DIETARY FATTY ACID CONTENT CHANGES THE LONG-TERM ACCLIMATION RESPONSE OF A CAPTIVE BIRD (*TAENIOPYGIA GUTTATA*) TO AMBIENT TEMPERATURES

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Abstract

Dietary fatty acid content changes the long-term acclimation response of a captive bird (*Taeniopygia guttata*) to ambient temperatures

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Due to climate change and managed care, birds may face interacting effects of novel diets and environmental temperatures. I examined how captive zebra finches (*Taeniopygia guttata*) responded to a 6-month acclimation to either thermoneutral or cool temperatures when provided with saturated or unsaturated fatty acid enriched diets. Body mass was lower on the unsaturated fatty acid diet, but only in cool conditions. Individuals in cool temperatures and saturated fatty acid enriched diets had heavier organs, but pectoralis mass and metabolic rate did not display an effect of diet. While birds on each treatments illustrated similar initial increases in plasma fatty acid unsaturation, composition and concentration of constituent fatty acid varied based on their diet and temperature. Individuals on the unsaturated fatty acid enriched diet and cool treatments had lower survivorship. Collectively, my research will allow researchers to better predict how birds will be impacted by shifting or novel environments.

Keywords: fatty acids; temperature; chronic acclimation; metabolic rate; unsaturation; *Taeniopygia guttata*

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Table of Contents

List of Figures:

Chapter 2:

List of Tables

List of Abbreviations & Symbols

Abbreviation	Expanded Term
AIC_C	Corrected Akaike Information Criterion
CI	Confidence Intervals
FA	Fatty acid
Hct	Hematocrit
MUFA	Monounsaturated fatty acid
NEFA	Total non-esterified fatty acid and/or total free-fatty acid
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxidative species
SFA	Saturated fatty acid
SFA-diet	Bird seed mixed with coconut oil
$T_{Initial}$	Pre-experimental exposure recordings
TNZ	Thermoneutral Zone
UFA	Unsaturated fatty acids
UFA-diet	Bird seed mixed with sunflower oil
UIN	Unsaturation index

Chapter 1: Introduction

Introduction to seasonal variation:

As seasons change, organisms are exposed to gradual shifts in environmental conditions, including temperature, food availability, precipitation, and daily light exposure. Birds align their phenology with the changing environmental conditions by changing at multiple levels of biological organization, including morphology, physiology, and behaviour to maximize their survival (Helms 1968; West 1968; Stalmaster & Gessman 1984; Vézina et al. 2006; Wu et al. 2015). Seasonal fluctuations in daily light exposure impact the circadian rhythm of animals (Hintz & Dyer 1970; Tulp & Schekkerman 2008), and ecological factors such as food availability (Warner& Erwin 2005; Walker II et al. 2019). In the latter case, some granivorous birds, such as the American goldfinch (*Carduelis tristis*), have adapted a breeding phenology to breed only when their preferred seed crops are most available (Stokes 1950), to have maximal availability of optimal food for taking on the physiological load of raising their young (Tulp & Schekkerman 2008). Additionally, tropical granivores like the zebra finch (*Taeniopygia guttata*) will opportunistically breed after it rains; rain causes sudden increases in the availability of local seed crops (Zaan 1995; Zaan 1996 (as cited in Carey 2009)).

Fat and feather responses to temperature stress:

Across their annual cycle, birds experience temperatures above or below their thermoneutral zone (TNZ). To overcome stressors like low ambient temperatures, such as those experienced during cold winter months, birds must achieve two things: find ways to reduce relative heat loss, and to increase heat production to make up for lost heat. One way in which birds reduce relative heat loss is by increasing their insulative layers by altering feather density and structural qualities (Nord et al. 2023). Such changes can take months to occur due to the resource requirements to produce new plumage (Rothery et al. 2001). However, because feathers are metabolically inert, they require little energy to maintain once formed (Helms 1968, Houston et al. 1997; Nord et al. 2023). Additionally, birds can increase the mass of their insulative layers of adipose tissue to increase distance between the environment and their core, reducing the gradient for heat transfer, thereby reducing relative heat loss (Bech 1980; Houston et al. 1997; Bairlein 2002; Laplante et al. 2019). Increased fat stores have the added benefit of providing energy for sustaining higher metabolic rates when upregulating heat production (Helms 1968; Bech 1980; Broggi et al. 2019). However, use of fat as a source of fuel for increased heat production necessitates that individuals increase their daily food intake, inciting possible fitness costs (Houston et al. 1997). For example, augmenting daily food intake via foraging can increase an individual's perceived predation risk (Helms 1968; McNamara and Houston 1990). As well, carrying additional fat stores to fuel thermogenesis will increase wing loading, which in turn, decrease take off speeds and

flight efficiency (Witter et al. 1994; Rogers 2015), impacting predator avoidance and survival in the long-term (Burns & Ydenberg 2002). As a result, there are presumably balances between the benefits of carrying extra fuel and insulation and the likely costs that carrying additional mass entails (Helms 1968; McNamara and Houston 1990).

Thermogenic ways of handling cold stress:

In addition to increasing insulation via feather and fat, a complementary approach used by many species to cope with chronically low temperatures is an enhanced capacity for thermogenesis (Roussel et al. 1998; Pani & Bal 2022). Increased thermogenesis can come from passive heat production, induced by increased organ mass/functionality, and from facultative heat production. Many species increase visceral organ mass as a way to increase heat production, including overwintering species. For example, Chinese bulbuls (*Pycnonotus sinensis*) increase pectoralis muscle and heart dry mass when exposed to cold climates (Zheng et al. 2014). Similarly, white-breasted nuthatch (*Sitta carolinensis*), black-capped chickadees (*Poecile atricapuillus*), and house sparrows (*Passer domesticus*) increase the mass of the pectoralis muscle, heart, and digestive organs when exposed to cold winter climates (Liknes & Swanson 2011). Accompanying an increase in the amount of metabolically active tissue is an increase in heat production (Hulbert et al. 2005; Vézina et al. 2006; Milbergue 2018).

In terms of facultative heat production, most birds rely on shivering thermogenesis (West 1968; Bicudo et al. 2001). As muscle contractions are relatively

inefficient, the majority of energy (66-75%) used for these contractions is released as heat (Kushmerick & Davies 1969; Hochachka 1973 (as cited in Hohtola 2002); Block 1994; Ettema 2001). Additionally, a few select orders of birds (Sphenisciformes (penguins), Anseriformes (ducks), and Galliformes (chickens)) display a capacity for non-shivering thermogenesis (Barre 1985; Duchamp & Barré 1993; Eldershaw et al. 1997; Chãinier et al. 2000; Raimbault et al. 2001; Monternier et al. 2014; Roussel et al. 2020). This process is reliant upon dissipating the proton and ion gradients of the mitochondrial membranes and sarcoplasmic reticulum through futile cycling, thereby releasing energy in the form of heat (Barre 1985; Chãinier et al. 2000; Roussel et al. 2020).

Fatty acids and their modulation of cell membranes:

In vertebrate endotherms, the fatty acid composition of an individual's cellular membranes influences its metabolic rate and capacity for heat production (Block 1994; Else & Wu 1999; Wu et al. 2001; Hulbert et al. 2005). Cell membranes are composed of a large number of constituent parts, primarily phospholipids and transmembrane proteins. Fatty acids can be divided largely into two types, unsaturated fatty acids (UFA), which contain double bonded carbons, and saturated (SFA) in which such double bonds are absent. The number of double bonds can vary among fatty acids, from monounsaturated fatty acids (MUFAs) with their single double-bond, to polyunsaturated fatty acids (PUFAs) with multiple double bonds. When a cell membrane has a relatively high proportion of UFAs, particularly PUFAs, the cell membrane has an increased permeability to ions and

polar substances (Bicudo et al. 2001; Wu et al. 2001). This propensity for leakage is reflected in the unsaturation index (UIN), defined as the ratio between the number of double bonds and the total number of fatty acids.

Many ions and molecules may cross the cellular and mitochondrial membranes through the use of transport proteins (Roussel et al. 1998; Saier 2000; Ledesma et al. 2002; Hollenstein et al. 2007; Pani & Bal 2022). However, ion leakage (which is largely controlled by the composition of the fatty acids) uncouples the ion gradients of the cell membranes and requires individuals to upregulate the activity of active transport proteins (e.g., Na^+/K^+ ATPase; sarco/endoplasmic reticulum calcium ATPase) to restore ion gradients (Roussel et al. 1998; Pani & Bal 2022). The increased membrane leakage and activation of transporters causes release of energy stored as adenosine triphosphate (ATP), resulting in an increase in obligatory heat production and an elevated basal metabolic rate (Roussel et al. 1998; Pani & Bal 2022).

Dietary preferences in energetically demanding conditions:

The diets of birds are a major regulator of tissue composition (Pamplona et al. 2002; McCue et al. 2009; Maulucci et al. 2016). Although many fatty acids can be produced/converted from fatty acids that are consumed, a few essential fatty acids must be obtained fully-formed from the diet, including linoleic and α-linolenic acid. These two PUFAs are essential fatty acids required as building blocks for the synthesis of other larger PUFAs (Pamplona et al. 2002; Maulucci et al. 2016). When birds are faced with

physiologically demanding periods, such as cold-stress, they will preferentially consume UFA-rich diets (McWilliams et al. 2002) to ensure an optimal level of tissue unsaturation. Such trends have been reported in willow ptarmigans (*Lacopus lacopus*; Isaev & Borisov 2016) and Iberian bullfinches (*Pyrrhula pyrrhula iberiae*; Hernández 2022), who seek out such food sources, even when such sources are scarce (Renner et al. 2012). Additionally, some species of temperate migratory birds, such as common crossbills (*Loxia curvirostra*) or great spotted woodpeckers (*Dendrocopos major*) will delay migrating to warmer wintering environments, so long as their preferred diets remain available (Newton 2006; Lindén et al. 2011). By maintaining higher dietary UFA consumption, they presumably allow their bodies to increase membrane leakage, and elevate basal heat production.

While UFAs are ideal for handling cool temperature by allowing for higher metabolic rates, they impose potential risks in the tissues for extended periods of time (Pamplona et al. 2002; Buttemer et al. 2010). As birds increase their tissue UIN (i.e., ratio between the number of double bonds and the total number of fatty acids), they increase the risk of incurring lipid peroxidation, a cascade of oxidative reactions which degrade UFAs, primarily PUFAs (Pamplona 2008; Zielinski & Pratt 2017; Su et al 2019). Lipid peroxidation only occurs in UFAs as the double bonds make these fatty acids a viable site for reactive oxygen species (ROS) to attach and be degraded, thereby producing more ROS (Zielenski & Pratt 2017). Birds exposed to ROS put themselves at risk of cellular dysfunction through oxidative damage which may lead to apoptosis (Su et al. 2019). To avoid and/or minimize the complications related to lipid peroxidation, birds may

preferentially select diets rich in SFAs and MUFAs, instead of PUFAs, if they do not require an immediate source of mobilizable energy (McWilliams et al. 2002; Jensen et al. 2020). Individuals may also reduce the risk of peroxidation via increased consumption of antioxidants (Catoni et al. 2008; Senar et al. 2010; Bolser et al. 2013).

If individuals are exposed to temperatures outside their TNZ and they do not have access to diets that would allow them to survive such conditions, they may move to more favourable environmental conditions (Osland et al. 2021). In the tropics, multiple species of birds are expanding their ranges into higher latitudes and altitudes to stay within their historic temperature niche (Freeman & Freeman 2014; Osland et al. 2021; Girish & Srinivasan 2022). These changes in locale may provide birds with the ideal temperatures, but may create new challenges, including limited access to foods with appropriate fatty acid content (Girish & Srinivasan 2022; Tekwa et al. 2022).

There are multiple cases where birds have been exposed to temperature stresses, and a diet they were not be able to control. For example, multiple species in the Eastern Himalayas and New Guinea have entered new geographic ranges, but their rates of dispersal varied with dietary niches (Osland et al. 2021; Tekwa et al. 2022). For example, omnivorous species in the Himalayas and New Guinea have shifted their ranges faster than frugivores or granivores. Frugivores and granivores rely on food sources that take longer to disperse, and as a result, the birds are not able to keep up with shifting environmental temperatures (Freeman & Freeman 2014; Osland et al. 2021; Girish & Srinivasan 2022; Tekwa et al. 2022).

Dietary plant unsaturation gradient

The membrane fatty acid composition of plants shows geographic variation. For example, plants native to higher latitudes and altitudes often rely on greater UFA concentrations, to resist freezing in low temperatures (Zhang et al. 2015). Therefore, any birds that shift their ranges to co-exist with these species may be forced to consume diets rich in UFAs, with resultant increases in tissue UINs. These elevated UINs may impose oxidative challenges on the birds in their new locations as increased UFA concentrations would put these species at further risk of oxidative damages (Grundy 1997; Hulbert 2002; Maulucci et al. 2016; Zielinski & Pratt 2017). However, elevated UIN could be more beneficial for long-term survival because UFAs allow birds to elevate heat production, by increasing membrane leakage, and are more mobilizable sources of energy than SFAs (Bairlein 2002; Hulbert 2002; Guglielmo 2010; McWilliams et al. 2004; Maulucci et al. 2016; Zielinski & Pratt 2017). There are few studies to date that have explored how individuals may manage the competing demands of temperature and dietary fatty acid composition, at least outside of the context of migration biology (see, for example, McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018).

Managed care/conservation:

Currently 13% of bird species have been identified as “threatened” (International Union for the Conservation of Nature 2014) and in need of human intervention to stop

their populations from further declining. In these at-risk species, a sampling of animals is often brought into managed care in hopes of providing favourable conditions that promote the species' recovery. Captive breeding programs are integral components of Species Survival Plans, which are developed to maintain genetically healthy populations under human care in the hopes of supporting the recovery of threatened species within a protected and human managed space (Gippoliti & Carpaneto 1997). However, often when species are first brought into human care from the wild, comprehensive knowledge of the optimal husbandry conditions are not always known. Even in some cases where the knowledge is available (i.e., certain pet species like zebra finches (*Taeniopygia guttata*) and budgerigars (*Melopsittacus undulatus*)), people may not be able to provide the birds with their natural temperatures due to physical limitations of technology. This may result in many captive tropical species living in cool/cold stress temperatures. For the success of captive breeding programs, it is essential to gain a better understanding of how various interacting factors may impact individual physiology.

Multiple environmental stressors to consider:

Negative health effects can occur when animals are not housed under the environmental conditions to which they are adapted (Watanabe 2007). Limited literature has explored how diet and temperature interact to impact the physiology of birds (McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018). To date, dietary fatty acids have been shown to impact several physiological processes related to how

birds respond to temperature acclimation, including metabolic rate, organ size, fat deposit accumulation, membrane fluidity/permeability, and fatty acid composition of blood plasma (Bairlein 2002; Hulbert 2002; McWilliams et al. 2004; McCue et al. 2009; Weber 2009; Maulucci et al. 2016; Carter et al. 2019). However, diet has rarely been examined alongside temperature, and when it has been, focus has typically been on the acute acclimation phase and acute stress response (McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018), rather than the long-term impacts that managed care or changes in geographic climate may impose.

Across acute time periods of exposure to changes in diet and temperature, birds incorporate fatty acids from their diets into their tissues and fat stores (McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018). In previous research examining metabolic rate and body mass of birds consuming UFA versus SFA-enriched diets, there have been contrasting results of how diet can impact the birds. In great tits (*Parus major*; Andersson et al. 2018), a SFA-diet increased BMR in birds housed in the cold. In contrast, Japanese quails (*Coturnix japonica*; Ben-Hamo et al. 2011) held under cold conditions had higher metabolic rates when fed an UFA-enriched diet. Although both studies demonstrated an increase in metabolic rate in cold conditions, the directionality of the impact of diet was conflicting. Additionally, patterns of mass change in response to diet are also conflicting. When responding to cold temperature acclimation, SFA-fed yellow-rumped warblers (*Dendroica coronate*; McWilliams et al. 2002) lost mass until they were returned to an UFA enriched diet, while Japanese quail displayed little variation in mass

regardless of their diet (Ben-Hamo et al. 2011). Due the inconsistent results from previous studies, and the lack of literature examining chronic time frames, further research is required.

Thesis overview and structure:

In my thesis, I tested the general hypothesis that consumption of diets enriched in either saturated or unsaturated fatty acids will alter the physiological response of zebra finches during a temperature acclimation. Over the course of six-months, I provided zebra finches with continuous exposure to either thermoneutral or cool temperatures while offering them diets enriched with either SFAs or UFAs. I examined various morphological and physiological markers at key points in the acclimation process. My thesis is divided into 3 data chapters, described below.

In Chapter 2, I focused on the morphological and physiological mechanisms by which birds maintain thermoneutrality. I hypothesized that body mass, body composition (organ and fat mass) and metabolic rate of birds will change in response to cool or thermoneutral temperatures, and will change differently based on whether an individual's diet is supplemented with SFAs or UFAs.

In Chapter 3, I aimed to identify how birds altered the composition of their available fatty acids and how their blood plasma unsaturation changed over time. I hypothesized that while the fatty acid composition of the blood plasma is initially dependent on the dietary fatty acid composition and temperature, as the experiment

extends into a chronic time frame, the available fatty acids should provide lower risk of oxidative damage. To test this hypothesis, I examined the blood plasma fatty acid composition of the birds at 1–2-month intervals over the course of the experiment.

Finally, in Chapter 4, I provide a narrative of how diets and temperatures may impact the survivorship of birds over the course of the experiment. Since unsaturation of fatty acids have been linked to declines in survivorship (Pamplona et al. 2002; Furness & Speakman 2008; Galván et al. 2015) and cold ambient temperatures can increase metabolic rate (Bicudo et al. 2001; Arens & Cooper 2005; Swanson 2001; Milbergue 2018), the combination of both factors may negatively impact survival more greatly than either diet or temperature in isolation. In this chapter I looked at mortality rate differences across treatments, and describe health implications of the diets. This chapter is directed toward animal welfare, with the goal to inform animal care initiatives.

In Chapter 5, I provide some general conclusions of how birds responded to their diets and temperatures by integrating the findings of the three data chapters. Additionally, I compared the results of my study to the previous acute-time span studies and discussed the similarities/differences. Finally, I provide some recommendations on how to manage captive populations while promoting longevity and minimizing the physiological stress/load of their housing conditions.

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Chapter 2: Dietary fats alter the physiological acclimation of zebra finches (*Taeniopygia guttata*) to housing temperatures by adjusting organ masses, but not metabolic rate

Abstract:

Climate change is forcing many avian species to adjust their geographic ranges, resulting in possible exposure to novel diets. Consumption of novel diets may alter how individuals would respond physiologically to environmental temperature. In this study I performed a chronic acclimation study of zebra finches (*Taeniopygia guttata*) exposing individuals to environmentally-relevant cool or thermoneutral temperatures and diets rich in either unsaturated or saturated fatty acids. Similar to previous studies over acute time frames, body mass initially changed due to an interaction between dietary fats and temperature (unsaturated fatty acid fed birds decreased in mass at cool temperatures compared to saturated fatty acid fed birds; this was not seen at thermoneutral temperatures). However, by the end of six months, the impact of diet and temperature diminished. I found that exposure to cool conditions increased basal metabolic rate (at 3-4 months), and the mass of body fat, pectoral muscle, and visceral organs. Additionally, the mass of the visceral organs was dependent on diet, where birds on unsaturated fatty acid diets had smaller organs. My study illustrates how unsaturated fatty acids in the diet impact the body mass of birds when exposed to cool temperatures, and identifies the potential mechanisms that cause changes in body mass. These physiological changes

allow us to make inferences about possible impacts on survival as habitable temperatures and food ranges continue shifting.

Introduction:

Across taxa, food availability can have profound effects on the behaviour and physiology of individuals. For example, certain temperate species of birds have shifted their natural ranges at the population level in search of their preferred food sources and environmental temperatures (as seen in ptarmigans (*Lagopus*; Lagerholm et al. 2018) and *Charadiiformes* (Maclean et al. 2008)). Meanwhile, other species may delay migration and remain as residents while enduring cold winter temperatures, so long as their preferred food remains available (MacArthur 1959; Newton 2006; Lindén et al. 2011; Velásquez-Tibatá et al. 2013; Princé & Zuckerberg 2015). Irrespective of their response, individuals will be required to balance nutrient and energy intake against their energy expenditure (Dawson et al. 1983; Stalmaster & Gessaman 1984).

With climate change, populations that shift their ranges will be increasingly exposed to novel and more variable seasonal environmental conditions (Valladares et al. 2014; Zettlemoyer & Peterson 2021). For non-migratory species, range shifts that avoid high summer temperatures may come with greater exposure to cold stresses and increased snowfall in winter months (Wang et al. 2011; De Frenne et al. 2013). For populations undergoing range expansion, timing and rates of dispersal may differ among organisms at different trophic levels, resulting in a trophic mismatch between consumers and their food/prey (Berg et al. 2010; Damien & Tougeron 2019, Zettlemoyer & Peterson 2021; Tekwa et al. 2022). Recent studies have highlighted that geographical mismatch may apply to specific aspects of animals' diets (Berg et al. 2010). For example, in the dispersal of multiple species of birds from New Guinea and the Eastern Himalayas, rates

of dispersal have varied based on the dietary niche of the species, where more generalist consumers (omnivores and carnivores) have been shifting their ranges faster than herbivores or insectivore specialists (Freeman & Freeman 2014; Girish & Srinivasan 2022). To predict population-level response to climate change it is necessary to understand the interacting effects of diet and temperature.

Birds have relatively high mass-specific metabolic rates (Hudson et al 2013), which in many species are fueled by lipids, especially during periods of high metabolic demand (Barlein 2002; Devost et al. 2014). For example, non-migratory granivorous species often rely on the high fat density of seeds and nuts to fuel energy-demanding shivering thermogenesis during winter (Pulliainen 1974; Sprenkle & Blem 1984; Greig-Smith & Wilson 1985, Barlein 2002). Seeds contain a variety of unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs), the relative proportions of which can vary across species of plants (Shahidi 2006; Bartkiene et al. 2016). As a result, the fatty acid composition of the seeds can impact seed selection by consumers, depending on the environmental conditions they are facing (Pierce & McWilliams 2005; Renner et al. 2012).

Greater unsaturation of dietary fatty acids enhances lipid mobilization (Mustonen et al. 2009), and as a result, birds living under cool conditions may preferentially consume diets rich in UFAs (McWilliams et al. 2002). Over time, the composition of fatty acids in an individual's tissues will tend to reflect that of their diet. If, for example, the diet is rich in UFAs, there may be an increase in membrane fluidity and passive ion

leakage, as more UFAs are incorporated into cell membranes (Bairlein 2002; Hulbert 2002; McWilliams et al. 2004; Maulucci et al. 2016). However, long-term elevation of UFAs in body tissues can also increase the susceptibility of these tissues to lipid peroxidation, because the double bonds of UFAs are prone to decay (Pamplona 2008; Zielinski & Pratt 2017; Su et al 2019). Thus, higher concentrations of UFAs in the tissue and plasma of birds, especially polyunsaturated fatty acids (PUFAs), can result in the production of reactive oxidative species (Hulbert 2002; Gaschler & Stockwell 2017; Zielinski & Pratt 2017).

Migratory birds can derive metabolic benefits from consuming PUFA-rich food while minimizing long-term risks (Guglielmo et al. 2002; Maillet & Weber 2006; Weber 2009) because they use up fat reserves in a matter of days (Battley 2000; Warnock 2010). In contrast, non-migrating species will instead preferentially consume foods with higher amounts of monounsaturated fatty acids (MUFAs) (McWilliams et al. 2002; Jensen et al. 2020). If MUFAs are not available, individuals may consume foods rich in SFAs and convert the SFAs into MUFAs to maintain the necessary elevated unsaturation within their tissues (Andersson et al. 2015; Jensen et al. 2020). MUFAs reduce the risk of lipid peroxidation, when compared to PUFAs (Zielinski & Pratt 2017), while providing the thermoregulatory benefit of elevated metabolic rates to non-migratory birds (Jensen et al. 2020).

When acclimating to changes in temperature, birds initially adjust their body mass via changes in fat stores (West & Meng 1968; Dawson et al. 1983; Li et al. 2020). In

the cold, these fat stores allow individuals to retain heat (Bech 1980) and to serve as energy reserves for the night (Blem 1976; Dawson et al. 1983; Cooper 2007). However, the type of fats consumed in the diet can impact the relative ease with which individuals can maintain fat stores over the long-term. For example, UFAs have lower melting points and are mobilized at faster rates than SFAs (Mustonen et al. 2009). As a result, birds on an UFA-rich diet may to need to consume food at higher rates or otherwise risk diminishing their fat stores (Guglielmo 2010; Zielinski & Pratt 2017). Additionally, maintaining larger fat stores for long periods of time increases the risk of oxidative stress in UFA-rich cell membranes and adipose tissue (Grundy 1997; Hulbert 2002).

Although birds can use their fat stores to help insulate themselves from the cold in short-term daily cycles (Houston et al. 1997; Dubois et al. 2016), acclimation to ambient temperatures typically takes between 1-3 weeks (McKechnie 2008; Dubois et al. 2016; Thompson et al. 2016), and involves changes to the size of visceral organs (Liknes & Swanson 2011; Zheng et al. 2014) and an increase in basal metabolic rate (BMR; McKechnie 2008; Swanson 2010). BMR can be influenced by membrane fatty acid composition (Hulbert et al. 2005), which in turn may be affected by a bird's diet (Guglielmo et al. 2002; Hulbert et al. 2005; McCue et al. 2009; Carter et al. 2019). Diets rich in UFAs can increase an individual's metabolic rate because they have an elevated ratio of double bonds to the number of fatty acid chains (unsaturation indexes; UIN; Pamplona 2008). By increasing an individual's UIN, membrane leakage increases, which can lower the efficiency of cellular processes and result in an increased release of heat

(Roussel 2020). As such, there may be benefits to consuming UFA-rich diets in the cold because these diets allow for greater passive heat production (McGuire et al. 2013). But what if such diets are not available? Understanding the interactive effects of diet and temperature is of increasing importance as species undergo range expansions, yet the combined physiological consequences of potential mismatches remain largely unknown.

In this study, I used captive zebra finches (*Taeniopygia guttata*) to explore the interactive effects of dietary fat and temperature acclimation on metabolic rate and body composition. Zebra finches are a well-characterized, non-migratory granivore, native to Australia and Indonesia. I exposed individuals to either cool or thermoneutral ambient temperatures over the course of six months, while supplementing their diet with either SFAs or UFAs. I monitored changes in body mass over the entire six months, and examined metabolic rate and body composition after three and six months, respectively.

Experimental Methods:

Animal care:

Use of animals was approved by the Trent Animal Care Committee following the guidelines of the CCAC (Animal Use Protocol # 26447). Zebra finches were housed in flight cages (45 cm × 45 cm × 90 cm) in the Trent University Animal Care Facility. Individuals were housed in one of 16 single sex cages, with 6 individuals per cage. Cages were within one of two environmental chambers set to 28°C. Each cage contained five perches, one of which was wrapped in paper towel to act as a form of enrichment, and

to provide additional way for birds so clean themselves aside from using the sand bath on the floor of each cage. All individuals were provided with *ad libitum* grit, water and "Canary/Finch Food" seed mix (containing plain canary seed, canola, nyjer, flax, and red villet; Essex Topcrop Sales Limited). Initially, all birds received cooked egg daily and lettuce twice per week, but approximately two months into the experiment egg provisioning was reduced to every second day (as birds stopped eating as much egg as they previously had been consuming at the start of the experiment). The reduction of dietary egg provisioning may have impacted the consumption of the main constituent fatty acids found in egg (order of highest concentration (oleic, palmitic, linoleic, and stearic acid); Juhaimi et al. 2017). However, since both diet treatments displayed this reduction in egg consumption and both then had egg mutually reduced in their weekly feedings, there should not have been any skewing of fatty acid consumption between treatments.

Pilot study:

Prior to performing our main experiment, I performed a pilot study to ensure that birds accepted the dietary change and manipulation of environmental temperature. Prior to the start of the pilot, all individuals (N=96) were weighed (±0.001g) and a 100ul blood sample was collected. I used a 2x2 design encompassing the following treatments:

Cool temperature x UFA-enriched diet (UFA-cool)

Cool temperature x SFA-enriched diet (SFA-cool)

Thermal neutral temperature x UFA-enriched diet (UFA-TNZ)

Thermal neutral temperature x SFA-enriched diet (SFA-TNZ)

All individuals were initially housed at 28 °C. Once blood sampling was completed, I began supplementing their standard seed by mixing in either sunflower oil (UFA diet: PC Organics Organic Sunflower Oil) or coconut oil (SFA diet: Nature's Way Premium Coconut Oil) at a seed to oil ratio of 5:1 (w:w), following Andersson et al. (2018). To compensate for the higher level of vitamin E (an antioxidant) found in sunflower oil, α-tocopherol (Sigma Aldrich (±) α-tocopherol, T3251-25G) was added to the coconut oil at 50 mg/100 g oil (Shahidi 2006; Andersson *et al.* 2018). On the fourth day of having access to their food (Day 4), the initial housing temperature of 28 °C was gradually changed 3 °C/day to either a cool temperature (18 °C, UFA-cool or SFA-cool) or a thermoneutral temperature (35 °C, UFA-TNZ or SFA-TNZ; Calder 1964; Wojciechowski *et al*. 2020). The cool temperature is the average maximum temperatures wild zebra finches experience in winter (Zann *et al.* 1995).

On the third day of birds having received their diets (day 3) one bird from the UFA diet treatment (temperature treatment had not yet been assigned) was found dead (without any notable symptoms). On day 7, I removed a single UFA-cool bird from the experiment because it had oily feathers and swollen eyes. On day 12 of the experiment, two more individuals from the UFA-cool treatment (out of 24) died, and seven individuals displayed ruffled feathers and squinting eyes. The pilot was immediately stopped on day 12, all birds were placed on the pre-experimental seed diet, and over the

span of two days, returned to 28 °C. All birds were kept in their respective treatment groups (SFA-cool, SFA-TNZ, UFA-cool, UFA-TNZ) while exposed to the standard diet, housing temperature, and conditions for 14 days.

Experimental diet and temperature manipulation:

At the start of the experiment (day 0), housing temperatures were adjusted by 4 °C/day to either 35 °C (TNZ) or 20 °C (cool; rather than 18 °C as in the pilot). On day 14, birds were provided a new diet with a seed to oil ratio of 11:1 (w:w), following McCue *et al.* (2009). Approximately 1 month after the beginning of the experiment (day 34), some birds in the UFA-cool temperature treatment again began to display similar roused feathers and squinted eyes as noted in the pilot, so I raised the temperature for the two cool treatments (UFA-cool and SFA-cool) to 24 °C. No further adjustments were made. All treatments (20 °C for 1 month plus 5 months at 24 °C = cool; 35 °C=TNZ; oil to seed ratio = 1:11; sunflower oil = UFA; coconut oil =SFA) remained unchanged until the end of the experiment. Sample sizes at the beginning of the experimental treatments were: SFA-cool: 12 females, 11 males; SFA-TNZ; 12 females, 12 males; UFA- Cool: 10 females, 8 males; UFA-TNZ: 11 females, 12 males. Number of individuals varied slightly among cages, but at the start ranged from 4-6 per cage.

During the pilot and experimental treatments, a total of 39 (5 & 34 respectively) birds were removed or died [See Chapter 4], the majority of which were from the UFA diet × cool stress treatment (20/39). To increase the sample size of the UFA-cool

treatment, an additional cage of females (n=4) and males (n=6) were added on days 30 and 35, respectively.

Blood draws & body mass:

Blood samples and morphometric measurements were taken from each individual prior to any experimental manipulations ($T_{Initial}$, approximately 30 days prior the exposure to the experimental treatment) and at 4 subsequent time points once the experiment had begun: (T_1) 27-30 days, (T_2) 55-58 days, (T_3) 159-161 days, and (T_4) 189-203 days (Fig 2.1). Blood samples (30-100 µL) were taken using a 25G 5/8 Precision Glide® needle and 75 µL heparinized capillary tubes. Prior to each blood draw, birds were fasted for 1.5 to 3.0 hours to ensure they were in a post-absorptive state. Blood was centrifuged (Thermo IEC MICRO-MB capillary tube centrifuge) and plasma and red blood cells were separated and stored in individual micro-centrifuge tubes at -70 °C for a subsequent study [see Chapter 3].

After each blood draw, body mass (± 0.001 g, Sartorius LE 2202), flattened wing chord length (± 1mm, with ruler), and head-to-bill length (± 0.01 mm, with manual calipers) were recorded and individuals were then returned to their home cage. The delayed start birds from the UFA-cool treatment (n = 10) maintained the same respective sampling times from the start of their experimental treatment as the earlier started cages, until the third blood draw (T_3), which occurred at 4 months (rather than 5). Birds this size take approximately 3 months to experience 95% fatty acid turnover in their body

(Carter et al. 2019); therefore, I reasoned they should have displayed chronic acclimation whether sampled at 4 or 5 months.

Basal metabolic rate:

Between 3-5 months (between T2 and T3), birds had their basal metabolic rate (BMR) measured using flow through respirometry. As an estimate of BMR, oxygen (O_2) consumption and carbon dioxide (CO_2) production were measured. Methods were based on those of Burness et al. (2010) and Andersson, et al. (2018). O_2 consumption was recorded for 12 hours overnight starting between 19:00 and 21:30, as the birds are expected to be less active overnight and therefore providing more accurate BMR readings. To ensure individuals were post-absorptive they were fasted for 1.5 hours prior to being placed into one of three 1 L plexiglass chambers, within a temperature-controlled cabinet set to 35 °C to keep all birds within their TNZ (Calder 1964; Wojciechowski *et al*. 2020). Pre- and post-respirometry body masses of each bird were recorded for each trial.

During respirometry, water vapor and CO_2 were scrubbed prior to reaching the chambers using columns of indicating Drierite (W.A. Hammond Drierite Company LTD), soda lime (Alfa Aesar, ID:24005), and Ascarite (Acros Organics, Arthur H.Thomas Company, ID Ascarite (II) CO_2 absorbent 20-30 mesh). Air flow to the 3 chambers and a baseline (containing no chamber) were directed automatically by a multiplexor (TR-RM, Sable Systems, Las Vega, NV, USA), with a flow rate 500 mL/min, regulated using mass

flow controllers (Sierra Instruments Inc). Air leaving the chamber (controlled by the multiplexor) was subsampled and scrubbed of water vapor prior to reaching the CO_2 analyzer (CA-10, Sable Systems, Las Vega, NV, USA), and then scrubbed of CO_2 prior to entering the O_2 analyzer (FC-10, Sable systems, Las Vegas, NV, USA). The system was calibrated before every run using >99% N_2 gas (PRAXAIR, N799109801), 1.00% CO_2 gas (PRAXAIR, W787407011), and regular room air scrubbed of water vapor and CO_2 (20.95% O_2). The accuracy of the respirometry system (± 3%) was verified every second run via burning a known amount of methanol.

Each chamber was sampled for 20-minutes and then switched to a baseline for 20 minutes to allow for the analyzers to be flushed with new air and to account for any drift in values over time. O_2 and CO_2 concentrations were recorded every 5 seconds, with the BMR for each bird being determined as the average of the lowest consecutive 50 samples (4 minutes 10 seconds) through the whole night. Because water was scrubbed before both analyzers and CO_2 was scrubbed before the O_2 analyzer, equations 10.1 and 10.7 (Equations: Lighton 2008) were used to calculate VO_2 and VCO_2, respectively.

Lighton 2008: 10.1:

$$\dot{V}o_2 = FR_i(F_iO_2 - F''_eO_2)/(1 - F''_eO_2)$$

Lighton 2008: 10.7:

$$\dot{V}co_2 = FR_i[(F'_eCO_2F'_iCO_2) - F'_eCO_2(F_iO_2 - F'_eO_2)]/(1 - F'_eCO_2)$$

Body composition:

After birds completed their T_4 blood draws (range:161-202 days, mean = 194.0 days) and had their morphometrics recorded, each individual was returned to its cages for a minimum of 30 minutes with *ab libitum* access to food and water. Seven random individuals per sex per treatment were then removed one at a time, taken to a separate room, anaesthetized with isoflurane gas, and then euthanized via decapitation. Immediately after euthanasia, trunk blood was collected into heparinized capillary tubes, centrifuged to isolate plasma from red blood cells, which were then stored separately at -70 °C.

Within 5 minutes of euthanasia, the left pectoralis muscle, heart, and liver were dissected and placed in pre-weighed air-tight cryotubes, temporarily frozen in liquid nitrogen, and then transferred to -70 °C for storage. The gizzard and small intestine were removed, cut open longitudinally, flushed of debris using tap water, and patted dry with paper towel. Wet mass for right pectoralis muscle, kidneys, gizzard, and small intestines were measured on a pan balance (± 0.001 g, SI-124 Denver Instrument). After weighing, organs (right pectoralis, kidney, gizzard, and small intestine) were returned to the carcass and stored at -20 °C for fat and water content analysis. Mass of the cryotubes containing heart and liver were later recorded; mass of the heart and liver was then determined using the difference between the mass of the vials containing the heart and liver and the pre-determined mass of the vials.

Carcasses (minus the left pectoral muscle, heart, and liver) were placed in pre-weighed ashless filter paper envelopes and freeze dried to a constant mass for 72 hours (Labconco Freeze Drier). Dried carcasses were weighed prior to fat extraction to provide a pre-fat extraction mass. To estimate fat content, dried carcasses were placed into a Soxhlet apparatus containing petroleum ether (Thermo Scientific Petroleum ether 40/60, L14649.0F) for 8 hours, left to air dry in the fume hood for 8 hours (to evaporate any remaining solvent) and then re-weighed. Fat content was calculated as the difference between pre-extraction mass and post-fat extraction mass.

Statistical Methods:

Statistical analyses were carried out using the "MuMIn", "lme4", "AICcmodavg", and "factoextra" packages in R (v.4.2.2 (2022-10-31)) through RStudio ("Cherry Blossom", 2023-03-09).

Information theoretic analyses:

Our statistical models often had 4 main effects (e.g., diet, temperature, sex, time [as an ordinal variable]), plus 2- and 3-way interaction terms. To identify which models explained the most variation in the dependent variable, I used an information theoretic approach used (Burnham et al., 2010). Models were evaluated using corrected Akaike Information Criterion (AIC_C). Models with a ΔAIC_C of < 2 were considered to hold strong support, while models between 2 and 4 ΔAIC_C provided moderate support (Burnham &

Anderson 2004). R^2 was used with AIC_C and evidence ratios to provide information on the goodness of fit for each set of models. If models were run as linear mixed models through the AIC_C analysis, marginal (not accounting for variation due to the random effect) and conditional R^2 values (accounting for random effects) were reported (Nakagawa & Schielzeth 2013). In the cases where simple linear regressions were used, adjusted R^2 values are reported, as increasing the number of predictor variables between models could otherwise inflate the R^2 provided in each model. To display the directionality of the trends, 95% confidence intervals (CI) and associated coefficients were reported.

Body mass, organ mass, and fat mass:

Prior to the analyses, residuals of the data were examined for outliers. Body mass residuals were based on a relationship between body mass and wing chord length; organ masses and body composition residuals were based on the relationship between each organ mass or fat mass and the mass of the birds at the last time point measured (T4). To avoid part-whole correlations (Christians, 1999), organ mass was subtracted from an individual's T_4 body mass prior to analyses. Residuals for body mass, organ masses, and fat mass were natural log transformed and scrutinized to four standard deviations of the mean. No residuals were beyond ±4 SD, and therefore all data points were used for analysis.

To explore factors contributing to variation in body mass, linear mixed models including random effects of cage, and individual identity nested within cage were run (Schielzeth & Nakagawa 2013). Models included combinations of temperature, diet, time (as an ordinal variable), and sex. Additionally, models including the two and three-way interactions of diet, temperature, and time were examined.

To explore the effects of diet and temperature on fat content and the mass of various organs, linear mixed models were used with cage identity as a random effect. In analyses of organ mass, cage identity explained less than a thousandth of the variation from the residuals of each organ, therefore cage identity was excluded, and I ran all analyses of organs as linear regressions instead. For analyses of fat content and organ mass, models included the main effects of diet, temperature, body mass (minus fat content or the organ of interest), sex, the two-way interaction of body mass with all other main effects, the two-way interaction of diet and temperature, and the three-way interaction of diet, temperature, and body mass. To reduce the masses of the visceral organs (heart, liver, gizzard, and small intestine) into 1-2 orthogonal axes, I performed a Principal Component Analysis (PCA) using a correlation matrix to standardize the data. Kidney mass was not included in the PCA because I was unable to collect mass of 6 kidneys; pectoralis muscle mass was also analyzed separately from the organ PCA as it is functionally distinct, contributing largely to shivering thermogenesis and peripheral movement.

Basal metabolic rate:

Prior to analysis, data were examined for possible outliers using respiratory exchange ratios (RER) and O_2 consumption. Six of the readings (from the same 2 nights of recordings) had biologically improbable RER for a bird at rest (negative values or >1.0; RER = -0.89, 1.55, 1.64, 1.84, 2.31, and 2.55). Four of these birds were run a second time and I used the second reading for the rest of the analysis; the other two birds were excluded as outliers as they did not have second trial run. In the end, I had 77 individuals for which I had reliable estimates of overnight rates of O_2.

In the analysis of O_2 consumption, statistical models included cage identity and respirometry chamber identity as crossed random effects. O_2 consumption models included the main effects of diet, temperature, and sex as fixed effects, as well as the interaction between diet and temperature. The body mass of birds (averaged from before and after a respirometry trial) was used as a covariate on its own, in two-way interactions with diet, temperature, and sex, and in a three-way interaction with diet and temperature.

Results:

Effects of temperature on body mass varied with diet and length of temperature acclimation:

Throughout the experiment, body mass was best predicted by the type of dietary fats being consumed (Diet) and the length of time (Time) individuals were on their

specific diet, as indicated by the terms Diet and/or Time appearing in each of the models with strong support ($\Delta AIC_C < 2.0$; Table 2.1). Despite expectation that temperature would be one of the main effects, temperature only appeared as a fixed effect in the lowest ranking top model (ΔAIC_C=1.94; Table 2.1). However, there was strong support for a three-way interaction between diet, temperature, and time, which explained approximately 78% of the variation in body mass (R^2_{con} = 0.779, Table 2.1). During T1 and T2, birds displayed significantly higher body mass when in the cold conditions and fed a SFA rich diet, but not when fed an UFA-rich diet (p<0.001). However, the interaction between diet and temperature diminished in significance as exposure to the treatments persisted into T3 and T4 (Fig. 2.2; $p_{Diet:Temp:Time3}$=0.2179, $p_{Diet:Temp:Time4}$=0.0392 vs $p_{Diet:Temp:Time1}$<0.001, $p_{Diet:Temp:Time2}$<0.001, SS Table 2.1). In the TNZ treatment, individuals had similar body mass across time, irrespective of diet remaining significantly lighter than cool acclimated birds (Fig. 2.2TNZ). Sex contributed little to explaining changes in body mass, and did not appear in any of the top four models (Table 2.1).

Fat content was not impacted by diet or ambient temperature:

According to AIC, after six months of exposure to their respective treatments, the amount of fat on each individual (Carcass Fat) was best predicted by a two-way interaction between the sex of the birds and their lean mass at the time of measurement, when temperature was included as a fixed effect (Table 2.2; Model 2: $\Delta AICc$=0.10, R^2c=0.342). In the cold, finches had less carcass fat; males also displayed a

different trend in carcass fat mass than females, where heavier males had higher levels of fat mass, females remained relatively stable in fat mass (Fig. 2.3). Model 1 explained only 1.05 times the variance in carcass fat that Model 2 could, and Model 2 had 10% higher goodness of fit according to adjusted R^2 (Model 2 R^2_{adj}= 0.346 vs Model 2 R^2_{adj}=0.234; Table 2.2). A sex by mass interaction appeared in the second and third top models (0.1 and 0.9 ΔAIC_C). Sex appeared in most of the models with strong support (Table 2.2). Although sex, mass, and temperature all repeatedly appeared in the strongly supported models, based on model averaged estimates no fixed effects significantly impacted fat content of the birds (all $p>0.05$, Table 2.5). Diet did not appear in any models with strong support, indicating that an individual's fat levels were not impacted by the type of fatty acids in their diet (Table 2.2).

Pectoralis muscle was larger following chronic acclimation to cool conditions:

Pectoralis muscle mass was best predicted by body mass and temperature, which appeared in all models with strong support (Table 2.2). The main effects of sex and diet occurred in the second and third models, but appeared to have little impact on pectoralis muscle mass. Including either sex or diet increased the AIC_C score by nearly two (ΔAIC_C=1.84 & 1.91; Table 2.2) and explained 2.5 times less variance than the model containing just body mass and temperature (ER=2.51 &2.59; Table 2.2). Pectoralis-free body mass and temperature were the only terms to significantly predict pectoral muscle

mass (both $p<0.01$; Table 2.5). The pectoral mass of cool acclimated individuals was greater than the pectoral mass of birds living in TNZ (Fig. 2.4).

Visceral organ mass was affected by diet, temperature, and body mass:

PC1 explained 59.7% of the variation in organ size of birds after a chronic acclimation to diet and temperature (Table 2.3), while all other dimensions explained less than the minimum expected amount of variance (25%; PC2=16.4%, PC3=13.9%, PC4=10.0%). According to PC1, all organs were positively correlated with each other (-0.7 to -0.8), but negatively correlated to PC1. To simplify data interpretation, I negatively transformed PC1 so that increases in the reported data directly reflects increases in the 4 organs examined.

Ambient temperature, diet, and mass of the birds explained most of the variance in the four organs (Table 2.4). Birds in cool conditions had significantly larger visceral organs than those acclimated to thermoneutral conditions ($p<0.001$; Table 2.5). Additionally, birds which were acclimated to UFA-rich diets displayed significantly smaller organ masses irrespective temperature when compared to birds fed SFA-enriched diets ($p=0.042$, Table 2.5, Fig. 2.5). Not surprisingly, as body mass increased, organ masses increased as well ($p=0.008$, Table 2.5). Although sex appeared in the second top model (Table 2.4), it did not significantly impact organ mass ($p = 0.6006$, Table 2.5).

Kidney mass after a chronic acclimation was related to an interaction of diet and temperature:

Kidney mass was best explained by the interaction of ambient temperature and dietary treatment (Table 2.4). The interaction of diet and temperature on its own was ranked as the top model according to AIC_C (Table 2.4, Fig. 2.6), which also had the highest R^2_{adj} of any models with strong support (R^2_{adj} = 0.376). However, according to model averaging using the 7 models with strong support, temperature was the only independent variable which significantly impacted kidney mass ($p<0.001$, Table 2.5), with kidneys being significantly heavier in cool temperature acclimated birds. Diet was not a significant predictor of kidney mass ($p=0.808$, Table 2.5). Body mass appeared in four of seven models with strong supports (Table 2.4), but was not a significant predictor of kidney mass ($p = 0.494$, Table 2.5). Sex did not appear in any models with strong support (i.e., $\Delta AICc < 2$).

Basal metabolic rate was highest among individuals acclimated to cool temperatures, independent of diet:

According to AIC_C, BMR rate was positively related to body mass and was higher in birds acclimated to the cool treatment; temperature and mass were found in all models with strong and moderate support (Table 2.6). The model containing temperature and mass was at least 1.87 times more likely to be the top model than one containing either diet or sex (ER=1.87 & 2.21, Table 2.6). Both temperature and mass

significantly impacted BMR (both $p<0.001$, Table 2.7), with birds acclimated to cool temperatures consuming more O_2 for a given body mass than birds acclimated to the warmer TNZ (Fig. 2.7). Despite diet and sex appearing in models with strong support (Table 2.6), according to model averaging neither diet nor sex significantly impacted O_2 consumption ($p=0.279$ & 0.362 respectively, Table 2.7).

Discussion:

I analyzed morphological and physiological parameters of zebra finches experiencing a chronic acclimation to different ambient temperatures and to diets of differing fatty acid composition. I hypothesized that diet and temperature would interact to change how birds typically respond to either physiological stressor. I predicted that birds acclimated to cool temperatures would increase their body mass. Mechanistically, this would likely be due to increased size of flight muscles, visceral organs, and fat mass. Additionally, I predicted that birds fed UFA-rich diets would have smaller organs. Accompanying the changes in visceral organ and pectoralis mass, metabolic rate was predicted to be higher in the cool treatment, and higher in the UFA-fed birds.

Body mass:

As predicted, I detected a significant three-way interaction between diet, temperature, and time. Body mass was lower in birds when acclimating to thermoneutral temperatures (Fig. 2.2), while their responses to the cool treatment

varied depending on which dietary treatment they were assigned. Similar to previous literature, there was an interaction between diet and temperature during the initial acclimation phase (Andersson et al. 2018, Ben-Hamo et al. 2011).

Individuals in the cool conditions displayed a significant drop in mass between $T_{Initial}$ and T_1 if fed a UFA-rich diet ($p_{Diet:Time1}<0.001$; Table SS2.1); this trend was not noted in the SFA diet. One of the early phase responses to cool temperatures is an increase in fat mass, which relies on the dietary fats being consumed (West & Meng 1968; Carey et al. 1978; Dawson et al. 1983). UFAs have lower melting points, are easier to mobilize compared to equally long SFA carbon chains (Blem 1976; Hulbert et al. 2005), and break down due to lipid peroxidation (Zielinski & Pratt 2017). These molecular properties of UFAs likely caused UFA-cool birds to have difficulty maintaining energy reserves, resulting in their initial mass loss, which never fully recovered to levels seen in the SFA-fed birds, despite ad lib food. In contrast, SFA-cool individuals maintained a relatively constant mass, which presumably acted as a thermal buffer while they were undergoing tissue-level acclimation to cool temperatures (Fig. 2.2).

Once the UFA-cool birds completed the acute acclimation to the conditions, their bodies likely developed mechanisms for acclimating to chronically cool temperatures that are slower to establish, such as an increase in mitochondrial density and function in active tissues (Milbergue et al. 2022) or an increase in visceral organ mass (Fig. 2.4). Once birds reached six months of acclimation, the differences in the properties of the

fatty acids they consumed and the ambient temperatures they inhabited led to only marginally significant differences in body mass between treatment groups (Fig. 2.2).

Songbirds can acclimate to temperatures in relatively short amounts of time (1-3 weeks; McKechnie 2008; Dubois et al. 2016; Thompson et al. 2016). In contrast, responding to changes in dietary fat composition may take months before birds fully acclimate their tissues to reflect the diet being consumed (Carter et al. 2019). Therefore, at the beginning of a chronic acclimation (Fig. 2.2: T_1 & T_2), birds presumably experienced changes in their physiology before reaching a steady state at about 3-4 months. Future studies could explore differences at the mitochondrial level in muscles or visceral organs following long-term acclimation to diet and temperature.

Body composition; fat content:

Birds living in colder temperatures typically increase subcutaneous fat stores, which act as both energy reserves and as a form of insulation against heat loss (Houston et al. 1997; Bairlein 2002; Dubois et al. 2016; Thompson et al. 2016). Although, I noted that temperature appeared in the top two models for explaining trends in fat deposition (ΔAIC_C= 0.00 & 0.10, Table 2.2), based on model averaging, neither diet nor temperature significantly predicted fat mass ($p_{Temperature}$=0.5596, Table 2.5; diet was not in any of the top models for carcass fat, Table 2.2). The reason why our results differ from expected is not clear. However, since most of the literature linking consumption of dietary fats to fat mass in birds are from migratory studies (Mac Arthur 1959; Bairlein 2002; Guglielmo et

al. 2002; McWilliams et al. 2004; Maillet &Weber 2006; Weber 2009; Lindén et al. 2011), typically reflecting very short-term changes, perhaps it was because I used a non-migratory species over a longer time frame. Migratory species of birds are adapted to rapidly increase their body mass by consuming unsaturated fatty acids (especially PUFAs) and storing them in enlarged fat stores, then oxidizing them rapidly during migration (Pierce & McWilliams 2005; Maillet & Weber 2006). By rapidly exhausting their fat stores, migratory species limit their exposure to UFAs and lower their risk of lipid peroxidation.

Body composition; organ mass:

One method for acclimating to the cold is increasing the mass of organs (Liknes & Swanson 2011), especially organs with have higher tissue specific-metabolic rates (Daan et al. 1990; Scott &Evans 1992; Weber &Piersma 1996; Chappell et al. 1999; Wang et al. 2001). Since birds in the cold require greater heat production to maintain homeostatic conditions, they develop larger visceral organs which allow for greater total heat production (Zheng et al. 2008; Zheng et al. 2010; Liknes & Swanson 2011; Zheng et al. 2013; Milbergue et al. 2018). Our results are consistent with these previous studies – birds acclimating to cool conditions had heavier organs (Table 2.5).

Visceral organ mass was related to the dietary treatment birds were provided with (Table 2.5, $p=0.0422$), with the UFA-fed birds having smaller organs. Tissues with a greater UIN, such as those resulting from a prolonged exposure to an UFA-enriched diet, have elevated membrane fluidity, membrane leakiness (Hulbert 2002; McWilliams et al.

2004; Maulucci et al. 2016), and metabolic rate (Roussel et al. 1998, Pani & Bal 2022). By increasing levels of UFAs in their cellular membranes, birds may decrease mitochondrial efficiency, thereby increasing heat production (Chaînier et al. 2000; Ben-Hamo et al. 2011; Pani & Bal 2022). As a result of changes in mitochondrial efficiency, birds fed the UFA-rich diets in the cold may have not needed to increase their organ mass as much as the SFA-diet birds, and instead relied on improvements to their heat production due to the integration of dietary UFAs into their organ tissues over time (Ben-Hamo et al. 2011). Additionally, birds in the warmth likely decrease their organ mass as a way to reduce BMR and lower risk of ROS production caused by the increased concentrations of the UFAs in their cellular membranes (Pamplona et al. 2002; Maulucci et al. 2016; Zielinski & Pratt 2017).

Individuals exposed to cool conditions had heavier kidneys than individuals in the TNZ; that is, kidneys were responding to environmental temperatures in a similar way to the other visceral organs, increasing in size during cold exposure. Impacts of temperature on kidney size are expected, since kidneys have a high metabolic rate/heat production (Burness et al. 1998; Williams & Tieleman 2000). However, diet and body mass did not impact kidney mass (Table 2.5; in contrast the other visceral organs). Mechanistically, the reason is not clear. However, in the PCA of the visceral organs, diet had a smaller impact on visceral organ mass than did temperature, suggesting the influence of diet may be weaker (p_{Diet}=0.0422, β=-0.689 vs $p_{Temperature}$=0.0084, β=-1.539; Table 2.5). A lack of an effect of diet on kidney mass may also reflect the decreased ability to accurately weigh

the kidney. The kidney is small relative to the other organs, and more difficult to extract from the carcass making measurement error more likely.

The pectoralis major was larger in zebra finches exposed to cool conditions than those held at thermoneutrality (Table 2.5). This is a common response in birds following cold acclimation/acclimatization, as the pectoralis is related to shivering thermogenesis (Vézina et al. 2006; Liknes & Swanson 2011). Further a large pectoralis functions as a thermal buffer to reduce heat loss during the cool acclimation (Bech 1980). In contrast with the visceral organs, the mass of the pectoralis was not affected by diet (Table 2.2). I may have expected a statistical interaction between temperature and diet if incorporation of UFAs into the muscle membranes of cool acclimated birds reduced the need for an increase in muscle size. This was not the case. It appears that muscles of zebra finches responded to combined effects of temperature and diet by increasing the number of building blocks, more so than by adjusting the membrane composition.

Basal metabolic rate

The data indicated that zebra finches acclimated to cool temperatures had higher BMR than those acclimated within their TNZ. Changes in BMR following chronic temperature acclimation has been widely reported (Liknes & Swanson 1996; McKechnie 2008; Dubois et al. 2016). BMR has also been shown to interact with dietary fat content during an "acute" time frame (Andersson et al. 2018). During exposure to a cold stress, great tits increased their BMR while fed SFA rich diets, but decreased their BMR at warm

temperatures even more than great tits which fed upon UFA rich foods (Andersson et al. 2018). This was attributed to the birds on the SFA-diet birds converting SFAs into MUFAs when in their cold stress to ensure they had sufficient UFAs to keep up with elevated energy consumption, and the BMR of the UFA fed birds being elevated by auto-oxidation (Andersson et al. 2018). Although I showed that chronic exposure to cool temperatures led to a higher BMR, diet had no effect on BMR (Table 2.7). A lack of an effect of diet on BMR was surprising given that diet did impact the size of visceral organs, and these organ masses are thought to be directly related to BMR (Burness et al. 1998; Williams & Tieleman 2000).

The lack of an effect of diet on BMR may be explained by the increased tissue integration of UFAs into their cellular membranes, making up for the smaller visceral organ size noted in the UFA-diet treatment (relative to the SFA-diet birds). Elevated UFA integration increases membrane fluidity which results in reduced efficiency of oxidative phosphorylation, due to increased calcium ion futile cycling, producing elevated heat production (Roussel et al. 1998, Pani & Bal 2022). This increase in heat production from cell membranes would then potentially reduce the need for larger organs in maintaining homeostatic internal temperatures in the cold. Additionally, the integration of UFAs into the cell membranes may be maladaptive by increasing BMR at thermoneutral temperatures. Therefore, birds potentially decrease the mass of their visceral organs to decrease wasted energy expenditure and as they would not need as much tissue to achieve stable internal temperatures.

Conclusions:

The primary goal of our study was to identify how a tropical bird, the zebra finch, responds to an acclimation to either thermal neutral or cool temperatures when exposed to diets with different fatty acid content. In contrast to previous studies, which have primarily focussed on acute time frames (Ben-Hamo et al. 2011; Andersson et al. 2018), I explored responses over a 6-month chronic time frame. I demonstrated that the body mass of birds in the early stages of their acclimation responded differently to ambient temperatures depending on the diet they consumed, similar to findings from previous acute studies. However, by the end of their chronic acclimation, the mass and physiology of individuals was due mostly to fixed effects of temperature (cool conditions increased body mass, organ mass, pectoralis mass, and BMR) and diet (UFA conditions decreased the mass of most visceral organs). As predicted, birds increased their mass of visceral organs and pectoral muscles by the end of the experiment, providing them with a long-term sustainable way of regulating body heat. Interestingly, only the size of the visceral organs was impacted by diet (not including the kidney), being smaller when fed the UFA-enriched diet; the pectoralis muscle and kidney, as key sources of heat production in birds, were only impacted by temperature, and not diet. Lastly, BMR was greater in birds housed in cool conditions versus TNZ housed birds, but interestingly, was not directly related to the changes in mass of the visceral organs. The lack of an effect of diet on BMR, despite the noted differences in visceral organ mass (UFA birds having

smaller visceral organs) was potentially due to the membranes compensating for their lower tissues' mass through diet induced futile ion cycling. The futile ion cycling caused by UFA rich membranes, increasing leakage, increases their metabolic rate to levels that would allow birds to maintain stable internal temperatures without requiring for as great of an organ mass as seen in the SFA-diet. This study illustrated that while it is important to gain an understanding of how isolated ecological factors can alter physiology of birds, it is essential to consider the impacts of multiple environmental factors at once, as multiple factors may change the "expected" response that birds may have to environmental stimuli.

Range shifts may cause species to experience long-lasting changes in food availability, either encountering novel food sources or losing major staples that they rely on for survival (Gawlik et al. 2002; Robb et al. 2008; Lu et al. 2012; Tekwa et al. 2022). Although our study provides insights into the long-term physiological consequences of differing diets and temperatures, future studies should examine the potential impacts of daily and seasonal fluctuations (Zann et al. 1995; O'Connor et al. 2009; Renner et al. 2012). Daily fluctuations in weather, or changes in the demands and availability of food over season time frames, may disrupt a bird's capacity for acclimation (Wu et al. 2015). As a result, future studies should examine how body mass, body composition, and metabolic rate respond over chronic time frames when diet and temperature vary in an unpredictable way.

Table 2.1: Model selection results for factors explaining variation in body mass. The table displays only those models with strong support (<2 ΔAIC_C). Models are ordered by ascending ΔAIC_C, with the model with strongest support based on AIC score and goodness of fit (R^2c) bolded. All models were run as a linear mixed models using cage and individual identity nested within cage as random effects.

Body Mass	K	AICc	ΔAICc	W_i	Acc W_i	R^2m	R^2c	ER
Diet+Time	9	1500.11	0.00	0.20	0.20	0.056	0.760	1.00
Time	8	1500.78	0.66	0.15	0.35	0.020	0.760	1.39
Temp*Diet*Time	**23**	**1501.12**	**1.01**	**0.12**	**0.47**	**0.099**	**0.779**	**1.66**
Temp*Diet+Time	11	1502.02	1.91	0.08	0.55	0.081	0.759	2.59
Diet+Time+Sex	10	1502.05	1.94	0.08	0.63	0.058	0.760	2.63
Temp+Diet+Time	10	1502.05	1.94	0.08	0.70	0.061	0.760	2.64

Note: K = number of parameters, AIC_C= corrected Akaike Information Criterion, Δ AIC_C = difference of focal model AIC_c compared to the lowest model AIC_C; W_i = Akaike weight; Acc. W_i = Accumulated weight of models; R^2_{mar} = Marginal R squared; R^2_{con}= Conditional R squared; ER = Evidence ratio. Models with interaction terms also include all internal fixed effects.

Table 2.2: Model selection results for carcass fat and pectoral mass for models with strong support (<2 ΔAIC_C). Models for carcass fat were run as linear mixed models, using cage identity as a random effect, and pectoral mass models were run as linear regressions. Models are ordered by ascending ΔAIC_C, with the model with the strongest support, based on AIC score and goodness of fit, bolded.

	K	AICc	ΔAICc	W_i	Acc W_i	R^2m	R^2c	ER
Carcass Fat Mass								
Temp+Mass+Sex	6	144.20	0.00	0.13	0.13	0.203	0.234	1.00
Sex*Mass+Temp	**7**	**144.30**	**0.10**	**0.12**	**0.25**	**0.239**	**0.342**	**1.05**
Sex*Mass	6	145.09	0.90	0.08	0.34	0.180	0.346	1.57
Sex	4	145.44	1.24	0.07	0.41	0.092	0.148	1.86
Temp+Sex	5	145.62	1.42	0.06	0.47	0.139	0.158	2.04
Mass+Sex	5	145.67	1.47	0.06	0.53	0.129	0.224	2.09
Temp*Mass+Sex	7	145.97	1.78	0.05	0.58	0.220	0.230	2.43
Temp+Mass	5	146.00	1.80	0.05	0.64	0.113	0.260	2.46
Pectoral Mass							**R^2adj**	
Temp+Mass	**4**	**20.40**	**0.00**	**0.29**	**0.29**		**0.499**	**1.00**
Temp+Mass+Sex	5	22.24	1.84	0.11	0.40		0.494	2.51
Temp+Diet+Mass	5	22.31	1.91	0.11	0.51		0.494	2.59
Sex*Mass+Temp	6	22.36	1.96	0.11	0.62		0.508	2.66

Note: Adjusted R^2 = R^2adj, abbreviations are as in Table 2.1

Table 2.3: Correlation between each organ mass and principal component one (PC1). PC1 was derived using a principal component analysis on the organ masses of zebra finches after a 6-month exposure to either a diet enriched in saturated fatty acids or unsaturated fatty acids while living in either thermoneutral (35 °C) or cool (24 °C) temperatures.

	PC1	
Eigenvalue	2.387	
Percent of variance explained	59.67	
	Corr	QuRe
Liver	-0.779	0.606
Gizzard	-0.792	0.628
Heart	-0.795	0.631
Small Intestines	-0.722	0.522

Note: Corr. = Correlation of each variable to their respective principal component. QuRe= Quality of Representation, the relative amount that principal component 1 contributes to explaining the variation of each organs mass. Sample size, n = 51 individuals.

Table 2.4: Model selection results for PC1 of the visceral organs principal component analysis and kidney mass displaying models with strong support (<2 ΔAIC_C). PC1 models and kidney models were run as linear regressions and ordered by ascending ΔAIC_C, with the model with the model with strongest support, based on AIC score and goodness of fit, bolded.

	K	AICc	ΔAICc	W_i	Acc W_i	R^2 adj	ER
Visceral organs PC1							
Temp+Diet+Mass	**5**	**168.07**	**0.00**	**0.24**	**0.24**	**0.425**	**1.00**
Temp+Diet+ Mass +Sex	6	169.09	1.02	0.14	0.38	0.430	1.67
Kidney mass							
Temp*Diet	**5**	**-210.02**	**0.00**	**0.15**	**0.15**	**0.367**	**1.00**
Temp+Diet	4	-209.46	0.56	0.11	0.27	0.338	1.33
Temp+Diet+Mass	5	-209.16	0.87	0.10	0.36	0.355	1.54
Temp+Mass	4	-208.97	1.05	0.09	0.45	0.331	1.69
Temp*Diet+Mass	6	-208.46	1.57	0.07	0.52	0.367	2.19
Diet*Mass+Temp	6	-208.33	1.70	0.06	0.59	0.365	2.34
Temp	3	-208.31	1.71	0.06	0.65	0.300	2.35

Note: Adjusted R^2 = R^2adj, abbreviations are as in Table 2.1

Table 2.5: Conditionally averaged carcass fat model estimates of predictor variables impacting changes in body mass using models with strong support ($\Delta AIC_C<2$). Fat mass was analyzed via linear mixed models, using cage identity as a random effect, while pectoral mass, and kidney mass were examined as linear regressions. Square brackets indicate the category being referred to for comparisons. Bolded p-values denote statistical significance.

Predictor Variable	Estimate	95% CI	z value	Pr(>|z|)
Carcass Fat Mass				
(Intercept)	-0.110	-3.212, 2.993	0.069	0.9447
Temp [TNZ]	0.628	-1.446, 3.168	0.583	0.5596
Mass	0.127	-0.139, 0.476	0.826	0.4091
Sex [Male]	-0.020	-2.997, 2.956	0.013	0.9895
Mass:Sex	0.064	-0.149, 0.676	0.416	0.6775
Mass:Temp	-0.025	-0.640, 0.227	0.243	0.8077
Visceral PC1				
(Intercept)	-2.002	-4.554, 0.551	1.537	0.1243
Temp [TNZ]	**-1.539**	**-2.211, -0.868**	**4.493**	**<0.001**
Diet [UFA]	**-0.689**	**-1.354, -0.024**	**2.032**	**0.0422**
Mass	**0.230**	**0.059, 0.402**	**2.634**	**0.0084**
Sex [Male]	-0.147	-1.056, 0.271	0.524	0.6006

Kidney Mass				
(Intercept)	**0.117**	**0.051, 0.183**	**3.494**	**<0.001**
Temp [TNZ]	**-0.032**	**-0.051, -0.014**	**3.370**	**<0.001**
Diet [UFA]	-0.007	-0.074, 0.055	0.243	0.8080
Diet:Temp	0.007	-0.006, 0.049	0.557	0.5773
Mass	0.001	-0.001, 0.007	0.683	0.4944
Diet:Mass	0.001	-0.011, 0.002	0.254	0.7993
Pectoral Mass				
(Intercept)	0.188	-0.102, 1.550	0.679	0.4970
Temp [TNZ]	**-0.202**	**-0.420, -0.094**	**3.058**	**0.0022**
Mass	**0.140**	**0.063, 0.185**	**8.093**	**<0.001**
Sex [Male]	-0.048	-1.753, 0.741	0.262	0.7931
Diet [UFA]	0.001	-0.220, 0.104	0.021	0.9832
Mass:Sex	0.002	-0.024, 0.165	0.150	0.8805

Note: Visceral PC1 scores reflect data negatively transformed. Due to the negative correlation that PC1 had with all visceral organs, the negative transformation allows the values displayed in the above table reflect a positive relationship of the independent factors with visceral organ mass.

Table 2.6: Model selection results for factors explaining variation in BMR. Models with strong support (<2 ΔAIC_C) are displayed. BMR models were run as linear mixed models using housing cage and respirometry chamber identity as random effects. Models were ordered by ascending ΔAIC_C, with the model with strongest support, based on AIC score and goodness of fit, is bolded.

O_2 consumption	K	AICc	ΔAICc	W_i	Acc W_i	R^2m	R^2c	ER
Temp+Mass	**6**	**-184.49**	**0.00**	**0.28**	**0.28**	**0.585**	**0.692**	**1.00**
Temp+Diet+Mass	7	-183.23	1.25	0.15	0.43	0.591	0.691	1.87
Temp+Mass+Sex	7	-182.91	1.58	0.13	0.55	0.590	0.694	2.21

Table 2.7: Conditionally averaged model estimates of predictor variables impacting BMR using models with strong support (ΔAIC_C <2). Models were examined as linear mixed models using cage identity and respirometry chamber as random effects. Square brackets indicate the category being referred to for comparisons. Bolded p-values denote statistical significance.

O_2 Consumption	**Estimate**	**95% CI**	**z value**	**Pr(>|z|)**
(Intercept)	**0.328**	0.214, 0.443	**5.610**	**<0.001**
Temp [TNZ]	**-0.132**	-0.166, -0.098	**7.652**	**<0.001**
Mass	**0.020**	0.013, 0.026	**5.626**	**<0.001**
Diet [UFA]	0.018	-0.015, 0.051	1.082	0.2794
Sex [Male]	-0.015	-0.049, 0.018	0.912	0.3620

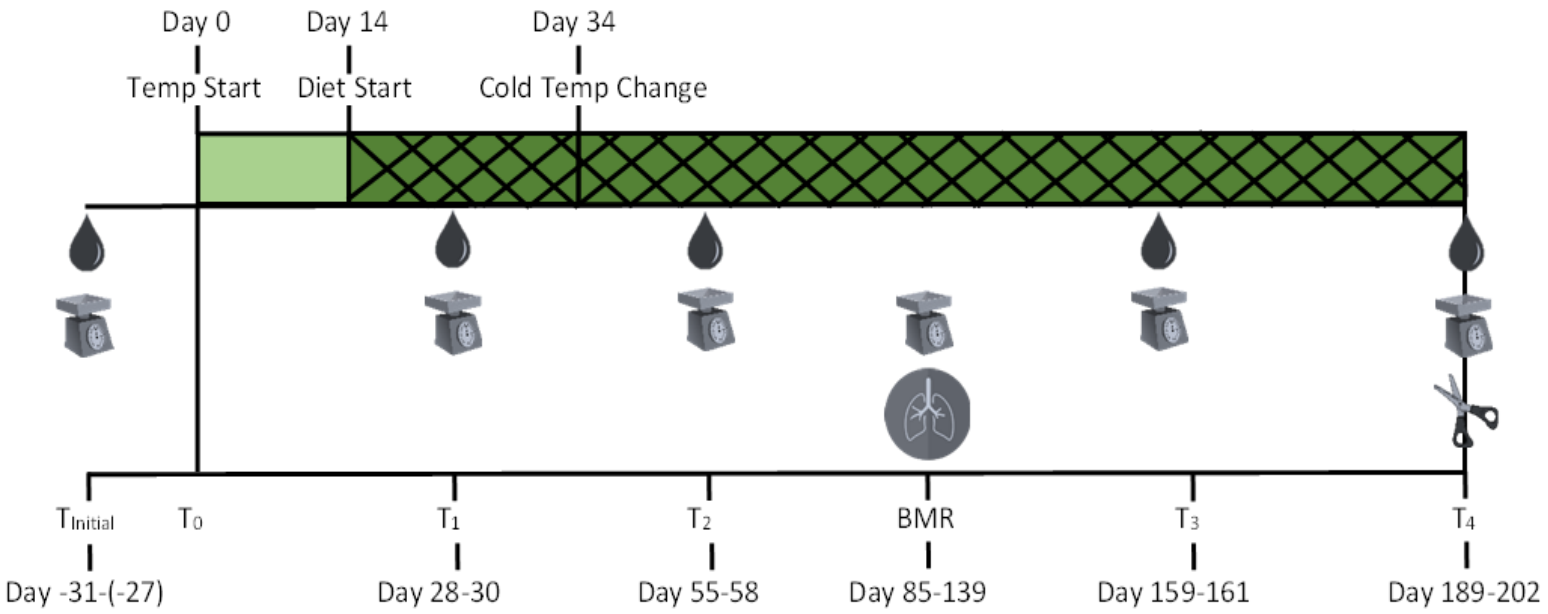
Day 0
Day 14
Day 34
Temp Start
Diet Start
Cold Temp Change
$T_{Initial}$
T_0
T_1
T_2
BMR
T_3
T_4
Day -31-(-27)
Day 28-30
Day 55-58
Day 85-139
Day 159-161
Day 189-202

Figure 2.1: Experimental timeline. Birds were initially housed at 28 °C, weighed, and blood sampled approximately 30 days prior to the experiment. Temperature changes began on day 0 (T_0) with birds being shifted from 28 °C to either 20 or 35 °C (at 4 °C/day). On day 14 birds were placed on diets enriched with either unsaturated fatty acids or saturated fatty acids. On day 34, cool temperatures were raised to 24 °C to improve animal welfare [see Chapter 2 Methods: Experimental diet and temperature manipulation]; the thermoneutral treatment remained at 35 °C. Temperatures were maintained until the end of the experiment. Due to required removals of birds through the experiment, I added cages of females (n=4) and males (n=6) to the UFA cool treatment on days 30 and 35 respectively. An initial baseline blood sample and weighing occurred before these new birds were exposed to any treatments. Experimental blood samples were taken at (T_1) 28-30 days, (T_2) 55-58 days, (T_3) 159-161 days, and (T_4) 189-202 days post initial temperature changes (the 10 late started UFA cool treatment birds had T_3 and T_4 done on days 125-130 and 161-160, and 4 other birds had their initial blood draw done at Day 0). Between T_2 and T_3, respirometry occurred to examine basal metabolic rate (BMR). Morphometrics were recorded whenever blood samples or respirometry was performed. A subgroup of birds was euthanized at the last time point (T4) for analyses of body composition.

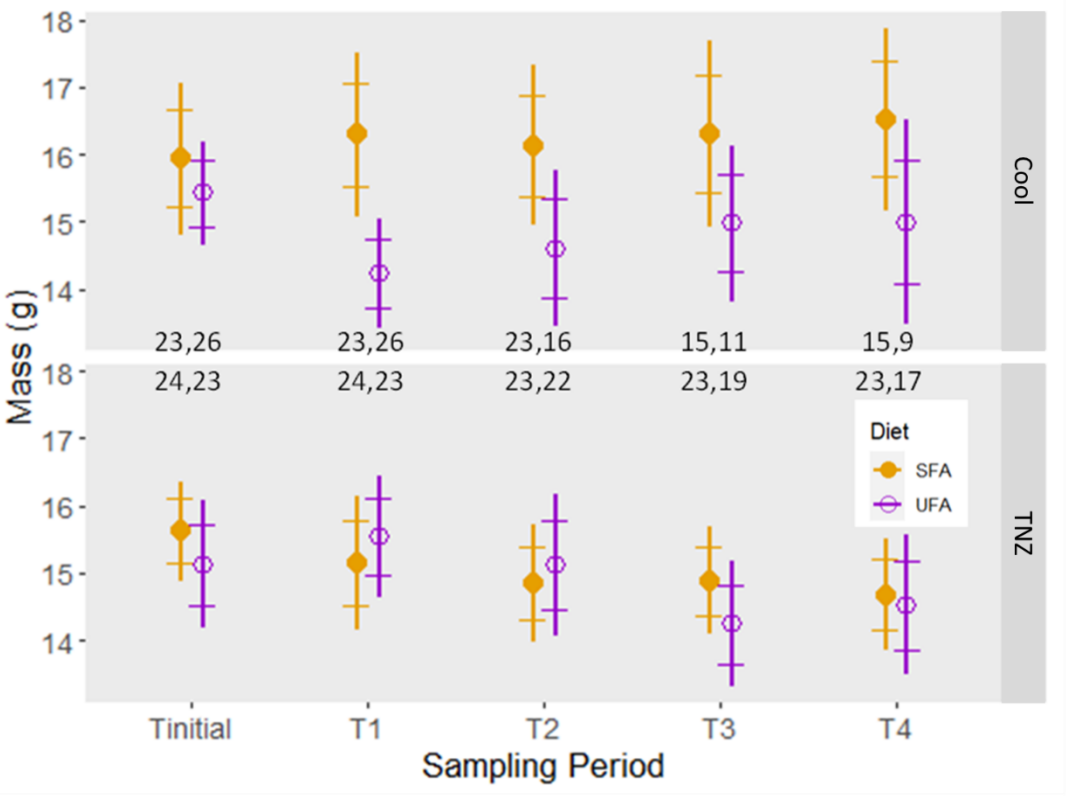

Figure 2.2: Body mass of zebra finches over the course of a multi-month acclimation to diets enriched in either saturated (SFA) or unsaturated (UFA) fats and held at either cool (top panel) or thermoneutral (TNZ; bottom panel) ambient temperatures. $T_{Initial}$ sampling period represents the body mass of birds prior to beginning any experimental treatment while other sampling periods reflect birds at approximately 1 (T_1), 2 (T_2), 5 (T_3), and 6 (T_4) months on treatment. Sample sizes, with sexes, pooled are indicated in each panel at each time point. Error bars depict 95% confidence intervals to their full bounds and 80% confidence intervals to within the bounds of the whiskers.

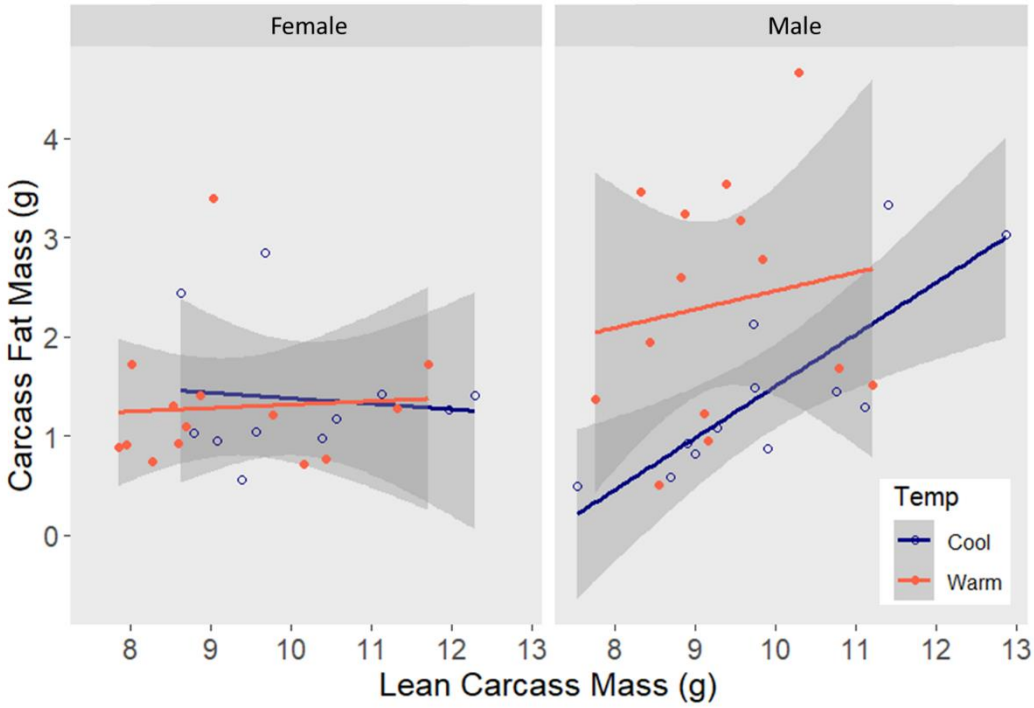

Figure 2.3: Carcass fat mass of zebra finches at the end of a 6-month acclimation to a diet enriched with either saturated or unsaturated fats while living at thermoneutral (TNZ; 35 °C) or cool (24 °C) housing temperatures. Sample sizes for carcass fat had diets pooled ($n_{coolFemale}$=11, $n_{CoolMale}$=14, $n_{TNZFemale}$=12, $n_{TNZMale}$=14). 95% confidence intervals are depicted as grey areas around each treatment's respective line of best fit. According to model averaging of models with strong support, neither diet nor temperature significantly impacted fat mass.

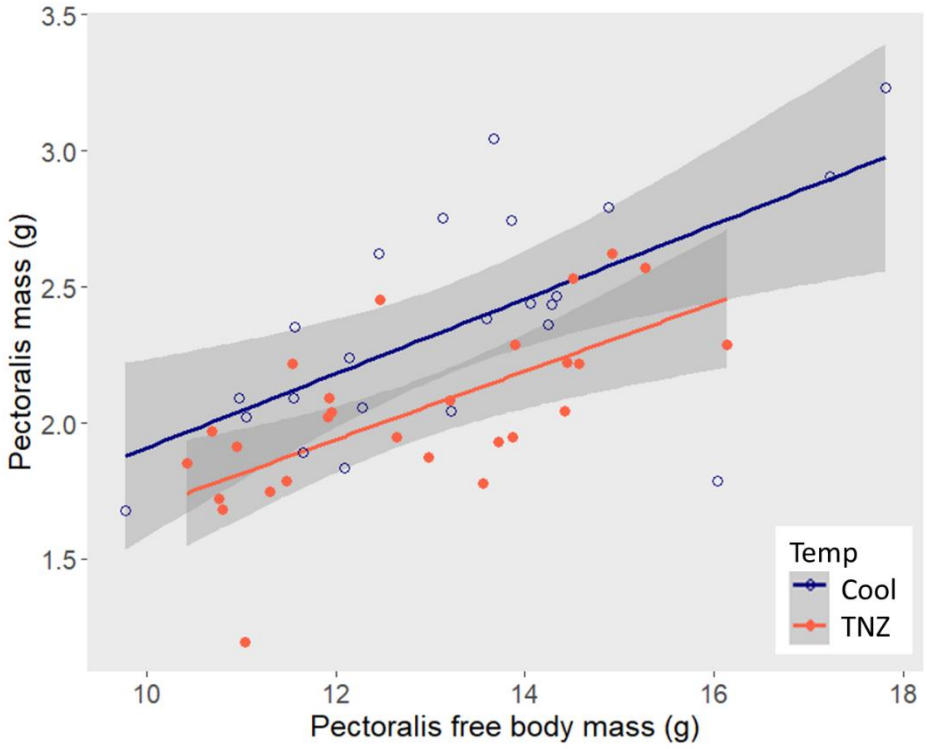

Figure 2.4: Pectoralis muscle mass of zebra finches at the end of a 6-month acclimation to a diet rich in either saturated or unsaturated fats while living at thermoneutral (TNZ; 35 °C) or cool (24 °C). Birds living in cool temperatures had significantly heavier pectoral mass for a given body mass regardless of dietary treatment according to model averaging. Sample sizes with sexes and diets pooled (n_{cool}=23, n_{TNZ}=27). 95% confidence intervals are depicted as grey areas around each treatment's respective line of best fit.

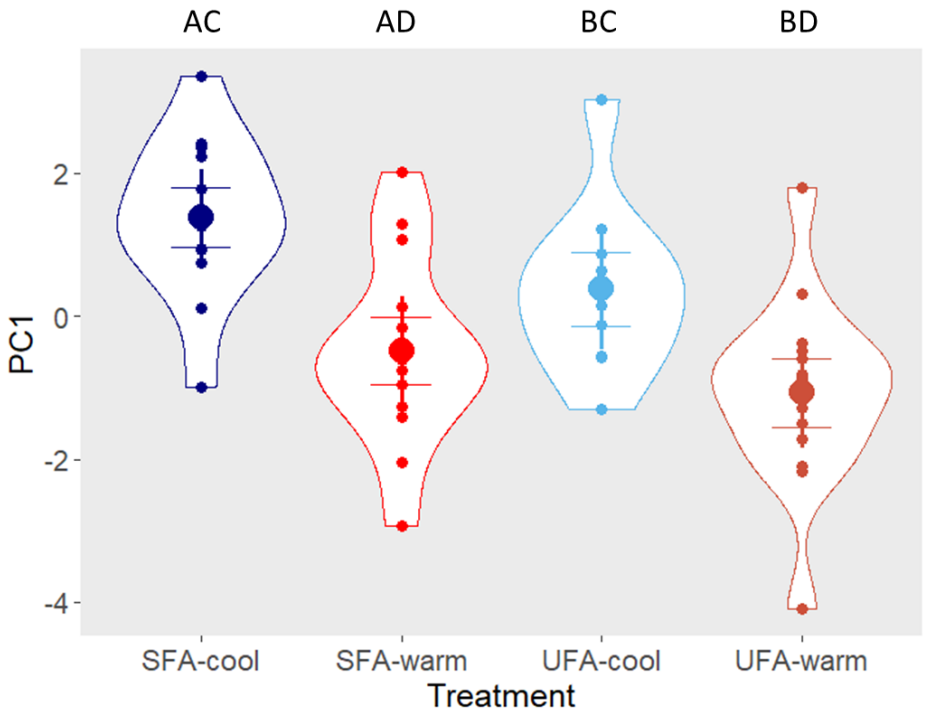

Figure 2.5: Variation in the mass of heart, liver, gizzard, and small intestines of zebra finches at the end of a 6-month acclimation exposed to a diet enriched with either saturated (SFA) or unsaturated fats (UFA), while living at thermoneutral (TNZ; 35 °C) or cool (24 °C). PC1 was negatively correlated to the mass of all visceral organs and were therefore negatively transformed. While having greater organ free body mass had a significant positive relationship with organ mass ($p=0.0084$), living in cool conditions ($p<0.001$) and being fed an SFA-rich diet ($p=0.0422$) was related to greater visceral organ mass than living in a bird's TNZ or being fed an UFA-rich diet. Sample sizes with sexes pooled: $n_{SFAcool}=13$, $n_{UFAcool}=10$, $n_{SFATNZ}=14$, $n_{UFATNZ}=14$. The enlarged point in the middle of the error bars represents the mean, while the error bars represent both the 80% confidence interval (to whiskers) and 95% confidence interval (full range of error bars). Treatments with significant differences are indicated by different letters.

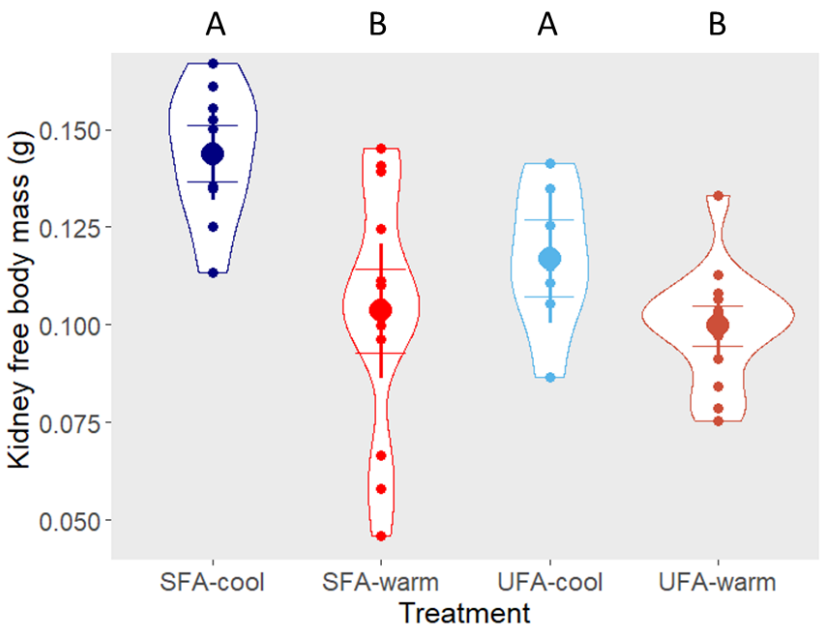

Figure 2.6: Kidney mass of zebra finches at the end of a 6-month acclimation to either a diet enriched with saturated (SFA) or unsaturated fats (UFA) while living at thermoneutral (TNZ; 35 °C) or cool (24 °C) temperatures. According to AIC_C, there was an interaction between diet and temperature on kidney mass. Birds housed in cool temperatures appeared to have heavier kidneys than those in the TNZ, but only when on an SFA diet. However, when examining model averaging, there was only an impact of temperature on kidney mass, but not diet, body mass, or the interaction of diet with temperature. Sample sizes with sexes pooled: $n_{SFAcool}$=10, $n_{UFAcool}$=7, n_{SFATNZ}=14, n_{UFATNZ}=14. The enlarged point in the middle of the error bars represents the mean, while the error bars represent both the 80% confidence interval (to whiskers) and 95% confidence interval (full range of error bars). Treatments with significant differences according to model averaging are indicated by different letters.

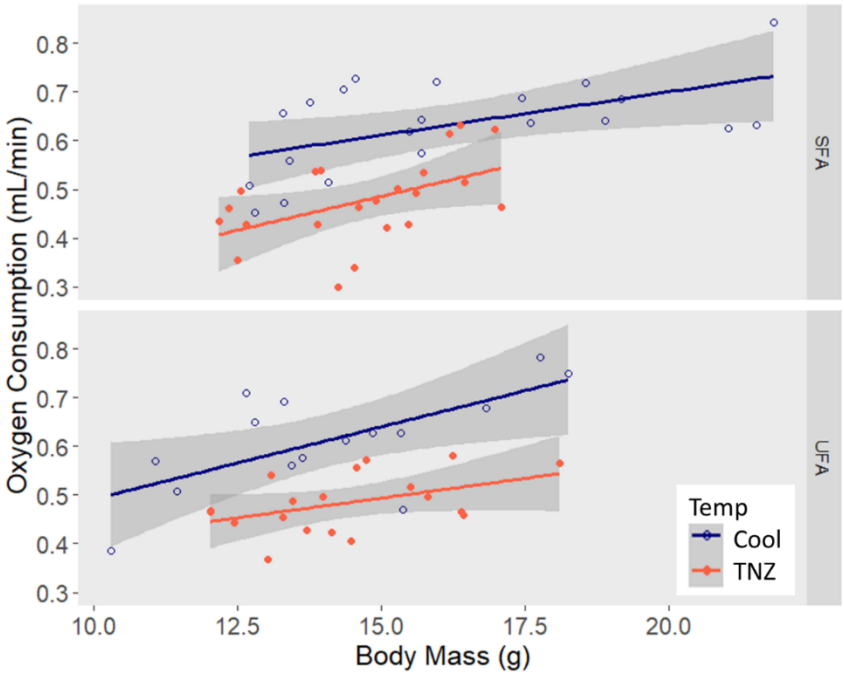

Figure 2.7: Basal metabolic rate (BMR), displayed as oxygen consumption rate (mL/min), of zebra finches after a 3-months of acclimation to a diet enriched in saturated (SFA) or unsaturated fats (UFA), while living at thermoneutral (TNZ; 35 °C) or cool (24 °C) conditions. Birds living in the cool conditions had higher BMR than those at thermoneutrality, irrespective of dietary treatment. Sexes were pooled: $n_{SFAcool}$=21, $n_{UFAcool}$=15, n_{SFATNZ}=22, n_{UFATNZ}=19. 95% confidence intervals are depicted as grey areas around each treatment's respective line of best fit.

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Chapter 3: Duration of exposure and unsaturation of dietary fats alter the plasma fatty acid composition of captive birds (*Taeniopygia guttata*) during temperature acclimation.

Abstract:

During periods of high metabolic demand birds may seek out foods containing higher concentrations or proportions of easily metabolizable nutrients, such as unsaturated fatty acids. However, birds that are exposed to different diets or to physiologically stressful conditions for extended periods of time, primarily use monounsaturated fatty acids to fuel metabolic processes, instead of polyunsaturated fatty acids, likely because this lowers the risk increased oxidative stress. With climate change, birds may become geographically mismatched with their ideal food sources. To explore the physiological consequences of such a mismatch I tested how blood plasma fatty acid compositions change in response to a diet rich in either unsaturated or saturated fatty acids, while individuals were acclimating to thermoneutral or cool temperatures. Birds maintained similar ratios of double bonds per fatty acid regardless of dietary or temperature treatments, but plasma fatty acid composition varied depending on their diet and duration of exposure. The results illustrate how birds chronically exposed to different diets or temperatures may alter their available fatty acids, and allows us to infer how future shifts in species range may shape how birds acclimate to their environments.

Introduction:

Climate change:

Climate change can expose species to temperatures and other environmental stressors that are outside of their preferred conditions (Osland et al. 2021; IPCC 2022). In the short term, individuals may have the capacity to acclimatize to these stressors through physiological mechanisms (Zheng et al. 2008; Zheng et al. 2010; Liknes & Swanson 2011; Milbergue et al. 2022), while over longer time periods populations may shift their ranges, allowing them to remain within their ideal ambient temperature range (Maclean et al. 2008; Kwon et al. 2019; Osland et al. 2021). However, for many species, the capacity to survive and establish a population in a new area may be impacted by their dietary niche; if preferred foods are not available, successful shifts in distribution may not be possible. Highlighting the interactive effects of diet and temperature, work on common cross-bills (*Loxia curvirostra*) and great spotted woodpeckers (*Dendrocopos major*) showed that they can better endure unfavourable conditions and may remain in place instead of migrating when they have access to their desired food sources (fatty acid rich cone crops) (Newton 2006; Linden et al. 2011). Due to the importance of lipids for fueling metabolic processes in birds, an increased fatty acid (FA) concentration from the diet should provide individuals with a greater fuel supply to cope with metabolically taxing periods.

Fatty acids and their importance in maintaining thermoneutrality

When birds experience long-term exposure to temperatures below their thermoneutral zone (TNZ), they generally increase insulation, mitochondrial density, and activity of their cells, thereby increasing their resting metabolic rate (Bech 1980; Wu et al. 2015; Milbergue et al. 2022). To fuel their high metabolic rates, birds rely heavily on lipids which they either obtain directly from their diet or synthesize endogenously within their tissues (Bairlein 2002; Weber 2009; Guglielmo 2010; Devost et al. 2014). Consumption of lipid-rich foods increases the available concentrations of total non-esterified fatty acids (NEFAs, Blem 1976; Bairlein 2002; Weber 2009). NEFAs represent the total concentration of all free FAs; the combination of all saturated, monounsaturated, and polyunsaturated fatty acids. Due to the importance in birds of lipids for fueling metabolic processes, an increased NEFA concentration should provide individuals with a greater fuel supply to cope with metabolically taxing periods (Guglielmo et al. 2002).

The specific FAs that individuals integrate into their tissues and lipid stores typically reflect the relative availability of these FAs in an individual's diet (Maillet & Weber 2006; McCue et al. 2009; Weber 2009). Under environmentally stressful conditions birds may adjust their dietary preference toward diets rich in specific FAs (Roussel et al. 1998; McWilliams et al. 2002; Weber 2009; Guglielmo 2010; Pani & Bal 2022). For example, during periods requiring increased heat production (McWilliams et

al. 2002; Jensen et al. 2020), individuals may shift their diet preferences toward those with an increased proportion of unsaturated fatty acids (UFAs).

UFAs are a type of NEFAs which contain one or more double bonded carbons (mono- and polyunsaturated, respectively). Integration of UFAs into tissues results in greater membrane fluidity and permeability (Roussel et al. 1998, Pani & Bal 2020), which lowers metabolic efficiency and increases heat production (Roussel et al. 1998; Ledesma et al. 2002). Illustrating the importance of relying on FA unsaturation to increase membrane leakage and heat production (Hulbert 2002; McWilliams et al. 2004; Maulucci et al. 2016), birds overwintering in the cold will increase the proportion of UFAs in their fat stores, which is then reflected in plasma FA composition (Jensen et al. 2020). Although the birds may increase concentrations of particular UFAs, this shift in FA content may not be detected when examining total NEFA concentration in plasma (Jensen et al. 2020). This is because birds may adjust the relative ratio of their constituent FAs while maintaining the same NEFA concentration. This is because more mobilizable FAs, particularly short-chain PUFAs, are released from the triacylglycerols of their white adipose tissues (Raclot 2003; Price et al. 2008), potentially overcoming the need to increase NEFA. The relative number of bonds per FA (referred to as the unsaturation index, UIN) is impacted greatly by the relative amount of polyunsaturated fatty acids (PUFAs), which contain more than one double bond. Despite FA concentrations being impacted by both ambient temperature and the diets which birds consume, there is relatively little known about how fatty acid concentrations and

compositions are impacted when diet and temperature change together. To gain a more comprehensive view of energy stores and usage, analysis of both NEFAs and the UIN is required.

Importance of unsaturated fatty acids and maintaining an unsaturation index:

The PUFAs, linoleic and α-linolenic acid, are essential for the synthesis of other long-chain PUFAs. However, in most terrestrial animals these are the only abundant FAs that cannot be produced endogenously by converting other dietary FAs (Pamplona et al. 2002; Castro et al. 2012; Castro et al. 2016; Maulucci et al. 2016). In the case where birds lose access to key dietary staples that would provide them with these essential PUFAs, birds will rely on saturated fatty acids (SFAs), converting them into UFAs with a single double bond (monounsaturated fatty acids; MUFAs). Therefore, despite being exposed to a non-optimal diet, conversion of SFAs to MUFAs allows individuals to maintain an appropriate unsaturation index (UIN; relative number of double bonds to number of FAs), which will allow them to cope with the temperature stressors they face.

Unsaturation and the increased risk of oxidative stress:

FAs that contribute to a greater tissue UIN (i.e., PUFAs) have been linked to shorter lifespan (Galván et al. 2015). This link between UINs and lifespan is thought to exist because tissues with high UINs, and thus, a relatively high proportion of double-bonds, are susceptible to lipid peroxidation, resulting in an increased production of

reactive oxidative species (ROS) and oxidative damage (Pamplona et al. 2002; Hulbert 2007). High levels of ROS may also arise during periods of high metabolic activity caused by experiencing environmental stressors (Constantini et al. 2010; Jimenez et al. 2014; Jimenez 2018). As a result, the longer birds are exposed to high concentrations of dietary UFAs, especially during metabolically taxing periods, the more they should prefer SFAs or MUFAs; dietary FAs with a lower risk of peroxidation (Zielinski& Pratt 2017; Jensen et al. 2020).

To date, most studies exploring the interactions between dietary fats and environmental temperature have focused on short-term acclimation, over a period of days to weeks (McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018; Jensen et al. 2020). These studies found that in response to acute-acclimation to diets rich in UFAs or SFAs and different temperatures birds adjust their metabolic rate (Ben-Hamo et al. 2011; Andersson et al. 2018) and the FA composition of their plasma (Andersson et al. 2018). For example, in the cold, birds will increase the relative UFA content of their plasma (Jensen et al. 2020), but the particular FAs which they will incorporate will vary in response to the diets they can immediately consume (Maillet & Weber 2006; Andersson et al. 2018).

However, individuals may be exposed to dietary shifts or temperatures over much longer periods, such as birds in the wild or held in managed care. This leaves the question: how does FA composition of tissues and of energy stores change over chronic time frames, when birds are acclimating to combined effects of temperature and diets of

varying FA content? A previous study on adult great tits illustrated that resting metabolic rate responded differently to feeding of SFA or UFA rich diets at high and low temperatures over an acute exposure period (6-8 days; Andersson et al. 2018). However, in Chapter 2 of this thesis I showed that the basal metabolic rate (BMR) of captive zebra finches (*Taeniopygia guttata*) was impacted by temperature, but not diet after a chronic exposure. Additionally, visceral organ mass was lower in UFA-fed individuals regardless of temperature [Chapter 2], suggesting that chronically acclimated individuals on an UFA-rich diet may have had greater BMR per tissue mass. This greater heat production may have been due to greater UIN of their tissues, resulting in lower metabolic efficiency. Given this, I might expect to see differences in tissue FA composition, reflected in the blood plasma, when individuals are exposed to chronic temperature stress and diets differing in FA composition.

Experiment objectives and hypotheses

In this study, I used captive zebra finches to examine the changes in plasma FA composition over chronic time periods, as individuals were acclimated to either thermoneutral or cool temperatures and to diets enriched with either saturated or unsaturated FAs. Based on my results from Chapter 2, I hypothesized that temperature and diet will independently impact the total concentration of free fatty acids (NEFA) that birds will have in their plasma. To have sufficient metabolic fuel to ensure a constant body temperature, I predicted that birds living in cool conditions will have higher plasma

NEFAs than those in their TNZ, independent of diet. Additionally, I predicted that birds fed an UFA-rich diet will have higher NEFA levels than those fed an SFA diet, because UFAs are easily mobilizable/peroxidizable and would need to be replaced more rapidly to make up for their metabolic inefficiencies, irrespective of temperature.

Beyond effects of diet and temperature on plasma NEFA concentrations, I also hypothesized that the plasma FA composition would follow a three-way interaction of diet, temperature, and time. Based on results from acute studies (McWilliams et al. 2002; Ben-Hamo et al. 2011; Andersson et al. 2018), I hypothesize that there will be an interactive effect of diet and temperature at the beginning of a bird's acclimation to temperature and diet. However, I predict that as the length of the birds' acclimation prolongs, the interaction would disappear and leaving the primary driver of plasma FA composition to be the temperature at which individuals are held. I reasoned that birds will maintain concentrations of FAs that are at lower risk of oxidation (SFA and/or MUFA, versus PUFA). Birds in the cool conditions will have greater concentrations of UFAs than TNZ birds, and UFA-fed birds will have higher concentrations of PUFAs.

Furthermore, I hypothesize that UIN will be dictated by an interaction between time and diet, as well as a fixed effect of temperature. When birds are first exposed to their experimental conditions, their UIN shall differ between treatments, being higher in birds exposed to the UFA-diet and/or the cool temperatures. However, by the end of their long-term acclimation, the UIN of the plasma should be the same between diets, but higher among individuals held at cool temperatures.

Methods:

Animal care:

Use of animals was approved by the Trent Animal Care Committee following the CCAC guidelines (Animal Use Protocol #26447). Methods for experimental design are based on those described in Chapter 1 [Experimental methods]. Zebra finches were housed at the Trent University Animal Care Facility in one of 16 single sex flight cages (45 cm x 45 cm x 90 cm), split between two 28 °C environmental chambers on a 14:10 light: dark photoperiod, with 6 individuals per cage. All cages contained a sand bath, 5 perches (1 wrapped in paper towel), and provided *ad libitum* grit, water and "Canary/Finch Food" seed mix (containing plain canary seed, canola, nyjer, flax, and red villet; Essex Topcrop Sales Limited)". Initially, all birds received lettuce twice per week and egg meal daily, but approximately 2-months into the experiment egg meal was reduced to every second day for all cages (as all birds stopped eating as much egg as they were at the start of the experiment).

Experimental design:

A pilot treatment [see Chapter 2 for details] was initially run using standard seed mix at a 5:1 seed to oil ratio using either sunflower oil (PC Organics Organic Sunflower Oil) or coconut oil (Nature's Way Premium Coconut Oil; with 50 mg α-tocopherol (Sigma Aldrich (±) α-tocopherol, T3251-25G) /100 mL of oil to make up for differences in oil

antioxidant levels). After three days on these diets (day 3), the environmental chambers were raised to 35 °C or lowered to 18 °C by 3 °C/day. By day 12, 3 birds had died and others birds displayed signs of illness (in the cool UFA treatment). I immediately stopped the experiment and returned individuals to their pre-experimental diets and to 28 °C. Fourteen days after the pilot I set new diet and temperature treatments: birds were acclimated to either the thermoneutral (35 °C) or cool (20°C, which was later raised to 24 °C on day 35; see Chapter 2) treatment. All birds were assigned to the same treatment group which they had been previously assigned during their pilot treatment (all randomly assigned previously, while ensuring similar age demographics per cage of approximately three <1-year olds and three >1-year olds). After acclimating to their temperature treatments for 14-days, birds were provided with diets of standard seed, mixed with oil, but at a 11:1 ratio (following McCue et al. 2009). Through the course of the experiment, the experimental diets were prepared in batches every 1-2 weeks and stored at 24 °C until the next batch was made.

The environmental temperatures used in these treatment groups were selected due to their biological relevance. The cool temperatures represented the average maximum daily temperature zebra finches face during the winter (Zann et al. 1995), and those experienced by zebra finches in managed care (typically, 18-25 °C; Olson et al. 2014; Griffith et al. 2017. I selected 35 °C as the upper temperature because it is within the bounds of expected mean daily maximum temperature that zebra finches may face

during the summer (Zann et al. 1995), and falls within their TNZ (Calder 1964; Wojciechowski *et al*. 2020).

Over the course of the pilot/experiment, individuals were periodically removed if they were sick or died [see chapter 4: Table 1]. The majority of these individuals were from the UFA-cool treatment (24/40). To supplement this treatment group, I added an additional cage of females (n=4) and an additional cage of males (n=6) at 30 and 35 days into the experiment, respectively.

Proportion of time spent feeding:

To test whether individuals in each treatment had similar rates of food consumption, I video-recorded individual feeding events as a proxy for food consumption. Approx. 183-203 days after the start of the experiment (between T3 and T4), I placed a video camera (Activeon, CCA10W, Activeon Inc.; San Diego, CA, USA) in front of each cage and recorded movements within the cage for approx. 2-hrs at a time. Cages were randomly selected for recording and recordings were performed between 09:30 and 20:00 EST at randomly selected times between times T_3 and T_4.

To analyzed feeding rates, videos were replayed and the first 30, 60, or 90 seconds were discarded depending on the last time where human disturbance was noted (allowing time for me to leave the room while recording occurred). Every 30 seconds thereafter I scored whether individuals were at the feeder or not. If a bird was present at the feeder, I examined the 10 seconds before and after the 30 second mark to

assess whether the bird was feeding or not. Of the 69 birds examined, all but 7 could be individually identified using band colour and plumage. These 7 individuals were spread across three cages (two pairs and a triplet). When one of these individuals were observed at the feeder, they were added to a cumulative total of feedings for their group. For analysis, the twins or triplet birds were assigned a number of feedings equal to their cumulative number of feedings divided by 2 or 3, respectively.

Blood sampling:

Blood samples and morphometric measurements were taken before ($T_{Initial}$: 27-30 days prior) starting their experimental treatment and at (T_1) 27-30 days, (T_2) 55-58 days, (T_3) 159-161 days, and (T_4) 189-202 days after starting the temperature treatment (time frame of the experiment is in reference to the initiation of the temperature treatment) [Chapter 2; Fig. 2.1]. The 10 delayed-start birds from the UFA-cool treatment maintained the same respective sampling times from the start of their experimental treatment as the earlier started birds for T_1 and T_2. However, the third and fourth blood draw (T_3, T_4), occurred on 125-130 days and 160-161 days, respectively. For these 10 birds, the $T_{Initial}$ blood draws occurred 1 day prior to the experimental change in temperature began (day -1). I reasoned this would provide the plasma composition of the birds prior to temperature or diet manipulation. The last two blood draws were slightly earlier for these 10 individuals than for majority of the birds, but because birds this size experience

95% FA turnover in their body within approximately 120 days (Carter et al. 2019) they were expected to have been in a chronically acclimated state.

Blood samples (30-100 µL) were collected from the brachial wing vein of each individual using a 25G 5/8 Precision Glide® needle and 75 µL heparinized capillary tubes. Prior to each blood draw, birds fasted for between 1.5 to 3.0 hours to ensure they were post-absorptive. Blood was centrifuged (Thermo IEC MICRO-MB capillary tube centrifuge) to isolate plasma from red blood cells, which were separated and stored in individual micro-centrifuge tubes at -70 °C until analysis.

Blood plasma fatty acid composition:

Plasma samples from the 5 time periods were transported approximately 4 hours on ice to McMaster University where they were placed immediately into a -80 °C freezer. Plasma samples were thawed over ice and handled in batches of 4 samples (a total of 102 batches), randomly selected from within a sampling period. Methods for FA analysis followed Lyons et al. (2021). For every 1 µL of plasma used, 0.5 µL of 0.6 mg/mL heptadecanoic acid solution was added to the sample to act as an internal standard. The methylation of the FAs was initiated using 1 mL of dimethoxypropane and 40 µL of 12 M hydrochloric acid, which was then stopped after 1 hour using 20 µL of pyridine. The remaining solution was evaporated under nitrogen gas until half the solution remained, and then a mix of 0.75 mL isooctane and 0.5 mL distilled water was added. This solution was centrifuged at 5 °C at 10,000 g for 5 min until a distinct aqueous layer had separated

on top. The aqueous layer was collected and replaced with another 0.75mL of isooctane, centrifuged and then the layer was collected again. The aqueous layers were combined and evaporated dry under N_2 gas, then resuspended with 50 µL of isooctane and transferred to a gas chromatography vial, which was repeated. The gas chromatography vial was then evaporated dry and FAs were re-suspended in isooctane, the vial was immediately capped and loaded into an Agilent 6890 N gas chromatographer equipped with a 30 m fused silica capillary column (DB-23, Agilent Technologies), flame ionization detector and an automatic injection system (Agilent Technologies 7683B Series). Each constituent FA (Table 3.1) was identified by comparing the retention times of the FA methyl esters to a standard FAME mix (C8-24, Sigma). Then, FA concentration (Supplementary Table 3.1) was determined by taking the area under the curve for their respective FA's readings of ion per unit time, and then comparing back to the absorbance of the heptadecanoic acid internal standard.

Statistical Methods

All statistical analyses were carried out using the "MuMIn", "lme4", "AICcmodavg", and "factoextra" packages in R (v.4.2.2 (2022-10-31)) through RStudio ("Cherry Blossom", 2023-03-09). NEFA, UIN, SFA, MUFA, and PUFA (mol/L) were log10 transformed prior to analysis. I evaluated the interactive effects of diet and temperature on plasma FA composition: NEFAs (total quantity), concentration of FAs from each of the 3 groups of FAs (SFAs, MUFAs, and PUFAs), and UIN. NEFAs, SFAs, MUFAs, and PUFAs

were calculated using the sum of the molar concentrations of each individual FA. UIN was calculated based on the total molar concentration of double bonds from all FAs divided by the total molar concentration of FAs.

To explore the data for possible outliers, I scrutinized NEFAs and the UIN values greater than 4 standard deviations of the mean. Five log10 transformed NEFAs values were >4 standard deviations above the mean and were removed as outliers. Then I evaluated the unsaturation index (UIN) and found six samples were > 4 standard deviations above the mean, all of which were from the first two (out of 102) batches of FA processing. Therefore, I removed both batches (n=8) out of concern of inaccurate readings from the constituent FA concentrations. Data were then used in the analysis of FA composition. In the end, a total of 13 plasma samples of a total of 393 (N=97 individual birds) were eliminated as outliers.

In all statistical analyses for blood plasma FA composition and concentration, temperature, diet, and time (sampling period) were included as fixed effects, as well as their two and three-way interactions. Individual identity was used as random effect for all examined variables because individuals were measured at multiple points over the experiment. Since decreasing hematocrit (Hct) correlates to greater plasma volume in blood, I also included Hct in the models to account for any potential impacts of the dilutions of FAs (McClelland et al. 1994). My statistical models often had upwards of 4 main effects (e.g., diet, temperature, Hct, time, the interaction between diet and temperature, etc.). To identify which models explained the most variation in the

dependent variable, I took an information theoretic approach (Burnham, Anderson & Huyvaert, 2010). Models were evaluated using corrected Akaike Information Criterion (AIC_C). I considered models with a ΔAIC_C of < 2 to hold strong support, while models between 2 and 4 ΔAIC_C provided moderate support (Burnham & Anderson 2004). R^2 was used with AIC_C and evidence ratios to provide information of the goodness of fit for each set of models. To explain goodness of fit of models, I used conditional R^2 values (accounting for random effects) and marginal (not accounting for variation due to the random effects) if models were analyzed as linear mixed models (Nakagawa & Schielzeth 2013). In the cases where I ran simple linear models, I reported adjusted R^2 values, as increasing the number of predictor variables between models could otherwise inflate the R^2 provided in each model. To determine which main effects impacted the dependent variables, all models with strong support according to ΔAIC_C were used in model averaging. To display the directionality and strength of the trends, I reported 95% confidence intervals (CI), associated coefficients, and p-values.

Data on the proportion of time spent feeding was assessed as the proportion of observation periods where birds were feeding. For each individual, I calculated the total number of feeding occurrences as a proportion of the total number of observation periods (number of observation periods: mean=227.5, median=236, maximum=237, minimum=167). One individual (of 68) had an unusually high feeding frequency (> 4SD of the mean). This individual was omitted from further analysis, as a probable outlier. Data were arcsine square root transformed to improve model assumptions. I tested for

variation in proportion of time spent feeding, using a linear mixed model including temperature, diet, their interaction as fixed effects, and cage identity as a random effect.

Results:

Total non-esterified fatty acid concentration:

According to AIC_C, a model containing a three-way interaction between diet, temperature, and time was the only model which provided strong or moderate support to explain variation in the molar concentration of total NEFAs in blood plasma (Table 3.2; the next best model was an interaction between diet and time, with a ΔAIC_C=9.82). The top model on its own explains 99% of all expected trends (W_i=0.99; Table 3.2). Neither hematocrit nor sex appeared in the top model (Table 3.2).

Despite the SFA-TNZ birds having the highest NEFA concentrations prior to experimental treatment (Fig 3.1B), NEFA levels of SFA-TNZ birds dropped by T1, and remained significantly lower than those SFA-cool or UFA-fed birds in either cool or TNZ temperatures until the end of the experiment (Fig 3.1B; T_1 to T_4 (Temp:Time& Temp:Diet:Time) p-values <0.006, Supplementary Table 3.2 Time4). The UFA-TNZ birds had significantly greater NEFA concentrations than the SFA-TNZ treatment birds at all experimental time points ($p<0.05$ for T_1 to T_4; Supplementary Table 3.2 Referencing Temp:Diet:Time1-4; Figure 3.1A.

Monounsaturated fatty acid concentration:

As hypothesized, plasma MUFA concentrations were best described by a three-way interaction of temperature, diet, and time; no other models provided strong or moderate support ($\Delta AIC < 2$) for the trends in MUFA concentrations (Table 3.2; the next best model included a diet by time interaction with $\Delta AIC=11.47$). Furthermore, the three-way interaction model, on its own explained 99% of all trends (AIC_C Wi=0.99; Table 3.2). Sex did not appear in either model with strong or moderate support. In both temperatures, UFA treatment birds increased their MUFA concentrations from $T_{initial}$ to T1. However, UFA-fed birds significantly decreased their MUFA concentrations by T_3 in both temperatures (Fig. 3.2). In the cool conditions, the MUFA concentrations of the SFA fed birds gradually increased until the end of the experiment; while a decrease with time was detected in SFA birds held at thermoneutral temperatures (Fig. 3.2). Starting at T_1, MUFA concentrations were lower at all subsequent time points in TNZ birds than in cool birds ($p_{Temp:Time1-4}=0.0033$ to $p<0.001$, Supplementary Table 3.4). Additionally from T_2 – T_4, SFA birds had lower concentrations of MUFAs than UFA-fed birds in TNZ conditions, but not in cool temperatures (Fig. 3.2; $p_{Temp:Diet:Time2-4}<0.001$, Supplementary Table 3.4).

Saturated fatty acid concentration:

The top model for saturated FAs contained only a two-way interaction between diet and time, with a fixed effect of hematocrit (Table 3.2). The only other model with strong support was also an interaction of diet and time with hematocrit and temperature

as fixed effects. The top model explained 2.59 times more than the model which included temperature and hematocrit (ER=0.259, ΔAIC_C=1.90; Table 3.2). According to model averaging, neither temperature, hematocrit, nor diet had a significant impact on SFA levels (Supplementary Table 3.3). The only variable that displayed a significant impact with SFA concentrations was time, where SFA levels had decreased by T_4, irrespective of treatment (Fig. 3.3).

Polyunsaturated fatty acid concentration:

Plasma PUFA concentrations were best described by an interaction between diet and time, as the only model with strong support; the diet by time interaction explained 51% of all trends (W_i=0.51 Table 3.2). Temperature did not appear in models with strong support (ΔAICc < 2, Table 3.2), while sex and hematocrit did not appear in any of the models with strong or moderate support (ΔAICc < 4, Table 3.2).

Independent of temperature, birds displayed differences in their PUFA plasma concentrations based on how long they had been exposed to their respective diets. For example, birds on the UFA-diet had higher plasma PUFA concentrations at T_1 and T_2 than individuals on the SFA-diet (Fig. 3.4). However, by T_3 there was no significant difference between the diet treatments (Diet:Time3, p=0.6079; Supplementary Table 3.5). There was a substantial drop in PUFA concentrations at T_4 among all individuals, irrespective of diet or temperature (Fig. 3.4; Supplementary Table 3.5: Time4, p<0.001).

Unsaturation index:

Variation in the plasma UIN was best explained by time, which appeared in each of the models with strong and moderate support (Table 3.3). Although temperature and diet appeared in many of the models with strong support (Table 3.3), neither term significantly predicted UIN when the models were averaged (p_{Diet}=0.886, p_{Temp}=0.266; Supplementary Table 3.6). The only significant effect was that there was an increase in unsaturation index in all individuals at T_1 when compared with individuals sampled a $T_{Initial}$ (Supplementary Table 3.6, $p_{TInitial}$<0.001; Fig. 3.5).

Proportion of time spent feeding:

There was no effect of temperature (β_{TNZ}=0.028, n_{cool}=24, n_{TNZ}=40, p=0.619), diet (β_{UFA}=-0.069, n_{SFA}=37, n_{UFA}=27, p=0.288), nor an interaction between temperature and diet (p=0.895) on proportion of time spent feeding (Supplementary Table 3.7). According to a linear mixed model, proportion of time spent feeding displayed no significance for differences between diet or temperature treatments.

Discussion:

The main objective of this study was to determine how metabolic fuel varies in birds based on the temperatures in which they live, the types of dietary fats they consume, and the amount of time they are exposed to these conditions. I predicted that while birds are exposed to cool temperatures, they would display an increase in their

plasma NEFA concentrations, independent of diet. I reasoned that birds would increase their NEFA concentrations when exposed to cool conditions to increase their available and easily mobilizable fuel sources. The data illustrated there was partial support for this prediction. Although NEFA concentrations were higher in cool conditions when individuals were fed an SFA-rich diet, NEFA concentrations did not differ when fed an UFA-rich diet, regardless of housing temperature.

Additionally, I predicted that there would be an interactive effect of diet and time, with a fixed effect of temperature on the UIN of the plasma FAs. I predicted that there would be higher UIN in the UFA-diet treatment and cool treatment, but only the cool treatment at the end of the 6-month acclimation. I did not find this. Instead, all birds acted similarly by increasing their UIN initially during experimental exposure, but returning to pre-exposure levels after the acute exposure ended, at approximately 8 weeks.

Concentration and composition of constituent fatty acid groupings are regulated by an interaction between diet and temperature:

Cool-SFA birds maintained their NEFA levels at initial levels, presumably to balance their NEFA uptake at tissue with the supply available, because a decrease in circulating NEFAs would limit the metabolic fuel they would have available for thermogenesis (O'Connor 1995). Additionally, since SFA-cool birds received fewer dietary UFAs from their food, they likely converted SFAs into MUFAs (Ben-Hamo et al. 2011;

Maulucci et al. 2016), to maintain a greater concentration of MUFAs, since MUFAs are more easily mobilizable and increase membrane permeability/fluidity better than SFAs (Hulbert 2002; Mustonen et al. 2009).

In contrast to the SFA-diet treatment, the UFA-diet treatment, displayed no significant differences in NEFA levels over time between temperature treatments (Fig 3.1). For the UFA-fed individuals in the cool conditions, individuals likely were able to cope with their initial demands for increased BMR by using the elevated PUFA concentrations provided by their diet and therefore did not require an overall increase in total NEFA. Additionally, since the UFA-cool birds constantly had access to UFAs, they likely did not need to increase stored FA, since they could easily attain mobilizable fuel in the source of UFAs from their diet.

NEFA concentrations of TNZ individuals did not differ from cool acclimated birds when fed an UFA-rich diet, but were significantly lower for the SFA-diet treatment than when fed the UFA-diet (Fig. 3.1). Unlike SFA-fed birds in the TNZ, the UFA-TNZ birds maintained NEFA concentrations as they may have needed more NEFAs to make up for those which were consumed lipid peroxidation. Similar increases in NEFA concentrations were noted in a study which exposed Ross308 broiler chickens to UFA-enriched oils for 21 days (Benzertiha et al. 2019). In that study, authors found that broilers fed the poultry fat diet (i.e., rich in UFAs) had elevated levels of blood NEFAs compared to birds fed palm oil (Benzertiha et al. 2019). However, the effect of diet between their diets was not significant on NEFA concentrations (Benzertiha et al. 2019). In my study, once the zebra

finches had acclimated to their temperature conditions (T_3, chronic acclimation was based on previous literature for both temperature and dietary fatty acid turnover occurring before 4 months; McKechnie 2008; Dubois et al. 2016; Thompson et al. 2016; Carter et al. 2019), they decreased the PUFA concentrations of their available energy stores (Fig.3.4). This was presumably to counter the oxidative risks imposed by the increase in double-bonds per FAs. In fact, previous studies of miniature swine (*Sus scrofa*) exposed to diets enriched in SFA, MUFA, or PUFAs for a chronic period also displayed reduced the percent PUFA concentrations of their plasma (Seiquer et al. 1996).

However, I noted that as the acclimation to the UFA-diet extended from acute to a chronic time period, birds decreased their plasma PUFA concentrations, when compared to pre-experimental levels (Fig. 3.4), but maintained MUFA levels (Fig 3.2) irrespective of temperature. This shift in plasma FA composition from T_1 to T_3 with chronic acclimation, minimizes the number of double bonds found. Therefore, it may serve to reduce the risk of oxidative stress, since increasing relative number of double bonds per FA (increasing UIN) have been shown to increase ROS production (Pamplona et al. 2002; Maulucci et al. 2016; Zielinski & Pratt 2017). Similar trends for reducing UIN over a chronic acclimation were reported in miniature swine when they were consuming diets rich in the essential PUFA, linoleic acid (Seiquer et al. 1996). In swine, this reduction in PUFA concentrations over time was believed to be caused by dietary linoleic acids inhibiting elongase and desaturase activity ([Innis 1986; Martinez & Ballabriga 1987] as cited by Seiquer et al. 1996). Such inhibition would result in less saturated fatty acids

being converted into MUFAs, reducing the total SFA concentration required to maintain a low UIN.

It could be argued that any differences in NEFAs, and the constituent FA concentrations, among individuals in each treatment were caused by differences in the caloric intake/food consumption of the birds. However, I do not think that was the case. The sunflower oil and the coconut oil used in the experiments provide the same calories per serving (80 calories/10 mL of oil), and therefore the same calories between diet treatments. Since the two diet treatments were isocaloric, and I could detect no significant effect of diet, temperature, or their interaction on feeding frequency (Supplement Table 3.7), I assumed individuals had similar energy intake rates. Therefore, results I reported should reflect the impact of the differences in the composition of the fatty acids of the diet, rather than variation in energy intake rates.

In all metrics of blood plasma FAs examined, I found variation across time in how the birds responded to their treatments. Total NEFA and MUFA both displayed a three-way interaction with diet, temperature, and time; which indicates that birds acclimated to their respective temperatures differently based on the dietary fats available, and that the bird's acclimation responses changed over the course of the experiment. Similar to a previous study on great tits (*Parus major*; Andersson et al. 2018), plasma composition of fatty acids is controlled by a complex interaction between diet and temperatures. Similarly noted by Andersson et al. (2018) in their SFA-diet warm treatment, I found SFA-TNZ individuals had lower MUFAs. However, I detected no differences in MUFA

concentrations between individuals on the UFA-diets, held at either thermoneutrality or in cool temperatures. I also found no difference between the two UFA-diet treatments and the SFA-cool treatment. Additionally, the response of the birds on the UFA treatments from my study deviated from Andersson et al.'s (2018) study as my UFA-cool acclimated birds did not have lower concentrations of MUFAs (compared to TNZ) during their acute acclimation phase (T_1). I found that birds fed the UFA-rich diet significantly increased MUFA concentrations at the beginning of the experiment (T_1) irrespective of the temperature at which they were housed, but decreased to pre-experimental levels by 5 months (T_3-T_4; Fig.3.2). In contrast, birds on the SFA-rich diet maintained MUFA concentrations until the end of the experiment when living in the cold, but decreased them when held at thermoneutral temperatures.

Plasma PUFA content was not impacted by temperature, displaying significantly higher concentrations in the UFA diet treatment compared with the SFA diet treatment, independent of temperature. However, the elevated PUFA concentrations in the UFA-diet birds returned to pre-experimental levels, similar to those seen in the SFA treatment, by T_3 (159-161 days; Fig. 3.4). SFA concentrations were best described by an interaction of diet and time, without any impacts of temperature (similar to what was found for PUFA concentrations). There was no significant difference in SFA concentrations between diet treatments nor did the concentrations differ with time (except for at T_4; Supplementary Table 3.3). My findings align with previous literature on the retention and digestibility of fatty acids. For example, studies of broiler chickens showed that the whole body total

SFA-content remains largely unchanged by dietary treatment, as diets above a threshold in SFA concentration did not increase absorption of SFAs (Poureslami et al. 2010). Furthermore, bodily SFA-content was noted to not change despite an increase in the production of endogenously produced MUFAs (Poureslami et al. 2010). Therefore, the broiler chickens are able to absorb more SFAs than the expected maximum capacity, so long as a portion of the SFAs absorbed are instead endogenously transformed into MUFAs. I noted similar trends in the zebra finches where cool-acclimated birds had increased concentrations of MUFAs (Supplementary Table 3.4). In contrast, TNZ finches, which do not require as high of a concentration of mobile fatty acids, had lower concentrations of MUFAs when compared with cool-acclimated birds (Supplementary Table 3.4), yet the two temperature groups had similar concentrations of SFAs (Supplementary Table 3.3).

Composition of unsaturated fatty acids differs between diet and temperature treatments, but does not alter the UIN response to acute versus chronic acclimation:

The unsaturation index increased within the first month of exposure irrespective of diet or temperature (T_1, Fig 3.5). However, the UIN then returned to pre-experimental levels by days 55-58 (T_2) of the experiment, remaining this way until the end of the experiment. Similarly, Jensen et al. (2020) found that UIN increased in dark-eyed junco (*Turdus merula*) regardless of whether they were preparing for the metabolic stress of migration or acclimating to cooling winter conditions. High levels of UIN/greater

proportion of unsaturated fatty acids are associated with elevated lipid peroxidation (Maulucci et al. 2016; Zielinski & Pratt 2017). The similarity in response between juncos and finches suggests that both species use a mechanism which may minimize the risk of lipid peroxidation, while using the specific FAs which are made available to them in their diets. Although the unsaturation index of the zebra finches did not differ with diet or temperature, the dietary fatty acids consumed by the birds likely dictated how the UIN was achieved (Fig. 3.2 & Fig. 3.4). That is, individuals achieved a common UIN by adjusting the relative proportions of UFAs, with UFA-fed birds initially increasing plasma PUFA levels, while SFA-fed birds increased MUFAs.

When an individual is exposed to a new temperature, it requires additional energy to develop the physiological mechanisms that will allow it to cope with the stressor (Vézina et al. 2006; Sgueo et al. 2012). In cool conditions, a higher UIN in blood plasma is beneficial because it allows for greater capacity for FA mobilization and membrane permeability (Hulbert 2002; Mustonen et al. 2009; Maulucci et al. 2016). This would then contribute to increased basal heat production from futile ion cycling (Roussel et al. 1998; Ledesma et al. 2002; Pani & Bal 2020). However, these increases in tissue UIN can have negative long-term health effects as the greater concentration of double bonds per fatty acid increases the risk of lipid peroxidation and auto-oxidation, and thereby oxidative stress (Maulucci et al. 2016; Zielinski & Pratt 2017). As my experiment progressed beyond the acute acclimation phase (after 4 weeks), individuals decreased their UIN to pre-experimental conditions. They did this by lowering their plasma

concentrations of MUFAs and PUFAs by T_2 (8 weeks) to levels noted before their experimental acclimation (Fig. 3.2 & Fig. 3.4, respectively). Interestingly, the plasma SFA concentration did not significantly change over the course of the experiment to compensate for the increases in unsaturation in an individual's diet. As noted in Andersson et al. (2018), SFA concentrations in both great tits and zebra finches were higher when living in warm conditions, likely as a way to increase total unsaturation of their total fatty acid content. As predicted, while experiencing cool temperatures on the SFA-rich diet, zebra finches likely upregulated the synthesis of MUFAs to ensure sufficient concentrations of UFAs (Andersson et al. 2018). The SFA-diet zebra finches could not as readily attain UFAs from their experimental diet as individuals from the UFA-diet treatment could.

Conclusions:

When zebra finches acclimated to diets rich in different FAs and to new temperatures, they increased the relative number of double bonds per FA in their fat stores. This provided the birds with mobilizable FAs that could be oxidized to fuel metabolism. However, the NEFA concentrations and composition of their FAs varied among the treatment groups over time. Plasma FA of birds from both dietary treatments housed in cool temperatures remained relatively stable over the 6-month acclimation. In contrast, while the UFA-TNZ birds remained relatively stable over the 6-month acclimation, the SFA-TNZ treatment birds showed an initial decrease in NEFA which then

remained lower than all other treatments until the end of the experiment (Fig. 3.1). The lower concentration of plasma NEFAs in the SFA-TNZ treatment birds was likely because they were not living in a metabolically taxing temperature. Additionally, as individuals were not exposed to high concentrations of UFAs in their diet, they were at lower risk of lipid peroxidation (Zielenski & Pratt 2017) and apoptosis (Praticò 2002). As a result, SFA-TNZ birds maintained lower NEFA levels, when compared to cool acclimated or UFA-fed birds. This was because they required lower NEFA concentrations to maintain stable internal temperatures or to replace FAs they lost to lipid peroxidation.

With birds shifting their ranges toward higher latitudes and/or altitudes due to climate change, they may face challenges while acclimatizing to their new environmental conditions (Freeman & Freeman 2014; Osland et al. 2021; Girish et al. 2022). If they expand their ranges away from their typical food source, they may be forced to consume novel food with more "cold"-adapted FA content (Zhang et al. 2015; Wójtowicz & Wójtowicz 2020). As latitude/altitude increases, the FA content of plant seeds within and between species has greater UIN due to the greater required membrane fluidity to function at the cooler temperatures (Baskin and Baskin 2014; Linder 2000; Zhang et al. 2015). Alternatively, in warmer temperatures, the seed FAs may become richer in SFAs as a lower UIN would allow for more energy to be stored in the seeds without losses to oxidative damage (Zhang et al. 2015). Therefore, if birds expand into more Northern or higher altitudinal environments at a pace beyond the rate of dispersal of the primary

food species to which they are locally adapted, they may enter ranges with plants which are more cold-adapted than they are used to (Osland et al. 2021; Tekwa et al. 2022).

With northward range shifts, individuals may increase consumption of UFA rich foods, which will expose birds to more oxidatively risky lipids (UFAs). This increase in oxidative substrate may increase apoptotic cell turnover and increase BMR as they may more rapidly need to replace tissues (Pamplona 2008; Zielinski & Pratt 2017; Su et al 2019). I showed that birds kept in thermoneutral temperatures and fed UFA-diets had levels of FAs in their plasma similar to those of birds living in the more physiologically demanding cool temperatures. Whether these individuals all had increased levels of oxidative stress is unknown, but would be a future avenue for research. As literature continues to expand on the subjects on how ecosystems (Freeman & Freeman 2014; Osland et al. 2021; Girish et al. 2022), and the physiology of species within their bounds are changing (Pierce et al. 2005; Twining et al. 2016, Andersson et al. 2018,), it is essential to consider how multiple environmental factors may interact and how the physiological response may potentially change with time. Understanding the impacts of interacting environmental factors will allow us to better predict individual- and population-level responses to climate change.

Table 3.1: List of fatty acids examined using gas chromatography on the blood plasma of zebra finches. Molecular formulas and molecular weights are provided. Each fatty acid with no double bonds were used in the calculation of saturated fatty acid content. Fatty acids with one double bond were included in the analysis of monounsaturated fatty acid content. Polyunsaturated fatty acid content was determined using fatty acids with more than one double bond.

Fatty Acid	Molecular formula	Molecular weight (g/mol)
Palmitic acid	16:0	256.4
Stearic acid	18:0	270.45
Palmitoleic acid	16:1	254.414
Elaidic acid	18:1 n-9 trans	282.46
Oleic acid	18:1 n-9 cis	282.47
Linoleic acid	18:2	280.4472
Dihomo-y-linolenic acid	20:3	306.482
Arachidonic acid	20:4	304.47
Docosahexanoic acid	22:6	328.488

Table 3.2: Model selection results for factors explaining variation in NEFA and the three fatty acid subgroupings, found in the blood plasma for zebra finches. Table displays models with strong and moderate support ($\Delta AIC_C < 4$). Models for all four groupings of FA were run as linear mixed models using individual identity as a random effect. Models are ordered by ascending ΔAIC_C, with models with strongest support bolded.

Model	K	AICc	ΔAICc	W_i	Acc W_i	R^2m	R^2c	ER
NEFA								
Temp*Diet*Time	**22**	**-463.85**	**0.00**	**0.99**	**0.99**	**0.22**	**0.292**	**1.00**
MUFA								
Temp*Diet*Time	**22**	**-302.74**	**0.00**	**0.99**	**0.99**	**0.145**	**0.216**	**1.00**
SFA							R_{adj}	
Diet*Time+Hct	**12**	**-388.55**	**0.00**	**0.51**	**0.51**		**0.187**	**1.00**
Diet*Time+Temp+Hct	13	-386.65	1.90	0.20	0.71		0.185	2.59
Temp*Diet*Time+Hct	22	-385.12	3.42	0.09	0.80		0.207	5.54
PUFA								
Diet*Time	**12**	**-339.92**	**0.00**	**0.51**	**0.51**	**0.188**	**0.338**	**1.00**
Temp*Diet*Time	22	-337.91	2.01	0.19	0.69	0.223	0.375	2.73
Diet*Time+Temp	13	-337.78	2.14	0.17	0.87	0.188	0.339	2.92

Table 3.3: Model selection results for determining factors which impact blood plasma UIN models with strong and moderate support ($\Delta AIC_C < 4$). UIN was analyzed using linear mixed models with individual identity as a random effect. Models are ordered by ascending ΔAIC_C, with the model having the highest goodness of fit (R^2c) bolded.

UIN	K	AICc	ΔAICc	W_i	Acc W_i	R^2m	R^2c	ER
Temp+Time	8	-951.83	0.00	0.32	0.32	0.086	0.112	1.00
Time	7	-950.76	1.07	0.19	0.50	0.006	0.020	1.71
Diet*Time+Temp	**13**	**-950.38**	**1.45**	**0.15**	**0.66**	**0.076**	**0.124**	**2.07**
Temp+Diet+Time	9	-949.85	1.98	0.12	0.78	0.077	0.122	2.70
Diet*Time	12	-949.24	2.59	0.09	0.86	0.089	0.115	3.66
Diet+Time	8	-948.79	3.04	0.07	0.93	0.000	0.029	4.56
Temp*Diet+Time	10	-948.31	3.52	0.05	0.99	0.082	0.104	5.81

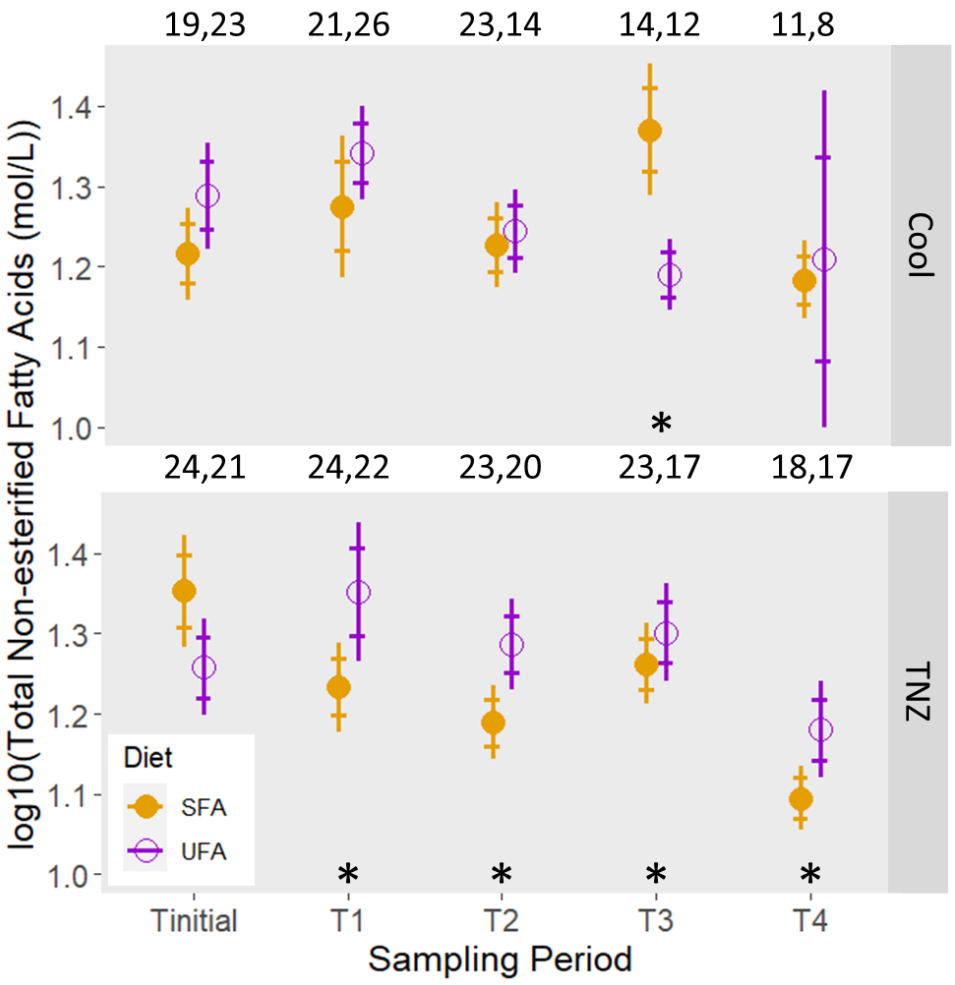
19,23
21,26
23,14
14,12
11,8
24,21
24,22
23,20
23,17
18,17
Cool
TNZ
log10(Total Non-esterified Fatty Acids (mol/L))
1.0
1.1
1.2
1.3
1.4
Diet
SFA
UFA
Tinitial
T1
T2
T3
T4
Sampling Period

Figure 3.1: Effect of dietary fatty-acid content and housing temperature on the concentration of total non-esterified fatty acids in zebra finches over 6-months. Birds were acclimated for 14 days at their respective temperatures before receiving their experimental diet. Blood samples were collected before birds were exposed to any treatments ($T_{Initial}$); subsequent blood samples were collected at (T_1) 27-30 days, (T_2) 55-58 days, (T_3) 159-161 days, and (T_4) 189-202 days post the initial temperature changes began. Sample sizes, with sexes pooled, are denoted in each panel at each time point with SFA diet individuals on the left and UFA diet individuals on the right. Error bars depict 95% confidence intervals to their full bounds and 80% confidence intervals to within the bounds of the whiskers. Late started UFA cool treatment birds (started on their treatments 28 days after other birds) had blood samples for T_3 and T_4 collected at days 125-130 (T3) and days 161-160 (T_4) (see methods for explanation). SFA-cool individuals had significantly greater NEFA concentrations than SFA-TNZ birds. This difference was not noted in UFA birds between temperature treatments; (*) denotes statistical significance at a time point.

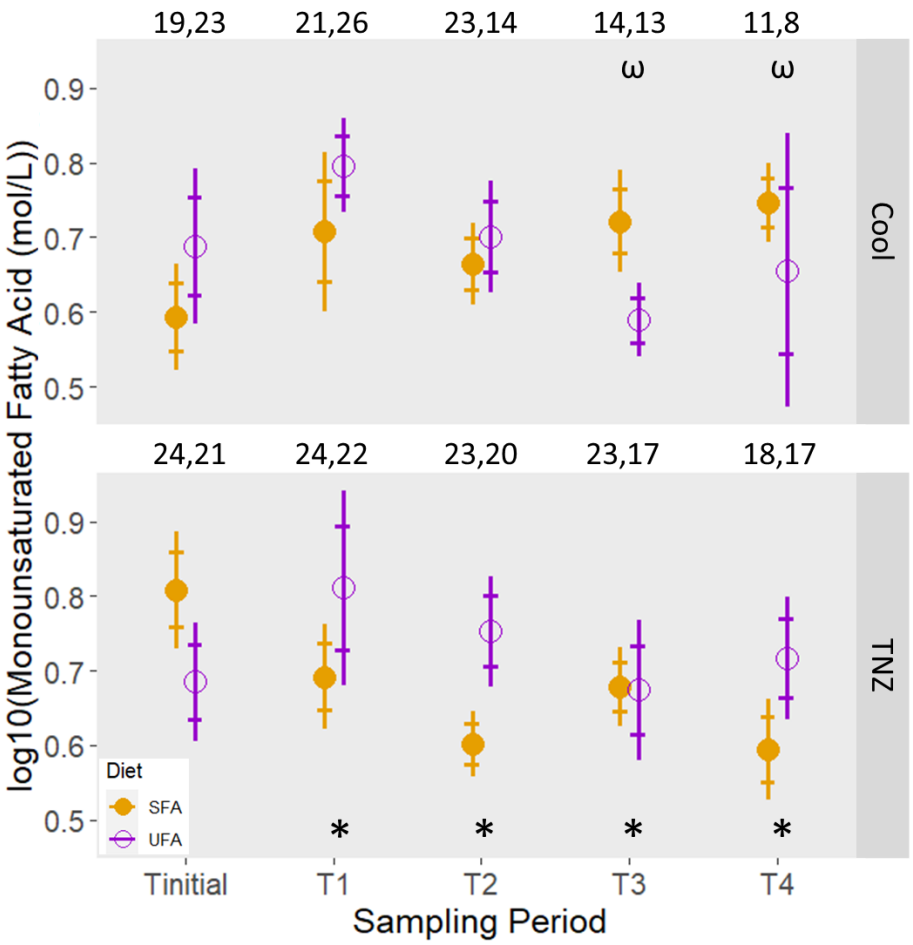

Figure 3.2: Effect of dietary fatty-acid content and housing temperature on blood plasma monounsaturated fatty acid concentrations of zebra finches over 6-months. Experimental conditions and timeline are described in Fig 3.1. Sample sizes, with sexes pooled, are denoted in each panel at each time point with SFA diet individuals on the left and UFA diet individuals on the right. Error bars depict 95% confidence intervals to their full bounds and 80% confidence intervals to within the bounds of the whiskers. Time points with a statistical interaction between diet and temperature are denoted using (*) and the independent impacts time and diet are denoted at the same time points as each other using (ω).

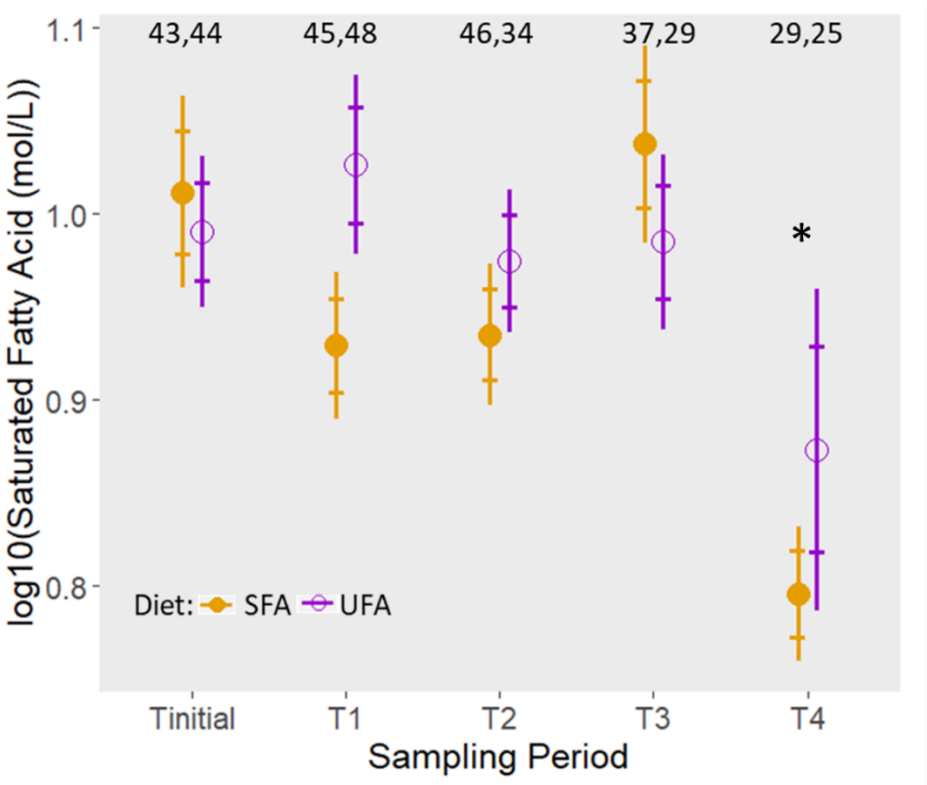

Figure 3.3: Effect of dietary fatty-acid content and housing temperature on blood plasma saturated fatty acid concentrations of zebra finches over 6-months. Experimental conditions and timeline are described in Fig 3.1. Sample sizes, with temperature and sexes pooled (neither had an effect on concentration), are denoted in each panel at each time point with SFA diet individuals on the left and UFA diet individuals on the right. Error bars depict 95% confidence intervals to their full bounds and 80% confidence intervals to within the bounds of the whiskers. Only significant (*) finding in SFA trends is a drop in SFA concentration at T_4.

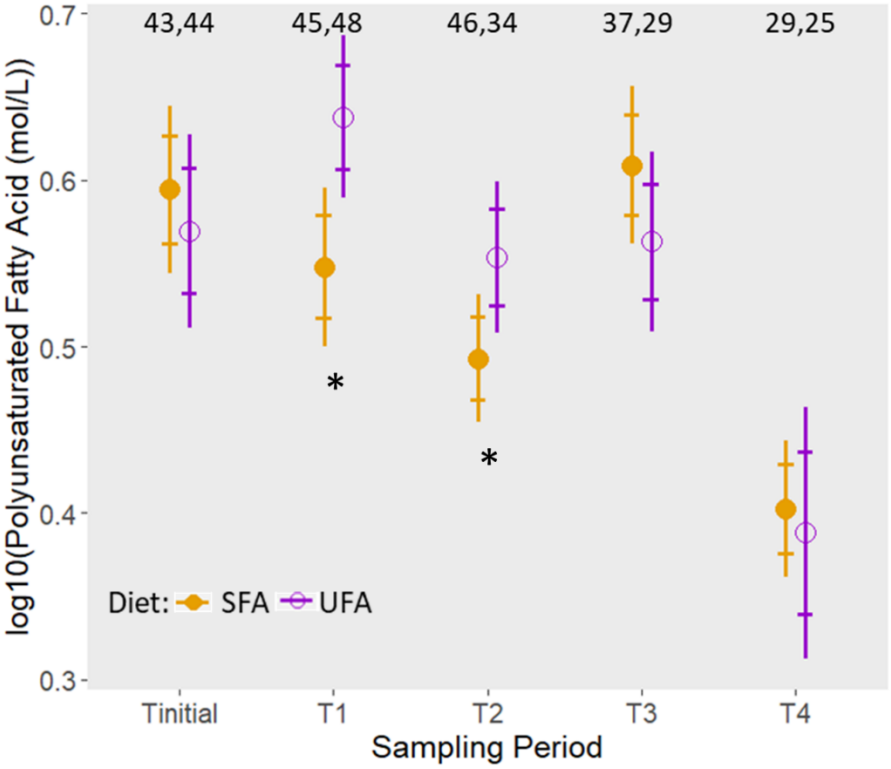

Figure 3.4: Effect of dietary fatty-acid content and housing temperature on blood plasma polyunsaturated fatty acid concentrations of zebra finches over 6-months. Experimental conditions and timeline are described in Fig 3.1. Sample sizes, with temperature and sexes pooled (neither had an effect on concentration), are denoted in each panel at each time point with SFA diet individuals on the left and UFA diet individuals on the right. Error bars depict 95% confidence intervals to their full bounds and 80% confidence intervals to within the bounds of the whiskers. There was a significant difference between diet treatments at T_1 and T_2 (*).

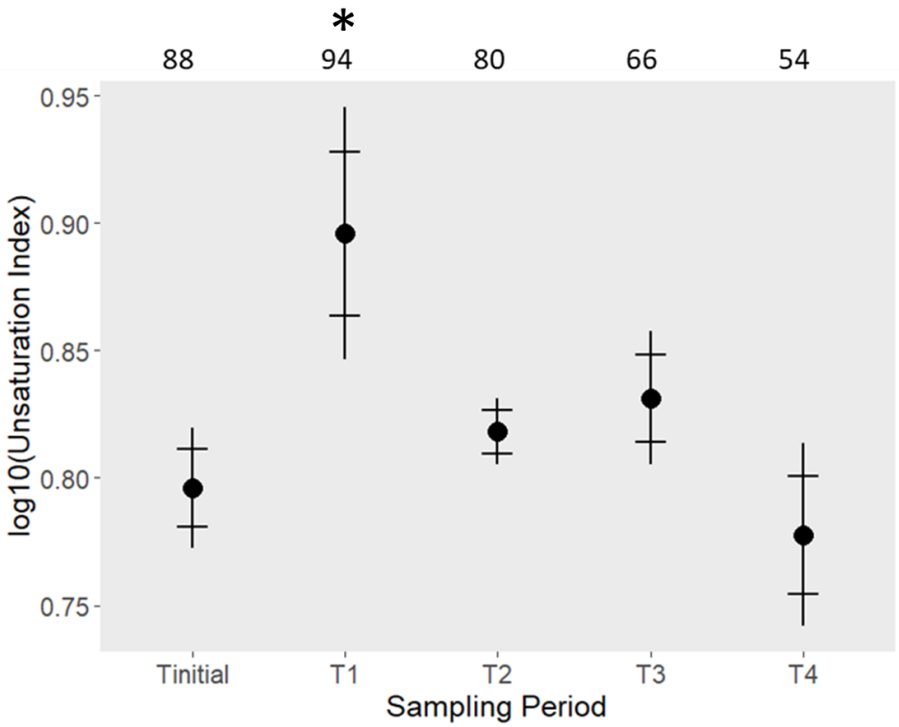

Figure 3.5: Unsaturation index (UIN) from zebra finches during 6-month acclimation to varying dietary fatty-acid content and housing temperature. The UIN is the relative ratio of double bonds to number of fatty acids for the blood plasma fatty acids. The experiment is described in Fig 3.1. Sample sizes, with diet, temperature, and sexes pooled, are shown over each time point. UIN was significantly higher at T_1 (*) than the UIN at $T_{initial}$; no other time points differed from $T_{initial}$.

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Chapter 4: Effects of diet and temperature stress on the health of a commonly housed tropical species of bird (*Taeniopygia guttata*)

Abstract:

Birds in the wild will adjust their diet daily and seasonally to best handle environmental stressors they face. However, birds in managed care are provided a set diet, and lack the freedom to select foods they prefer in specific environmental conditions. This may result in increased production of reactive oxidative species and oxidative damage, with consequences for health and/or survival. Since elevated metabolic rate (often associated with cool temperature challenges) and tissues rich in unsaturated fatty acids (often induced by unsaturated fatty acid rich foods) both increase reactive oxidative species production, I predicted that the combination of both environmental stressors will increase morbidity and mortality. While studying the acclimation response of zebra finches (*Taeniopygia guttata*) to ambient temperatures and diets enriched with either unsaturated or saturated fatty acids, I noted an increase in morbidity in one of the treatment groups; individuals held under temperatures in which they were typically housed (cool conditions) and fed a diet rich in unsaturated fatty acids. I examined the proportional hazard which dietary fat content, ambient temperature, and their interaction had on morbidity and mortality. While I did not detect an interactive effect between diet and ambient temperature, unsaturated fatty acid-enriched diets and cool ambient temperatures independently increased morbidity/mortalities. Improving

understanding of the impact of environmental factors on health will support the improvement of husbandry protocols for animals brought into managed care for species conservation initiatives.

Introduction:

Tropical birds are brought into managed care for reasons such as animal rehabilitation, head starting, research, or as pets. These birds go from environments that fluctuate daily and seasonally (Beaulieu 2016; Griffith et al. 2017) and to which the species is adapted, to fixed and unchanging conditions set and controlled by the caregiver. Thus, birds entering managed care lose some of their autonomy to self-regulate their physiological needs as they are constrained by the conditions being provided. In the wild, birds facing extreme seasonal weather can adjust their behaviour to minimize potential impacts on physiological systems (Bicudo et al. 2001; Cooper 2007), including adjusting their dietary preferences to consume foods that best suit the conditions in which they are living (Wansink & Tinbergen 1994; McWilliams et al. 2002; Renner et al. 2012; Isaev & Borisov 2016; Hernández 2022). Therefore, by provisioning wild animals entering managed care with non-optimal diets, health and survival challenges may be imposed, which they may not be able to overcome.

When exposed to temperature stressors, birds undergo physiological acclimation to keep their internal temperatures within a limited range. In the cold, for example, birds decrease heat loss by insulating their body with additional layers of fat (West & Meng 1968; Dawson et al. 1983; Li et al. 2020). To achieve a homeostatic balance, they need to increase their food consumption, particularly the consumption of foods containing saturated fatty acids (SFAs), as their high melting point and low risk of auto-oxidation allow birds to rapidly deposit fat stores (Guglielmo 2010; Zielinski & Pratt 2017).

However, these SFAs also pose a health risk of increasing cholesterol levels over extended periods of time, which then increases the risk of cardiac diseases like atherosclerosis (Grundy 1997; Petzinger & Bauer 2013).

For long-term survival in cool conditions birds often rely on unsaturated fatty acids (UFAs). The presence of double bonds in UFAs increase cellular membrane permeability and fluidity, with a resultant elevation of metabolic rate (Roussel et al. 1998, Pani & Bal 2020). However, as the number of double bonds relative to the number of FA chains (the unsaturation index; UIN) increases, so does the risk of producing reactive oxygen species (ROS) (Pamplona 2008; Zielinski & Pratt 2017; Su et al 2019). ROS are known to disrupt physiological processes by damaging proteins and DNA (Davies 2000; Maulucci et al. 2016; Zielinski & Pratt 2017), which can contribute to tissue dysfunction and cell death (Pamplona 2008; Su et al. 2019).

While UFAs may be related to oxidative damage, so is exposure to cold stress. ROS are produced as a by-product of metabolism, and elevated metabolic rates necessary for thermogenesis would then increase the likelihood of ROS production (Davies 2000; Jimenez 2018). While facing increased ROS levels, birds use antioxidants to limit oxidative damage (Davies 2000; Buttemer et al. 2010; Zhao et al. 2014). However, if individuals do not have enough dietary or internally-produced antioxidants, they will be at further risk of oxidative stress.

While conducting a study on the physiological and morphological impacts of the combination of dietary fats and ambient temperature on the tropical granivore, zebra

finch (*Taeniopygia gutatta*) [see Chapter 2 & Chapter 3], I noted adverse health effects in birds living in cool conditions when fed an UFA-enriched diet. I recorded all cases of birds dying or being removed from the 6-month experiment and the pilot study (preceding the experiment by one month) for animal welfare concerns, and report the findings below as a proxy of survivorship. Using these data, the potential interactive effects of diet and temperature on morbidity and mortality were examined. Since the methods for this chapter largely repeated those of the previous two chapters, I chose to place the methods at the end of this chapter to improve flow of this chapter.

Results:

Throughout the course of the experiment and pilot, I identified six potential symptoms that were used to identify when birds should be considered for removal from the experiment: leg inflammation, lethargy, oiled feathers, roused feathers, squinting eyes, and thin keel condition (Table 4.1). Typically, the first symptoms noticed in the birds would be oily feathers or having their feathers roused for extended periods of time. In response to cool temperatures, birds erect their feathers for extended periods of time to trap air close to their body, even if it reduces their ability to freely move around and feed. Piloerection was only noticed in birds which later displayed lethargy and thin keel conditions. The oily feathers were typically associated with birds displaying squinty/puffy eyes, both of which were likely related to birds not effectively cleaning themselves. These five symptoms (all but leg inflammation) were not noticed until birds had reached

approximately 28 days (14 days with diet and temperature combined) in the experimental treatment. Meanwhile, leg inflammation occurred uniquely during a short spell between 45-52 days into the experiment, and only affected four individuals (of 100).

Over the 6-month experiment, a total of 35 individuals were removed, with an additional 5 removed or dying during the pilot (Table 4.2). The UFA-cool treatment was responsible for 19 of all removals, including all 5 birds removed during the pilot (Table 4.2). The UFA-thermoneutral zone (TNZ) and the SFA-cool treatments had similar survivorship, with each having seven and eight removals/mortalities, respectively (Table 4.2). Interestingly, in contrast to the UFA-cool treatment's low survivorship, the SFA-TNZ treatment only noted one mortality (Table 4.2).

According to a multivariate cox regression analysis, diet and temperature displayed moderate (i.e., p = 0.05 - 0.01) to strong evidence (i.e., p=0.01-0.001) for impacting survivorship, with UFA enriched diets and cool temperatures independently lowering longevity of the birds (Table 4.3; p_{Diet}=0.0103, HR= 0.317; $p_{Temperature}$=0.0058, HR=0.2836). There was insufficient evidence to reject the null hypothesis regarding fixed effects of sex (p=0.9465) or age (p=0.7948) on survivorship. Additionally, there was insufficient evidence to infer a diet by temperature interaction (p=0.4160). Figure 4.1 highlights how an UFA-rich diet and cool temperatures can independently cause decreased survival, and how their inclusion together greatly increased the likelihood of birds being removed from the experiment.

Discussion:

An interaction between diet and temperature on survivorship was predicted due to the greater morbidity/mortality rates in the UFA-cool treatment. However, there was insufficient evidence to suggest that there was an interactive effect of the UFA diet and cool temperatures on morbidity/mortality rates when birds were exposed to both conditions (Fig. 4.1). Although the fixed effects of diet and temperature independently impacted the survivorship of the zebra finches, where the UFA-diet and cool temperatures lowered survivorship, this resulted in UFA-cool birds displaying the greatest morbidity/mortality.

Impacts of dietary fats on health:

A high concentration of dietary UFAs negatively impacted the bird's health. Of the total number of mortalities/removals (n = 35), 24 occurred in individuals who were on the UFA diet (Table 4.2). With birds constantly exposed to UFAs, they may have been exposed to more lipid peroxidation (Pamplona 2008; Zielinski & Pratt 2017). Lipid peroxidation can result in the production of ROS, which, if not eliminated by antioxidants (from the diet or internally sourced), can negatively impact cellular processes resulting in damage to proteins and DNA (Marnett 1999; Ren et al. 2018; Zhao et al. 2014). Additionally, ROS have been found to be related to the expression of pro-inflammatory

factors (Ren et al. 2018). Anecdotally, birds on an UFA-diet more often displayed inflammation, either on their face or legs, than individuals in the SFA treatment.

Impacts of cool temperatures on health:

While living at temperatures outside their TNZ, birds have an increased metabolic rate, causing them to expend more of their energy reserves. This increase in fuel expenditure can cause an elevation in ROS, which can cause tissue damage (Buttemer et al. 2010; Zhao et al. 2014; Jimenez 2018; Ren et al. 2018). Further, ROS can be related to inflammation and other metabolic diseases (Ren et al. 2018). To mitigate oxidative damage, the body will act in a pro-inflammatory manner by sending lymphocytes to target and eliminate damaged cells (Ren et al. 2018). Therefore, the increased frequency in cases of inflammation of the legs and around the eyes of the birds may have been directly caused by oxidative damage induced by an UFA-rich diet and cool ambient temperatures.

Further, while the body may fight back against oxidative damage by eliminating damaged cells, individuals would still need fully functioning tissues. Therefore, while these birds may be expending resources to eliminate affected cells, they must also replace those proteins and cells targeted for elimination (Mason & Rathmell 2011), which has an energetic cost beyond that of thermoregulation.

Combined impacts of unsaturated fatty acids and cool temperatures:

In this study, the combination of UFA enriched diets and cool temperatures may have exposed birds to an increase in ROS and likely an increase in oxidative damage. The combined effects of both treatments may have created an oxidative load beyond which many birds could cope. The UFA-cool treatment may have created a cycle where increased food consumption, due to the cool temperatures, caused the birds to increase their consumption, which then further integrated UFAs into their tissues (Guglielmo et al. 2002; Hulbert et al. 2005; McCue et al. 2009; Carter et al. 2019). This elevated tissue UIN would then result in a further increase in lipid peroxidation, and therefore, ROS production (Davies 2000; Pamplona 2008; Zielinski & Pratt 2017), with downstream effects on health and survival.

Interestingly, my results are counter to those of McWilliams et al. (2002), another study that evaluated the interactive effects of temperature and dietary FAs. While I primarily noted mortality/morbidity in the UFA-cool treatment, they noted mortality issues when individuals were provided a diet rich in SFAs (50% SFA compared to a diet composed of 10% SFA), rather than primarily UFAs, when exposed to cold conditions, (McWilliams et al. 2002). Although there is no clear explanation for these different outcomes, they may be related to species-specific responses to FA compositions impacting birds differently based on the geographic niche of the species. While I examined zebra finches, native to Australia and New Guinea, they studied yellow-

rumped warblers (*Dendroica coronate*), a temperate species native to North America (McWilliams et al. 2002).

Experimental diet planning:

My experimental treatments were based on previous studies (McCue et al. 2009; Andersson et al. 2018), neither of which reported similar occurrences of illness in birds on their treatments. Notably, the oils used in my experimental diets are commercially available oils; safe for human consumption. The UFA morbidity/mortality rates were not related to a specific container/batch of sunflower oil or seed because I used multiple bottles of oil and batches of seed over the course of the experiment with consistent results. Interestingly, in the SFA treatment (coconut oil) there was a single batch of feed used during weeks 18-19 which caused 6 birds in the cool conditions to display sickness behaviours, resulting in their removal from the study and replacement of the feed. Up to this point in the experiment (147 days), only one of the 18 birds removed from the cool treatments had been from the SFA group (Table 4.3).

Conclusions:

Birds in managed care are typically kept in stable conditions, and in the case of tropical birds, it can often be at temperatures well below thermoneutrality (Olson et al. 2014; Beaulieu 2016). One of the overlooked challenges in housing animals under controlled conditions is in establishing an appropriate diet, particularly in determining a

diet for the specific environmental conditions. When a species is first brought into managed care, its diet is typically based on what similar species or their closest living relatives consume (Crissey 2005). While overt deleterious effects are assessed, in many cases, diets may be maintained without consideration of their interaction with other environmental or introduced factors. My study highlights the importance of considering interacting factors – I showed that the combined effects of cool temperatures and a diet rich in unsaturated fatty acids resulted in increased mortality/morbidity of individuals.

Due to on-going concerns with biodiversity loss, there is potential need to increase the number of bird species in managed care for research or conservation initiatives. However, it is essential to consider the impacts of multiple environmental stressors, such as may occur in captive environments, on animal health. My initial research questions concerned the physiological and morphological response of zebra finches to an acclimation to both temperature and dietary fats [see Chapters 2 & 3]. However, investigating the health of a tropical non-migratory species can provide important insight into animal husbandry practices for birds already in or under consideration for managed care.

Methods:

Housing conditions:

Use of animals was approved by the Trent Animal Care Committee following the guidelines of the CCAC (Animal Use Protocol # 26447). The experimental design is

described in detail in Chapter 2 [Experimental methods]. Briefly, zebra finches (*Taeniopygia guttata*) were housed in flight cages (45 cm × 45 cm × 90 cm) in the Trent University Animal Care Facility. Birds were provided with *ab libitum* grit, water, and "Canary/Finch Food" (containing plain canary seed, canola, nyjer, flax, and red villet; Essex Topcrop Sales Limited) in one of 16 single sex cages, with 6 individuals per cage in one of two environmental chambers set to 28 °C. Diets were supplemented with egg meal and lettuce. Birds had access to a sand bath, and food and water were kept in dishes on the floor of each cage.

Pilot study:

An experimental treatment was initially run using my standard seed mix at a 1:5 oil to seed ratio supplemented with either sunflower oil (PC Organics Organic Sunflower Oil) or coconut oil (Nature's Way Premium Coconut Oil; with 50 mg α-tocopherol (Sigma Aldrich (±) α-tocopherol, T3251-25G) /100 mL of oil to make up for differences in oil antioxidant levels). After three days on these diets, the environmental chambers were either raised to 35 °C or lowered to 18 °C in increments of 3 °C/day (from 28°C). Twelve days after being on these diets I found 3 dead birds (N=96) and multiple other birds displayed signs of illness; I immediately stopped the diet and temperature treatments and returned all individuals to pre-experimental condition seed and temperature (28 °C) over a period of 2 days.

Experimental treatment:

Fourteen days after birds had been returned to standard husbandry conditions, I re-initiated the experiment with an adjusted diet and temperature regime. For 14 days, birds (N=90) were acclimated to either thermoneutral (35 °C) or cool (20 °C, which was later raised to 24 °C on the 35th day of treatment (day 35)) conditions. After the 14-day temperature acclimation, cages were then provided with experimental diets of standard seed mixed with oil (as described above) at a 1:11 ratio. This ratio was chosen as it was used in a previous study examining dietary FA enriched diets in zebra finches (McCue et al. 2009). Throughout the course of the experiment, the diets were prepared in batches every 1-2 weeks and stored at 24 °C until the next batch was made. All low temperatures used during the pilot and experimental phases reflected either natural or managed temperatures in which zebra finches are found, with the lowest temperature used being close to the average maximum daily temperature zebra finches face during the winter (18 °C; Zann et al. 1995); while most standard housing conditions for zebra finches range from 18-25 °C (Olson et al. 2014; Griffith et al. 2017).

Birds underwent blood sampling and morphological assessments prior to receiving any treatment and at four times throughout the experiment. On days 30 and 35, a cage of females (n=4) and a cage of males (n=6) were added to the UFA-cool treatment to ensure sufficient sample size (N=100). These 10 late start birds were recorded throughout the experiment with reference to their own start date, but had their third and fourth sampling periods done 28 days prior to the other treatments, as

fatty acid acclimation was expected to be 95% completed by this point (Carter et al. 2019). As part of another experiment [Chapter 2], a subsample of birds underwent overnight respirometry trials between 85-139 days from the start of the experiment.

Animal health and endpoints:

Throughout the course of the experiment, there was constant communication between researchers, veterinary care, and Trent University's animal care staff to ensure any birds showing signs of illness were removed from the experiments immediately at the earliest identifiable endpoints possible. To simplify decision making I created guidelines for the removal of individuals from the experiment (Table 4.1). Birds were monitored for any symptoms or signs of illness 1-3 times per day during the experimental treatment period. In cases were birds displayed any symptoms, depending on severity, they were either removed from the experiment immediately or allowed 24 hours to return to visibly healthy conditions and then I decided whether they should be removed from the experiment permanently. In cases where three or more birds from a single treatment appeared sick spontaneously, I discarded all remaining feed and created a new batch of feed, as a cautionary measure. When birds died during the experiment, they were predominantly found in the morning at the first assessment (between 0700 – 09:00).

Statistical Analysis:

In the analysis of survivorship, I examined birds from the experimental treatment (N=100), but not any of the birds that only participated in the pilot. Sample size is larger than previous chapters as we included birds in this analysis that were removed from the experiment prior to the first weight measurements/blood draws [See Chapter 2, Fig. 2.1]. The removal or death of birds from the experiment was considered as a proxy for mortality/survivorship. Statistical analyses were carried out using "survival" packages in R (v.4.2.2 (2022-10-31)) through RStudio ("Cherry Blossom", 2023-03-09). Cox proportional hazards models were used to analyze the impact of diet, temperature, sex, and age (less than or greater than a year old) of the birds used in the experiment. Assumptions of the proportionality of survival were verified graphically. P-values, Wald-statistics, coefficients, hazards ratios, and 95% confidence intervals are reported.

To determine which main effects impacted the dependent variables, model averaging was used on all models with strong support according to ΔAIC_C. Rather than use the all or nothing "significance" terminology typically claimed with p-values, and to maintain the gradient style of evaluation used in the information theoretic approach, the terms of "insufficient evidence" ($p>0.05$), "moderate evidence" ($p = 0.05 - 0.01$), "strong evidence" ($p=0.01-0.001$), and "very strong evidence" ($p < 0.001$) were used to describe trends (Sterne & Smith 2001; Muff et al. 2022).

Table 4.1: List of symptoms identified during the pilot and experiment which were used to determine whether zebra finches may need removal from the experiment.

Symptom	Description
Oiled feathers	The presence of oily feathers, particularly on the head but also other regions of the body.
Lethargy	Birds were considered lethargic if they spent large portions of their time on the ground, especially if they remained in the corners of their cages, and did not fly away from animal care personnel or researchers walking by the cages as expected in healthy zebra finches.
Squinting/puffy eyes	Area of the face around the eyes became inflamed resulting in the squinting of their eyes. The inflamed tissue was often dry or displayed irritated red skin.
Roused feathers	Erection of feathers for extended periods, which did not lower as the birds actively moved around. Rousing of the feathers is a cold response of zebra finches, but if maintained for prolonged periods, was considered it a sign of illness. Often paired with lethargy, or the squinting of the eyes.
Leg inflammation	Bruising of the foot and swelling of tissue surrounding the tibiotarsus and phalanges.
Thin keel condition	Keel condition refers to the fullness of the bird's keel, numbered 0-5+ with 0 being extremely low interstitial fat/minimal

	musculature creating a concave shape to the keel, and 5+ being overly conditioned with high levels of fat stores creating a convex/almost boxy feel to their keel. Keel scoring would require handling of birds that may appear sick, which could cause undue stress if they are already in a poor condition, and therefore, was only used when birds were displaying other symptoms above.

Table 4.2: Number of events where zebra finches were removed or died over a 6-month experiment (and from the 2-week pilot performed prior to the experiment) when fed either an unsaturated fatty acid (UFA) or saturated fatty acid (SFA)-enriched diet while exposed to thermoneutral (TNZ) or cool temperatures.

	UFA cool	UFA TNZ	SFA cool	SFA TNZ	Total
Unrelated removal	0	1	0	1	2
Removed/alive	4 (1)	3	3	0	10 (1)
Removed/dead	4 (2)	0	4	0	8 (2)
Dead	11 (2)	3	1	0	15 (2)

Note: Temperature treatments were 35 °C (thermoneutral) and 20 °C that was subsequently raised to 24 °C [cool; see methods]. Bracketed numbers refer to additional birds removed from the pilot study, which were not included in the analysis (N=100). Birds listed as dead were found during daily checks. Birds listed as removed displayed symptoms of sickness and were removed as they displayed symptoms listed in Table 4.1. Unrelated removals refers to incidents where birds were removed from the experiment due to factors unrelated to any experimental manipulation.

Table 4.3: Cox proportional hazards test results for the analysis of zebra finches in a diet by temperature acclimation over 6 months.

Dependent Variable	Coeff.	HR	95% CI	Z	p-value
Diet	**-1.148**	**0.317**	**0.132, 0.763**	**-2.566**	**0.0103**
Temperature	**-1.260**	**0.284**	**0.116, 0.694**	**-2.759**	**0.0056**
Sex	0.023	1.024	0.519, 2.021	0.067	0.9465
Age	0.099	1.105	0.522, 2.336	0.230	0.7948
Diet*Temperature	-0.939	0.391	0.041, 3.757	-0.813	0.4160

Note: Positive coefficients infer greater hazard in the SFA diet, thermoneutral temperature, female sex, and birds greater than one year old. HR = Hazards ratio, Z = Wald Statistic. Main effects showing strong evidence of an effect on survival are bolded.

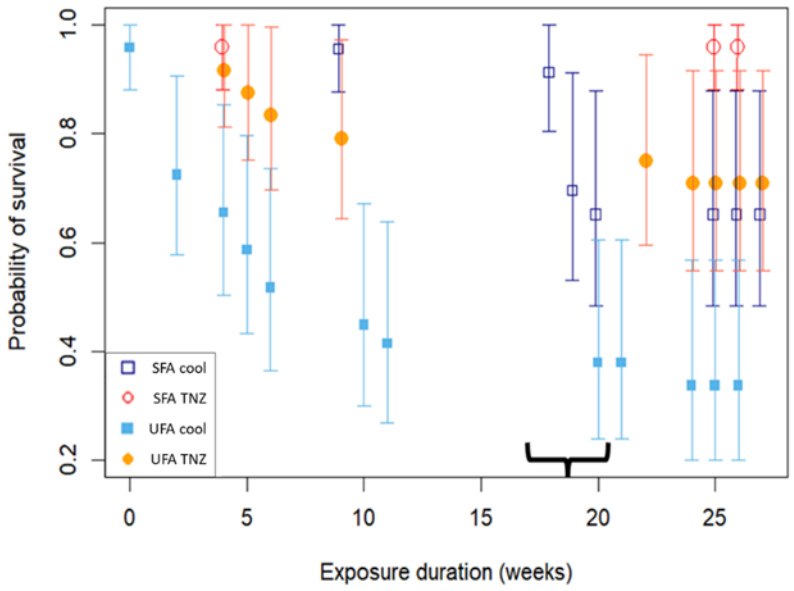

Figure 4.1: Probability of survival of zebra finches over 6-months of acclimation to thermoneutral (TNZ) or cool ambient temperatures and diets enriched with unsaturated or saturated fatty acids ($N_{SFAcool}$=23, $N_{SFA\ TNZ}$ =24, $N_{UFAcool}$=29, N_{UFATNZ} =24). Probability of survival was determined using a proxy of mortality/survivorship including birds that either died or were removed from the experiment out of concern for their health. Each point on the graph reflects an event in time which impacts the estimate of the probability of survival (when a finch died or was removed from the experiment), where all treatment groups started at 1.0 and then decreased with every removal or death. Error bars represent 95% confidence intervals of the estimate of the probability to survive. The bracket at weeks 18-20 indicate a timepoint when the SFA-diet had a singular bad batch of feed, which led to six individuals being removed (3 died) from the SFA-cool treatment.

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Chapter 5: General Conclusion:

Through this thesis, I determined how dietary fatty acid content may impact the capacity for temperature acclimation of zebra finches (*Taeniopygia guttata*) and how the acclimation response may change over the long-term. Below, I summarize my three data chapters and explore their implications, followed by some brief concluding remarks.

Chapter 2, morphology and metabolic rate:

In Chapter 2, I tested the impacts of diet and temperature on morphological markers and basal metabolic rate. I acclimated individuals to either cool or thermoneutral temperatures, and fed them diets rich in either unsaturated (UFAs) or saturated fatty acids (SFAs). Birds living in cool conditions and fed an UFA-rich diet initially lost mass at the start of their acclimation and remained lighter than their thermoneutral-counterparts through the duration of the experiment. This trend was not seen among birds on the SFA-diet; instead, these birds showed lower body mass once their experimental treatments began, irrespective of the temperature at which they were housed. By the end of the 6-month experiment, the effect of the statistical interaction between diet and acclimation temperature on body mass had largely disappeared. I cannot be certain whether the lack of significant was due to a reduction in statistical power due to decreasing sample size, or because of essentially a selection event, whereby members of the study population with less successful phenotypes were removed or died over the course of the experiment.

At the end of the 6-month experiment I dissected a subset of individuals to measure their body composition (i.e., organ, muscle, and fat mass). Visceral organ mass was significantly impacted by both temperature and diet independently. Individuals acclimated to cool temperatures had larger organs, compared to individuals acclimated to thermoneutral temperatures. Additionally, if individuals were fed an UFA-diet, they had smaller organs, when compared with individuals on the SFA diet. In contrast to the independent effects of diet and temperature on visceral organs, fat mass did not differ among individuals, irrespective of the diet or temperature they experienced.

My results align with the expected physiological mechanisms for chronic temperature acclimation. Maintaining elevated fat mass for insulation is beneficial for early acclimation to cool conditions (Helms 1968; Bech 1980; Bairlein 2002; Broggi et al. 2019; Laplante et al. 2019). However, increased fat mass may carry costs if maintained over long periods (Helms 1968; McNamara and Houston 1990; Burns & Ydenberg 2002). As a result, it is perhaps not surprising that all individuals had similar fat mass after 6-months, with any physiological differences reflected at the organ level.

Over long-term cold-acclimation, an increase in organ mass would be beneficial for increasing heat production, which is consistent with my results. Individuals undergoing acclimation to cool conditions had larger visceral organs than individuals held at thermoneutrality. Furthermore, individuals acclimated to cool conditions had higher metabolic rates when measured at thermoneutrality, reflecting their larger pectoralis muscles and visceral organs. Increasing the mass of visceral organs and the

pectoralis is a common strategy for birds undergoing acclimation to cold temperatures (Liknes & Swanson 2011; Zheng et al. 2014).

Interestingly, diet also impacted organ mass, independent of temperature. Individuals on the UFA-enriched diet had smaller organs than those on the SFA-diet. This was possibly because the dietary UFAs were integrated into the cell membranes of the organs. Integrating UFAs into cell membranes would increase membrane permeability (Bairlein 2002; Hulbert 2002), lower metabolic efficiency, and increase heat production (Roussel 2020; Pani & Bal 2022). As a result, individuals on the UFA likely did not need the same-sized organs as those on the SFA diet, to maintain the same total heat production (which is what I found – individuals on the two diets had the same BMR). That is, members of the two diet treatments achieved the same BMR via different physiological mechanisms; increasing total organ size in one group (SFA), and increasing organ metabolic intensity in the other (UFA).

The high mass-specific metabolic rates of visceral organs mean they contribute disproportionally more to BMR than does muscle (Zheng et al. 2008; Zheng et al. 2010; Liknes & Swanson 2011; Zheng et al. 2013; Milbergue et al. 2018) and visceral organ mass varied in size based on the diet the birds consumed, the metabolic inefficiency that an UFA-rich diet can induce may have increased the tissue specific BMR of the birds (Ben-Hamo et al. 2011; McGuire et al. 2013; Roussel 2020). Therefore, despite using less tissue to maintain heat, the organs may consume proportionally more energy per cell, resulting in similar BMRs between birds of either diet treatment, but not between

temperatures. To better understand the mechanisms for acclimating to isocaloric diets varying in fatty acid composition and ambient temperatures, future studies should examine how body composition and BMR varies at additional time points during their acclimation.

Chapter 3, plasma fatty acids & unsaturation:

In Chapter 3, I explored how birds altered their plasma fatty acids composition over time. When acclimating to different temperatures and diets differing in fatty acid content, birds initially increased the relative ratios of double bonds to fatty acids (unsaturation index; UIN). However, as the duration of their acclimation moved from an acute to chronic time span (by T_2, 8 weeks), the UIN of their plasma returned to the levels which individuals had prior to the start of the experimental acclimations ($T_{initial}$). This likely occurred because the tissue membranes had adjusted to their environmental stressors and did not need to rely as heavily on high UIN in their plasma to maintain metabolic processes (Raclot 2003; McCue et al. 2009; Jensen et al 2020). In contrast to patterns observed for UIN, the concentrations of the major constituent FAs in the blood plasma varied greatly based on the temperature (cool vs thermoneutral), dietary FAs consumed (UFA vs SFA), and the duration of their acclimation.

Plasma PUFA concentrations were affected by diet and the interaction between diet and time. UFA fed birds had a greater PUFA concentrations at T_1 (4 week), however, by T_2 (8 weeks), PUFA concentrations returned to pre-experimental levels ($T_{Initial}$). Beyond this time point, there no difference in PUFA concentrations between diet treatments.

Plasma SFA concentrations were not affected by the diet birds were fed or the temperatures at which they were housed. However, although there were no significant differences between diets,

Plasma NEFA concentrations remained the same as pre-experimental levels in all treatment groups, except for individuals fed a diet rich in SFA and held in their thermoneutral zone (TNZ); these individuals decreased their plasma NEFA levels, and kept levels low for much of the experiment. SFA-TNZ birds were acclimated to thermoneutral temperatures and would not have required high concentrations of mobile fatty acids to maintain stable internal body temperatures. As a result, they did not increase their MUFA production or storage of NEFAs (resulting in the lower NEFA levels I detected). In contrast, the higher concentration of NEFA noted in the UFA-TNZ birds, when compared with the SFA-fed birds, was likely because of the surplus PUFAs and MUFAs found in their diet (McCue et al. 2009). Additionally, the greater plasma NEFA concentrations detected in the SFA-cool birds (when compared to the SFA-TNZ birds) was likely because they converted SFAs from their diet into MUFAs. Similar trends were detected by Andersson et al. (2018), where relative MUFA levels increased in great tits when exposed to cold temperatures and an SFA-enriched diet. This increase in relative MUFA concentrations noted by Andersson et al. (2018) expectedly allowed individuals to attain a desirable UIN while they acclimated to the cool conditions (Maulucci et al. 2016; Jensen et al. 2020).

Although birds may have adjusted their fatty acid composition to minimize the risk of oxidative damage (Hulbert 2007; Andersson et al. 2018), I did not measure ROS or antioxidant concentrations/production. Therefore, I could not verify that the dynamic changes in fatty acid composition I noted over the course of the acclimations were due to oxidative stress or its potential damages. Future studies should explore indices of oxidative stress, especially in light of the treatment-specific morbidity and mortality I detected in Chapter 4.

Chapter 4, survivorship and health:

Birds exposed to either cool temperatures or UFA-rich diets, displayed more symptoms of poor health and had a greater risk of death.

Symptoms:

Most symptoms I identified in birds with declining health were consistent with oxidative damage (inflammation of tissues) and limited available free energy (lethargy and oiled feathers). Oxidative damage from ROS can be produced from high dietary UFA content (Pamplona 2002; Maulucci et al. 2016; Su et al 2019) and elevated metabolic rates (Buttemer et al. 2010; Jimenez 2018). Oxidative damage has been related to the expression of heat shock proteins which induce inflammation (Zhao et al. 2014; Ren et al. 2018). The presence of ROS can lead to protein and DNA degradation (Marnett 1999; Pamplona 2002; Schieber & Chandel 2014), which increases purine/oxidized purine by-product and amino acid concentrations (Stadtman & Levine 2003; Sautin & Johnson

2008; Huang et al. 2020); by-products that enter the uric acid cycle. The production of uric acid helps the birds in two ways, (1) by removing nitrogenous wastes, and (2) by acting as an endogenous antioxidant (Sautin & Johnson 2008). However, the process of producing additional uric acid requires the body to expend additional energy (Petrie et al. 2013). This expenditure of energy may limit available free energy and may explain the lethargy I noted among individuals in the UFA (particularly UFA-cool) treatments. Additionally, uric acid is related to and one of the leading causes for gout, an arthritic inflammation which is caused by uric acid crystallization in joints (particularly in the feet) (Sautin & Johnson 2008; Copur et al. 2022). Although it was not confirmed in the study population, due to the relevance of foot inflammation and swelling throughout the study and the potential oxidative damage induced by the UFA-diet and cool temperatures, uric acid could have been responsible for some of the noted symptoms.

Survivorship:

I identified greater mortality in birds exposed to combined cold and dietary UFAs. This contrasts with results reported by McWilliams et al. (2002). McWilliams et al (2002) is one of three previous studies which have examined the combined effects of dietary fatty acids and temperature acclimations. In contrast to my results, McWilliams et al. (2002) had higher mortality among individuals fed diets with a lower UIN (50% SFA vs 10% SFA diets) when in cold housing conditions. Differences between studies may, at least in part, be due to species-specific physiological adaptations. While McWilliams et al. (2002) studied a temperate species (yellow-rumped warbler; *Dendroica coronate*), I

examined a captive-bred, tropical species (zebra finch). Tropical birds typically have lower UFA content in their tissue (Furness & Speakman 2008; Galván et al. 2015). Because tropical species may not be adapted to maintain high levels of tissue-unsaturation, they may be unable to handle the ROS induced by an UFA-rich diet, at least when compared with more temperate species. Future studies should examine the level of lipid peroxidation and concentrations of ROS/antioxidants (particularly uric acid) during combined diet and temperature acclimation. This would allow for a better understanding of endogenous antioxidant dynamics, and their impact on the health and survivorship.

Closing remarks:

My thesis provided an examination of the interactions between dietary fatty acids and ambient temperature, over a longer time scale than previous studies. I confirmed previous studies and showed that dietary fats can alter the physiological response birds may experience during temperature acclimation, however, most of the acute effects/interactions reported previously diminished as the duration of acclimation increased. Furthermore, I illustrated the potential dangers that individuals may face when dealing with diet and temperature mismatches. This was exemplified by zebra finches exposed to cool temperatures and UFA diets experiencing various health issues and higher risk of mortality.

My thesis improves understanding of the impact fatty-acid-rich diets have at environmentally/captively relevant temperatures, which many tropical species experience under managed care. I illustrated the importance of determining the ideal

conditions to house birds in, and if aspects of these conditions cannot be acquired, then the importance of considering and regulating other aspects of their housing conditions to limit the physiological strain the birds may face. Further, as the natural ranges of birds shift to stay within their ideal temperature niche (Maclean et al. 2008; Lagerholm et al. 2017), knowledge of how diets can alter physiological responses to temperature will allow us to better predict how birds will be impacted by climate change.

Even outside of the context of geographic range shifts and/or birds living in managed care for head starting, it is essential to consider the impacts of multiple environmental factors. As these environmental/physiological interactions are further examined, it becomes even more evident of the importance for studies to report the fine details of experimental conditions. Since the diet of birds being examined can vary between experiments (micro- and macronutrients), these differences could have major impacts of the physiology of individuals and therefore be a responsible for some of the variation in results reported among studies.

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Supplementary materials:

Supplementary Table 2.1: Conditionally averaged body mass model estimates of predictor variables impacting changes in body mass using models with strong support (ΔAIC_C <2). All models were compared as linear mixed models using cage and individual identity (nested in cage identity) as random effects. Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values indicate statistical significance.

Predictor Variables	Estimate	95% CI	z value	Pr(>|z|)
(Intercept)	**15.919**	**14.893, 16.945**	**30.411**	**<0.001**
Time1	-0.132	-0.712, 0.448	0.446	0.6555
Time2	-0.344	-0.967, 0.278	1.084	0.2782
Time3	**-0.716**	-1.349, -0.083	**2.217**	**0.0266**
Time4	-0.659	-1.395, 0.077	1.756	0.0792
Diet [UFA]	-0.938	-2.242, 0.367	1.409	0.1588
Diet:Time1	**-1.516**	-2.363, -0.668	**3.505**	**<0.001**
Diet:Time2	**-1.435**	-2.364, -0.507	**3.029**	**<0.001**
Diet:Time3	-1.066	-2.176, 0.045	1.881	0.0599
Diet:Time4	**-1.317**	-2.488, -0.146	**2.204**	**0.0275**
Temp [TNZ]	-0.496	-1.996, 1.005	0.648	0.5173
Temp:Time1	-0.823	-1.677, 0.030	1.892	0.0585
Temp:Time2	**-1.028**	-1.888, -0.169	**2.345**	**0.0190**

Temp:Time3	-0.587	-1.519, 0.344	1.236	0.2165
Temp:Time4	**-1.004**	-1.935, -0.072	**2.112**	**0.0347**
Diet:Temp	0.647	-1.764, 3.057	0.526	0.5992
Diet: Temp:Time1	**2.402**	1.200, 3.605	**3.915**	**<0.001**
Diet: Temp:Time2	**2.239**	0.969, 3.509	**3.456**	**<0.001**
Diet: Temp:Time3	0.894	-0.528, 2.317	1.232	0.2179
Diet: Temp:Time4	**1.559**	0.078, 3.040	**2.063**	**0.0392**
Sex [Male]	0.210	-0.796, 1.215	0.409	0.6826

Supplementary Table 3.1: Constituent fatty acid concentrations (mg/mL) of all birds exposed to a temperature acclimation of either cool (20 °C) or thermoneutral (TNZ; 35 °C) temperatures, in combination with a diet enriched with either saturated fatty acids (SFA) or unsaturated fatty acids (UFA). Values reflect averaged values (±SD) of birds at each sampling period blood draws were taken; see Figure 3.1 for further information.

		Total	SFAcool	UFAcool	SFATNZ	UFATNZ
	T_{Intial}	1.74±0.85	1.51±0.49	1.73±0.55	2.06±1.32	1.59±0.62
	T_1	1.67±1.14	1.78±1.82	1.83±1.16	1.33±0.50	1.73±0.63
16:0	T_2	1.50±0.52	1.50±0.62	1.49±0.32	1.40±0.43	1.62±0.59
	T_3	1.79±0.70	2.35±0.97	1.37±0.29	1.66±0.56	1.79±0.55
	T_4	1.20±1.07	1.11±0.31	2.02±2.63	0.93±0.21	1.16±0.48
	T_{Intial}	0.52±0.28	0.37±0.18	0.50±0.28	0.68±0.29	0.52±0.27
	T_1	0.59±0.31	0.58±0.29	0.56±0.35	0.63±0.32	0.57±0.29
16:1	T_2	0.48±0.16	0.53±0.15	0.41±0.12	0.47±0.13	0.50±0.20
	T_3	0.57±0.38	0.55±0.17	0.49±0.11	0.56±0.15	0.63±0.71
	T_4	0.50±0.21	0.56±0.16	0.42±0.21	0.47±0.16	0.52±0.28
	T_{Intial}	1.07±0.48	0.94±0.26	1.07±0.32	1.26±0.76	0.98±0.30
	T_1	1.15±0.67	1.11±0.59	1.30±1.02	0.94±0.44	1.23±0.39
18:0	T_2	0.95±0.32	0.94±0.34	0.95±0.24	0.89±0.37	1.02±0.30
	T_3	1.08±0.43	1.39±0.61	0.86±0.20	0.98±0.33	1.12±0.36
	T_4	0.74±0.22	0.72±0.17	0.71±0.26	0.65±0.12	0.85±0.26

18:1n-9trans	T_{Intial}	0.65±0.68	0.55±0.30	0.57±0.41	0.86±1.06	0.59±0.60
	T_1	0.68±2.55	0.67±1.68	0.40±0.32	0.38±0.24	1.33±4.89
	T_2	0.25±0.13	0.27±0.13	0.21±0.11	0.25±0.11	0.27±0.17
	T_3	0.22±0.09	0.23±0.12	0.18±0.03	0.25±0.09	0.19±0.10
	T_4	0.49±0.30	0.46±0.28	0.65±0.44	0.34±0.16	0.58±0.31
18:1n-9 cis	T_{Intial}	0.36±0.40	0.22±0.32	0.46±0.47	0.40±0.44	0.31±0.32
	T_1	0.65±0.49	0.46±0.17	0.84±0.45	0.42±0.16	0.87±0.73
	T_2	0.63±0.45	0.51±0.41	0.82±0.38	0.39±0.19	0.89±0.56
	T_3	0.55±0.30	0.69±0.36	0.38±0.13	0.52±0.27	0.59±0.33
	T_4	0.38±0.27	0.51±0.35	0.30±0.27	0.30±0.20	0.40±0.26
18:2	T_{Intial}	0.76±0.48	0.65±0.22	0.75±0.30	0.88±0.67	0.74±0.57
	T_1	0.69±0.64	0.82±1.15	0.75±0.29	0.48±0.19	0.73±0.51
	T_2	0.58±0.26	0.57±0.30	0.59±0.15	0.49±0.17	0.69±0.31
	T_3	0.64±0.24	0.79±0.22	0.56±0.13	0.65±0.26	0.57±0.23
	T_4	0.52±0.20	0.58±0.14	0.65±0.38	0.48±0.13	0.48±0.18
20:3	T_{Intial}	0.27±0.12	0.24±0.09	0.24±0.08	0.34±0.15	0.25±0.14
	T_1	0.26±0.20	0.29±0.22	0.24±0.15	0.25±0.26	0.27±0.15
	T_2	0.21±0.08	0.20±0.07	0.17±0.06	0.21±0.06	0.24±0.10
	T_3	0.23±0.09	0.25±0.09	0.16±0.05	0.25±0.09	0.22±0.08
	T_4	0.19±0.06	0.18±0.04	0.19±0.08	0.18±0.05	0.19±0.07
20:4	T_{Intial}	0.14±0.23	0.10±0.05	0.21±0.42	0.12±0.08	0.12±0.07

	T_1	0.25±0.32	0.13±0.10	0.27±0.35	0.31±0.39	0.28±0.34
	T_2	0.14±0.09	0.14±0.11	0.13±0.07	0.14±0.08	0.15±0.09
	T_3	0.23±0.32	0.26±0.27	0.13±0.05	0.15±0.12	0.39±0.54
	T_4	0.01±0.03	0.02±0.03	0.02±0.04	0.01±0.03	0.00±0.01
	T_{Intial}	0.05±0.06	0.04±0.04	0.05±0.05	0.07±0.07	0.05±0.05
	T_1	0.13±0.16	0.15±0.26	0.14±0.12	0.12±0.13	0.12±0.07
22:6	T_2	0.09±0.04	0.09±0.05	0.08±0.02	0.08±0.02	0.10±0.04
	T_3	0.10±0.05	0.13±0.07	0.08±0.02	0.09±0.04	0.11±0.05
	T_4	0.04±0.04	0.03±0.03	0.04±0.03	0.04±0.06	0.03±0.03

Supplementary Table 3.2: Model estimates of predictor variables impacting changes in blood plasma NEFA for the three-way interaction of dietary fats, ambient temperature, and time (the only model ΔAICC <2). The three-way interaction was run and compared as linear mixed models using individual identity. Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values below to denote statistical significance. The intercept values are in references to SFA-cool birds at $T_{Initial}$.

NEFA	Estimate	95% CI	t value	Pr(>|t|)
(Intercept)	**1.220**	**1.164, 1.276**	**42.835**	**<0.001**
Temp (TNZ)	**0.133**	**0.058, 0.208**	**3.487**	**<0.001**
Diet (UFA)	0.065	-0.011, 0.140	1.679	0.0941
Time1	0.021	-0.053, 0.095	0.564	0.5729
Time2	0.007	-0.066, 0.079	0.185	0.8531
Time3	**0.150**	**0.067, 0.233**	**3.542**	**<0.001**
Time4	-0.040	-0.129, 0.050	-0.871	0.3841
Temp:Diet	**-0.158**	**-0.264, -0.053**	**-2.962**	**0.0033**
Temp:Time1	**-0.141**	**-0.241, -0.041**	**-2.774**	**0.0059**
Temp:Time2	**-0.171**	**-0.270, -0.072**	**-3.382**	**0.0008**
Temp:Time3	**-0.241**	**-0.349, -0.134**	**-4.415**	**<0.001**
Temp:Time4	**-0.217**	**-0.333, -0.102**	**-3.700**	**<0.001**
Diet:Time1	0.039	-0.061, 0.139	0.766	0.4444
Diet:Time2	-0.049	-0.157, 0.059	-0.884	0.3774

Diet:Time3	**-0.246**	**-0.364, -0.128**	**-4.087**	**<0.001**
Diet:Time4	-0.030	-0.163, 0.103	-0.441	0.6593
Temp:Diet:Time1	**0.142**	**0.002, 0.282**	**1.999**	**0.0465**
Temp:Diet:Time2	**0.238**	**0.091, 0.385**	**3.185**	**0.0016**
Temp:Diet:Time3	**0.379**	**0.222, 0.535**	**4.760**	**<0.001**
Temp:Diet:Time4	**0.205**	**0.036, 0.376**	**2.383**	**0.0177**

Supplementary Table 3.3: Conditionally averaged blood plasma SFA model estimates of predictor variables based on models with strong support (ΔAIC_C <2). Model averaging used linear mixed models with individual identity as a random effect. Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values denote statistical significance. The intercept values are in references to SFA-cool birds at $T_{Initial}$.

SFA	Estimate	95% CI	z value	Pr(>|z|)
(Intercept)	0.931	0.768, 1.095	11.169	<0.001
Diet (UFA)	-0.009	-0.091, 0.073	0.214	0.8304
Time1	-0.076	-0.164, 0.013	1.675	0.0940
Time2	-0.079	-0.159, 0.001	1.936	0.0529
Time3	0.012	-0.100, 0.123	0.203	0.8390
Time4	**-0.222**	**-0.310, 0.134**	**4.934**	**<0.001**
Hct	0.001	-0.001, 0.004	0.935	0.3500
Diet:Time1	0.092	-0.025, 0.208	1.542	0.1231
Diet:Time2	0.058	-0.063, 0.179	0.944	0.3454
Diet:Time3	-0.053	-0.210, 0.179	0.669	0.5035
Diet:Time4	0.081	-0.029, 0.191	1.438	0.1503
Temp (TNZ)	0.016	0.027, 0.187	0.393	0.6945
Diet:Temp	-0.021	-0.254, -0.024	0.386	0.6992
Temp:Time1	-0.026	-0.280, -0.055	0.398	0.6906
Temp:Time2	-0.020	-0.244, -0.019	0.384	0.7012

Temp:Time3	-0.031	-0.328, -0.078	0.402	0.6877
Temp:Time4	-0.018	-0.254, 0.019	0.358	0.7207
Diet:Temp:Time1	0.031	0.043, 0.367	0.389	0.6970
Diet:Temp:Time2	0.030	0.025, 0.370	0.382	0.7021
Diet:Temp:Time3	0.046	0.115, 0.485	0.402	0.6876
Diet:Temp:Time4	0.009	-0.141, 0.257	0.197	0.8437

Supplementary Table 3.4: Model estimates of predictor variables impacting changes in blood plasma MUFA concentrations for a three-way interaction of dietary fats, ambient temperature, and duration of the experiment (the only model ΔAICC <2). The three-way interaction was run and compared as linear mixed models using individual identity as a random effect. Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values denotes statistical significance. The intercept values are in references to SFA-cool birds at $T_{Initial}$.

MUFA	Estimate	95% CI	t value	Pr(>|t|)
(Intercept)	**0.599**	**0.530, 0.668**	**17.029**	**<0.001**
Temp (TNZ)	**0.210**	**0.117, 0.302**	**4.448**	**<0.001**
Diet (UFA)	**0.096**	**0.003, 0.190**	**2.030**	**0.0431**
Time1	0.071	-0.021, 0.163	1.512	0.1317
Time2	0.066	-0.024, 0.156	1.447	0.1489
Time3	**0.120**	**0.016, 0.223**	**2.282**	**0.0232**
Time4	**0.144**	**0.032, 0.255**	**2.542**	**0.0115**
Temp:Diet	**-0.219**	**-0.348, -0.088**	**-3.308**	**0.0010**
Temp:Time1	**-0.187**	**-0.312, -0.063**	**-2.967**	**0.0033**
Temp:Time2	**-0.274**	**-0.398, -0.151**	**-4.367**	**<0.001**
Temp:Time3	**-0.251**	**-0.384, -0.118**	**-3.701**	**<0.001**
Temp:Time4	**-0.357**	**-0.501, -0.214**	**-4.904**	**<0.001**
Diet:Time1	0.030	-0.094, 0.154	0.482	0.6305

Diet:Time2	-0.060	-0.195, 0.074	-0.886	0.3763
Diet:Time3	**-0.223**	**-0.370, -0.076**	**-2.990**	**0.0030**
Diet:Time4	**-0.171**	**-0.337, -0.006**	**-2.048**	**0.0413**
Temp:Diet:Time1	0.161	-0.013, 0.334	1.821	0.0696
Temp:Diet:Time2	**0.332**	**0.150, 0.515**	**3.581**	**<0.001**
Temp:Diet:Time3	**0.339**	**0.145, 0.534**	**3.435**	**<0.001**
Temp:Diet:Time4	**0.410**	**0.199, 0.623**	**3.839**	**<0.001**

Supplementary Table 3.5: Model estimates of predictor variables impacting changes in PUFA concentrations from zebra finch blood plasma for a three-way interaction of dietary fats, ambient temperature, and duration of the experiment according to the only model with strong support (ΔAICC <2). The three-way interaction was run and compared as linear mixed models using individual identity as a random effect. Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values denote statistical significance. The intercept values are in references to SFA birds at $T_{Initial}$.

PUFA	Estimate	95% CI	t-value	Pr(>|t|)
(Intercept)	**0.598**	**0.552, 0.644**	**25.683**	**<0.001**
Diet (UFA)	-0.028	-0.092, 0.036	-0.856	0.3925
Time1	-0.047	-0.105, 0.011	-1.598	0.1111
Time2	**-0.105**	**-0.163, -0.047**	**-3.571**	**<0.001**
Time3	0.009	-0.052, 0.071	0.293	0.7700
Time4	**-0.195**	**-0.261, -0.128**	**-5.772**	**<0.001**
Diet:Time1	**0.114**	**0.032, 0.196**	**2.750**	**0.0063**
Diet:Time2	**0.086**	**0.001, 0.172**	**1.981**	**0.0485**
Diet:Time3	-0.024	-0.114, 0.067	-0.514	0.6079
Diet:Time4	0.009	-0.087, 0.105	0.188	0.8508

Supplementary Table 3.6: Conditionally averaged UIN model estimates of predictor variables impacting changes in body mass using models with strong support (ΔAIC_C <2). Square brackets indicate the category being referred to for comparisons. Time values were all evaluated in reference to $T_{Initial}$ measurements. Bolded p-values denote statistical significance. The intercept values are in references to SFA birds at $T_{Initial}$.

UIN	Estimate	95% CI	z value	Pr(>|z|)
(Intercept)	**-0.108**	-0.126, -0.089	**11.390**	**<0.001**
Temp (TNZ)	0.010	-0.001, -0.001	1.112	0.2660
Time1	**0.044**	0.022, 0.066	**3.953**	**<0.001**
Time2	0.013	-0.009, 0.036	1.173	0.2407
Time3	0.017	-0.007, 0.041	1.400	0.1617
Time4	-0.011	-0.044, 0.022	0.662	0.5080
Diet (UFA)	0.001	-0.024, 0.033	0.143	0.8862
Diet:Time1	-0.002	-0.045, 0.032	0.160	0.8727
Diet:Time2	0.001	-0.036, 0.045	0.111	0.9119
Diet:Time3	0.002	-0.033, 0.052	0.198	0.8431
Diet:Time4	-0.013	-0.101, -0.010	0.500	0.6174

Supplementary Table 3.7: Results of a linear mixed model for the proportion of observations where birds were observed feeding. Data were arcsine square root transformed prior to analysis. A single individual with an unusually high proportion of time spent feeing was removed as a possible outlier (>4SD above the mean). Cage identity was included as a random effect. Reference treatments are in brackets. ($n_{SFAcool}$=15, n_{SFATNZ}=22, $n_{UFAcool}$=9, n_{UFATNZ}=19)

	Estimate	CI	T value	Pr(>|z|)
(Intercept)	**0.226**	**0.156, 0.296**	**5.782**	**<0.001**
Temp (TNZ)	0.028	-0.068, 0.124	0.513	0.619
Diet (UFA)	-0.069	-0.181, 0.043	-1.109	0.288
Temp:Diet	0.011	-0.136, 0.157	0.135	0.895