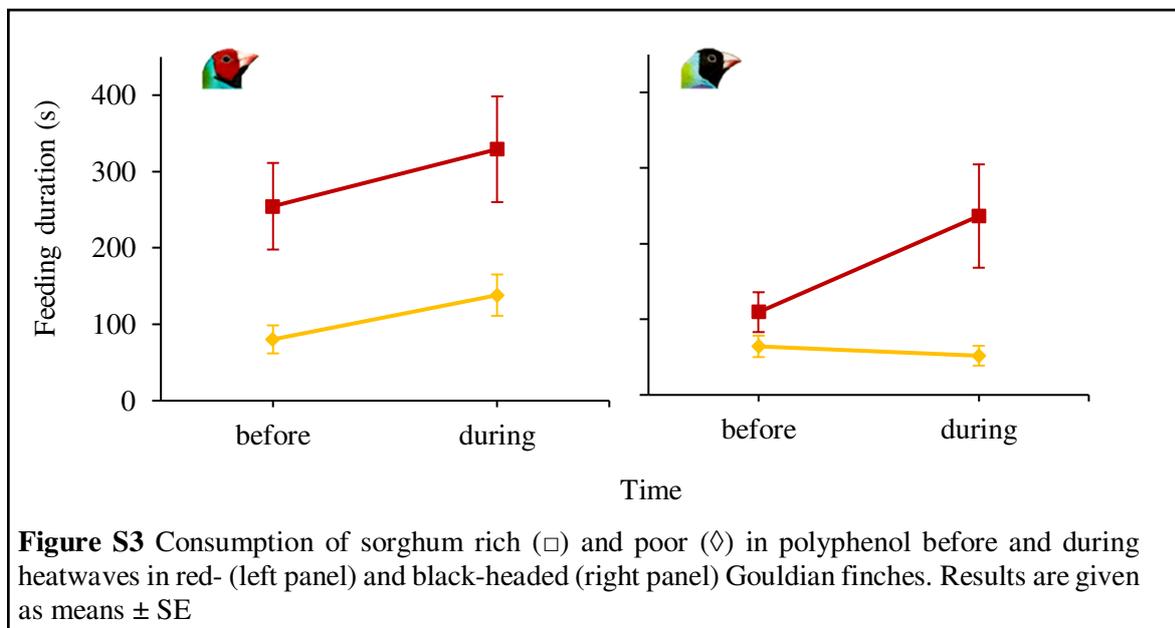
### Manuscript in preparation

During challenging conditions, animals may behaviourally respond similarly to avoid hyperthermia. Physiologically, they can maintain homeostasis, e.g. oxidative balance, through endogenous machinery (e.g. antioxidant enzymes). However, because exposure to stressors may provoke a disequilibrium of the homeostasis (e.g. a shift of the oxidative balance toward oxidative damage), these endogenous defences may not always be sufficient. Therefore, animals may also behaviourally chose food rich in antioxidant content present in their habitat (Beaulieu and Schaefer, 2013). To test the positive effects of antioxidants on temperature-induced oxidative imbalance, several studies expose organisms to different regimes (e.g. rich or poor in carotenoids). However, very few assessed whether organisms can actively take advantage of the antioxidant content in their food. In the Gouldian finch (*Erythrura gouldiae*), red-headed individuals need higher carotenoid levels due to the consumption of carotenoid for the synthesis of their red feathers. They are also more aggressive, less exploratory (Williams et al.,

2012) and have higher metabolic rate (Study 1) than black-headed individuals, suggesting alternative life-history strategies between these two morphs. The Gouldian finch could therefore be a good model to examine intraspecific variability in response to stressors linked to different oxidative status between morphs (Arnold et al., 2015; Beaulieu et al., 2014). We therefore expected red-headed to feed more in sorghum rich in antioxidants compared to black-headed individuals in baseline conditions, i.e. before the heatwaves, but also during the heatwaves. Similarly, because we previously found that black-headed females decrease plasmatic antioxidant defences during the heatwaves, we expected that black-headed females are unable to take advantage of polyphenol content in their food. In this study, we monitored the feeding duration in red- and black-headed pairs before and during heatwaves, and compared these durations between two types of milled sorghum differing in their polyphenol content (mean difference in polyphenol content between the two types: 32%), a sorghum rich in

antioxidants (i.e. red sorghum) and a sorghum poor in antioxidants (i.e. white sorghum) for 4 hours in the middle of the day (i.e. when energy requirements are minimal). As we previously found, red-headed individuals ate overall more than black-headed individuals (Morph:  $F_{1,433} = 9.28$ ,  $p = 0.002$ ), irrespective of the sorghum type (Morph\*Seed:  $F_{1,433} = 0.59$ ,  $p = 0.44$ ; Fig. S3). Furthermore, red- and black-headed individuals differed in their sorghum consumption before and during the

heatwaves (Time\*Morph\*Sorghum:  $F_{1,433} = 6.90$ ,  $p = 0.009$ ). After separating by time, we found that red-headed ate overall more red sorghum than white, irrespective of time (post-hoc tests: all  $p < 0.025$ ; Fig. 2a). However, black-headed individuals did not vary their white sorghum consumption during heatwaves (post-hoc tests:  $p = 0.76$ ) and consumed as much red as white sorghum before the heatwaves ( $p = 0.11$ ), but they ate more red sorghum during the heatwaves than white sorghum ( $p = 0.008$ ; Fig. S3).



Despite the fact that red-headed birds eat, in general, significantly more sorghum rich than sorghum poor in antioxidants, they did not ate more sorghum rich in antioxidants compared to black-headed birds. In accordance with our expectations, we found that black-headed individuals consumed more sorghum rich than sorghum poor in antioxidants during the heatwaves, whereas this consumption difference was not observed before the heatwaves. These results suggest that black-

headed birds seem to take advantage of the food properties and adapt their feeding behaviour in response to thermal conditions, whereas red-headed individuals were not able to do so. In a further step, we will relate these preliminary results of individual feeding choice to oxidative markers, and use the residuals as an index of individual oxidative status, and put this in relation to individual colour variability (beak, head, breast, abdomen, back and tail).





## Acknowledgements

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*Many people have helped me throughout these years in very different ways, leading to the successful completion of this thesis. Here, I would like (or try...) to express all my appreciation for them.*

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*A special thanks to Prof. Klaus F. for our work together, your scientific and personal advice, and for handing me with trust the two climate chambers during two consecutive years. At this point, I would also wish to thank Prof. Gabriele U. and Prof. Gerald K., and the people in their respective working groups, for adopting me, for the significant scientific and personal exchanges and input throughout my PhD.*

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*Thanks to the 112 Gouldian finches in the study and their nice collaboration without whom this work would not have been possible.*

*Thanks to all my students, Max B., Simon O., Sanju S., Mathilde B., Katrin L. and Dominique T. for helping me analysing the videos and assisting me during blood measurements and bird husbandry. Together with all the students of the practical classes, seminars and lectures, I would like to thank you all for being at my side during my first steps at supervising and teaching, for your trust and all the helpful discussions we had together.*

*Thanks to all my co-workers from the Animal Ecology group, with a special thanks to Josi, Anika and Anais for sharing the beautiful office with me, for these memorable evenings together, the numerous peals of merry laughter and your daily moral support. Huge thanks to the Animals@Work group, Ellis, Erika, Cristina, Yoran, Merijn, and Juan-Diego, and a special hug for Blanca, for this great stay in Groningen, for your warm welcome, the several*

*discussions, the huge scientific input and all your encouragement during conferences and in the end of my PhD. See you very soon ;)*

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*I could never get through these emotionally-challenging years without my two halves, on whom I could always count to recharge my batteries. Thanks **Alice & Batsheva** for just being you. You were my real balance and cure during the most difficult periods. You taught me how to stay calm, how to stay strong and how to continue believing in me. I am so grateful for having you two in my life.*

*Finally, I would like to thank and dedicate this work to **my family**, and especially to the two most important people in my life. Obrigada **Mimi**, obrigada **Nuno** para estarem sempre ao meu lado, obrigada de sempre acreditarem em mim, de me dar cada vez mais força para continuar, da vossa compreensão para os meus longos estudos, de me darem todo o suporte moral quando estava precisando, tirarem tempo para mim quando estava em baixo e sempre, **sempre**, tarem de braços abertos, que, sem falta, conduziram ao final a essa tese. Continuo a dizer: nao sei o que faria sem vocês.. Por isso, grando abraço! Amo-vos tanto.*

**Obrigada a todos!**

**Many thanks to everybody!**

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## Curriculum vitae

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# Curriculum vitae

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## Degrees

**2015- 2019: PhD thesis: Effects of simulated heatwaves on the behaviour, maintenance and reproduction in a tropical, polymorphic bird, the Gouldian finch.** University of Greifswald, Germany. Supervisor: Dr Michaël Beaulieu

- Behavioural measurements (Neophobia/Neophilia, Aggressiveness, Docility, Food choice, Diurnal feeding behaviour, Courtship behaviour, Parental care)
- Physiological measurements (Telomere dynamics using TRF method, Oxidative status, Body condition, Colour measurement with spectrometer)
- Reproduction survey of captive Gouldian finches (*Erythrura gouldiae*)

**2013-2015: MSc in Ecophysiology and Animal Behaviour.**

**MSc thesis: Telomere length determinants in early life: genetic and environmental aspects of a mechanism linked to individual fitness.** IPHC-DEPE Strasbourg, France. Supervisor: François Criscuolo. With honours

- Flight performances
- Telomere dynamics using qPCR and oxidative status measurements
- Blood sampling in zebra finches (*Taeniopygia guttata*)
- DNA extractions
- EIA hormone assays

**2012-2013: BSc in Cellular Biology and Physiology.** University of Strasbourg, France. With honours

**2010-2012: BSc in Life Sciences.** University of Luxembourg, Luxembourg. With honours, Prize of the best international mobility.

## Publications

**Fragueira R, Beaulieu M (2019)** Red does not always dominate black: morph-specific behavioral variation in response to heatwaves. *Anim Behav*, 148, 81-91.

**Fragueira R, Verhulst S, Beaulieu M (2019)** Morph- and sex-specific effects of challenging conditions on maintenance parameters in the Gouldian finch. *J. Exp. Biol*, 222, jeb196030.

**Fragueira R, Fischer K, Helfenstein F, Beaulieu M (submitted)** Reduced offspring quality despite increased parental investment after simulated heatwaves in a polymorphic bird.

## Scientific communications:

**10<sup>th</sup> International Congress of Comparative Physiology and Biochemistry**, August 2019 (Ottawa, CA)

Oral presentation requested "All losers! Polymorphism does not represent an evolutionary rescue in Gouldian finches coping with heatwaves."

**Fragueira R, Fischer K, Helfenstein F, Beaulieu M**

**27<sup>th</sup> International Ornithological Congress**, August 2018 (Vancouver, CA)

Oral presentation “To breed or to moult: an examination of morph-specific moult-reproduction strategies and costs in Gouldian finches.”

**Fragueira R**, Verhulst S, Beaulieu M

**European Conference on Behavioural Biology**, August 2018 (Liverpool, UK)

Oral presentation “Temperature-induced behavioural variation in the polymorphic Gouldian finch.”

**Fragueira R**, Beaulieu M

**110<sup>th</sup> Meeting of the German Zoological Society**, September 2017 (Bielefeld, DE)

Poster “Good vibrations: Are they involved in the courtship display of colourful birds?”

Treschnak D, **Fragueira R**, Beaulieu M, Eberhard M.

**DZG Graduate Meeting: Animal Behavior**, May 2017 (Celle, DE)

Oral presentation “Multimodal courtship communication in the polymorphic Gouldian finch (*Erythrura gouldiae*).”

Treschnak D, **Fragueira R**, Beaulieu M, Eberhard M.

**109<sup>th</sup> Meeting of the German Zoological Society**, September 2016 (Kiel, DE)

Poster “The Red and the Black: Differential effect of a heat wave on behaviour and reproduction in a polymorphic bird.”

**Fragueira R**, Fischer K, Beaulieu M

### Referee

**Articles:** (n=1) in Functional Ecology

### Teaching:

**Nov. 2018-March 2019:** Seminars in the module ‘Evolutionary ecology’ for Master students, University of Greifswald, Germany.

**Dec. 2018:** Teaching assistance (2 courses out of 12) in the module ‘Evolutionary ecology’ for Master students, University of Greifswald, Germany.

**Nov. 2015-2018:** Practical courses in the module ‘Evolutionary ecology’ for Master students, University of Greifswald, Germany.

### Supervision:

**2017:** Co-supervisor of Master student at the University of Greifswald: (1) Basin M ‘Effects of a heat wave on the behaviour of a polymorphic tropical bird, the Gouldian finch (*Erythrura gouldiae*)’; (2) Linzel K ‘Diurnal feeding behavior of a polymorphic species in relation to thermal conditions’; (3) Treschnak D. ‘Multimodal courtship communication in the polymorphic Gouldian Finch’

**2016:** Co-supervisor of Bachelor students at the University of Greifswald: (1) Ostermann S ‘Impact of a heat wave on the feeding behavior of a polymorphic tropical finch’; (2) Baumgarten M ‘Temperament differences in a polymorphic tropical finch’; (3) Shresta S. ‘Effects of temperament on reproductive performance in a tropical finch’

### Outreach:

**Annual Meeting of the “Gesellschaft für Arterhaltende Vogelzucht e.V.”**, October 2018 (Neu Ruppin, DE)

Oral presentation “Wirkung einer Hitzewelle auf das Verhalten eines polymorphen tropischen Vogels – am Beispiel der Gouldamadine”

**Fragueira R**





# Affidavits

## 1 Statutory declaration

---

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

*I hereby declare that I have submitted this work so far neither at the Faculty of Science and Mathematics at the University of Greifswald nor at any other university with the purpose to earn a PhD degree.*

*Furthermore I declare that I have written this work as an independent effort and did not use any other sources and guides than those cited in the work. I did not copy any paragraphs of a third author without marking them as a citation.*

Greifswald, den

Fragueira Rita

## 2 Statement in collaborative working

---

Hiermit erkläre ich, dass die in der folgenden Inhaltsübersicht mit meinem Namen gekennzeichneten Kapitel von mir selbständig verfasst worden sind:

**Abstract** Writing: **Fragueira, R**  
**Introduction** Writing: **Fragueira, R**  
**Results and discussion** Writing: **Fragueira, R**

### Publication list:

Study 1 Conceptualization & Methodology:  
**Fragueira, R**; Beaulieu, M  
Data curation & analysis: **Fragueira, R**  
Writing original draft: **Fragueira, R**  
Writing & Editing: **Fragueira, R**; Beaulieu, M

Study 2 Conceptualization & Methodology:  
**Fragueira, R**; Verhulst, S; Beaulieu, M  
Data curation & analysis: **Fragueira, R**  
Writing original draft: **Fragueira, R**  
Writing & Editing: **Fragueira, R**; Verhulst, S; Beaulieu, M

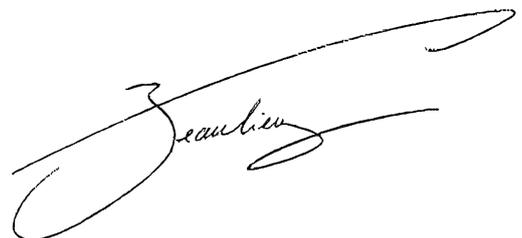
Study 3 Conceptualization & Methodology:  
**Fragueira, R**; Helfenstein F; Beaulieu, M  
Data curation & analysis: **Fragueira, R**  
Writing original draft: **Fragueira, R**  
Writing & Editing:  
**Fragueira, R**; Helfenstein, F; Fischer, K; Beaulieu, M

Greifswald, den

Fragueira Rita

Die Unterschrift weiterer Autoren kann aus technischen Gründen nicht eingeholt werden. Da die betreffenden Kapitel jedoch in Zeitschriften veröffentlicht sind, haben alle Mitautoren in die Publikation eingewilligt

Dr. Michaël Beaulieu (Wissenschaftlicher Betreuer)



### 3 Declaration on the submission of an electronic copy of the PhD thesis

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Mathematisch-Naturwissenschaftliche Fakultät Einverständniserklärung nach § 4 Abs. 1 Nr. c  
Promotionsordnung

*Faculty of Science and Mathematics Declaration of consent according to § 4 sect. 1 Nr. c  
Doctoral Degree Regulations*

Hiermit erkläre ich, dass von der Arbeit eine elektronische Kopie gefertigt und gespeichert werden darf, um unter Beachtung der datenschutzrechtlichen Vorschriften eine elektronische Überprüfung der Einhaltung der wissenschaftlichen Standards zu ermöglichen.

*I hereby declare my consent to produce and store an electronic copy of this thesis for the purpose to enable an electronic examination of the thesis with the goal to exclude plagiarism and to obey good scientific practices.*

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