Title:

Adaptation or plasticity? Effects of temperature on metabolic rate and life-history traits in the Australian Painted Dragon lizard.

Author names and affiliations:

Daniel J Ritchie[1,2]†, Christopher R Friesen[1,2]

1) School of Earth, Atmospheric and Life Sciences, The University of Wollongong, 2522 Wollongong, New South Wales, Australia
2) School of Life and Environmental Sciences, University of Sydney, Heydon-Laurence Bldg A08, Science Road, Sydney, NSW 2006, Australia

† email of the corresponding author: cfriesen@uow.edu.au

Abstract

The roles of adaptation and plasticity in shaping life-history traits have long been a subject of debate in evolutionary ecology, with the relevance of each varying by traits, populations, species, and clades. Ectothermic organisms, which obtain most of their metabolic energy from ambient heat, occupy wide geographic ranges where heat can be unevenly distributed. There is considerable potential for populations at opposite ends of a temperature cline to exhibit differing thermal adaptations, energy budgets, and phenotypically plastic responses to temperature. Using factorial laboratory experiments, we investigated the hypothesis that two populations of male painted dragon lizards (*Ctenophorus pictus*) would differ significantly in thermal physiological traits at opposite ends of a temperature-cline. After acclimatising both populations in warm and cool temperature treatments, we quantified differences in thermal performance curves, somatic growth (body size and weight) and reproductive investment (relative testes mass). We found that the Northern, warm-adapted population had a significantly higher metabolic rate at high temperatures and ina greater investment in body size and relative testes mass than the cool-adapted southern population, consistent with geographic variation of thermal traits across both populations. The effects of temperature treatment were negligible, except for body length (skeletal growth), which was greater in the cool treatment for both populations, suggesting similar thermal plasticity responses. Our results indicate that thermal adaptation and phenotypic plasticity both contribute to life-history variation in *C. pictus.*

Keywords: adaptation, plasticity, ectotherm, agamid, metabolic rate, life-history

2.1. Introduction

Geographic variation is a valuable tool for studying the fundamental processes of adaptive evolution (Pörtner, 2002). Life-history traits are essential components of fitness (Ricklefs & Wikelski, 2002). Changes in phenotypes along environmental clines result from variable trade-offs driven by finite resources and their effects on metabolic processes (Ricklefs & Wikelski, 2002). Temperature has a profound impact on all organisms' metabolism, but especially in ectotherms, whose metabolic rate is primarily driven by environmental sources of heat (M. J. Angilletta, 2009). Thus, ectothermic species spanning broad geographic ranges evolve contrasting thermal and metabolic adaptations to adapt to local conditions (M. J. Angilletta, 2009). When local environmental conditions are consistent, the optimisation of metabolic and cellular processes can be incredibly fine-tuned (Flukes, Wright, & Johnson, 2015). However, when conditions are highly variable, metabolic and cellular processes must be plastic, enabling individuals to survive in diverse conditions (Schulte, Healy, & Fangue, 2011). Both evolutionary pathways can influence the speed and efficiency of metabolic functions, ultimately leading to variation in life-history traits across a species' range as a function of temperature (M. Angilletta, Steury, & Sears, 2004).

Ectotherms are metabolically sensitive to changes in ambient temperature and have developed a host of thermal adaptations to optimise bodily function across various environments via behavioural and physiological mechanisms (M. J. Angilletta, 2009). One way of quantifying differences in thermal adaptations is by measuring the resting metabolic rate (RMR) of an organism (Marshall & McQuaid, 2011). Resting MR in ectotherms is defined as the metabolic rate of an organism in a quiescent, post-absorptive state; it is the basic energetic cost of self-maintenance at a given temperature (Burton, Killen, Armstrong, & Metcalfe, 2011). High RMR within individuals suggests increased capacity for energetically costly activities (metabolic scope), such as sprint speed, territorial defence, and somatic and reproductive investment, and is often linked to fitness (Cano & Nicieza, 2006). In ectotherms, thermal metabolic optimum should evolve to match the most prevalent temperatures a population experiences when resources are most available (Seebacher, 2005). However, high RMR is energetically costly, and the fitness benefits of maintaining high RMR depend on resource availability (Huey & Kingsolver, 2019). Therefore, when conditions are highly variable, plasticity in metabolic rate is advantageous (Seebacher & Franklin, 2011). However, not all species, populations or individuals are equally plastic. For example, cane toads from higher latitudes, living in greater seasonal and diel environmental variability, exhibit greater plasticity in metabolic rate than individuals from lower latitudes, where temperatures are more uniform (Winwood-Smith, Alton, Franklin, & White, 2015). Thus, selection will favour high and consistent RMR when conditions are stable, and plastic RMR when conditions are unpredictable (Seebacher & Franklin, 2011). Changes in optimal metabolic temperatures and plasticity due to environmental variability can alter selection between life-history traits such as somatic investment and reproduction (McGill, Enquist, Weiher, & Westoby, 2006).

Metabolism associated with somatic investment and growth forms a significant portion of an organism's energetic budget and therefore is a reliable indicator of inter-population differences in resource availability and allocation (Adolph & Porter, 1993). In ectotherms, a substantial proportion of growth rate and growth period is believed to be a heritable trait and an evolved adjustment to environmental conditions (Chandan, Bhattacharya, Rajkumar, Prince, & Chatterjee, 2019). Fast and consistent somatic growth directly correlates with body size, which scales to nearly all facets of the phenotype, such as morphological, behavioural, reproductive, physiological, and life history characteristics (Honěk, 1993; Johnsson, Nöbbelin, & Bohlin, 1999). Large body size is achieved through increases in skeletal, muscular, and organ weight, as well as fat stores throughout an organism's lifetime, which significantly increases longevity (Adolph & Porter, 1993). However, somatic growth rate in ectotherms is highly plastic; alterations in temperature and food consumption (i.e. changes in metabolic scope) in early life have strong influences upon growth efficiency (Sibly & Atkinson, 1994). Additionally, many ectotherms, such as fish, amphibians, and reptiles, exhibit indeterminate growth, maintaining plastic growth rates throughout an organism's life (Dumas & France, 2008). Due to the cost of growth, some reptiles are known to decrease in mass and skeletal length when conditions are unfavourable and increase when conditions improve (Chiari, Glaberman, Tarroso, Caccone, & Claude, 2016; Wikelski & Thom, 2000). Growth and somatic investment can reflect adjustments to resource availability over evolutionary time-scales and within individuals' lives (Adolph & Porter, 1993). Alterations in metabolic function caused by disparate resource availability may also influence life-history traits, such as reproductive investment in metabolic function (Pörtner, 2002).

Investment in current somatic growth versus investment in future reproduction is the most basic life-history trade-off in nature and varies between populations when resources are disparate (Pörtner, 2002). Investment in future reproduction increases the likelihood of successfully passing on genes; however, if reproduction is too energetically costly, somatic growth and repair mechanisms will be impaired (Cox et al., 2010). This trade-off is sensitive to environmental temperature changes, which can tip the balance by affecting energetic budgets (e.g., food availability and metabolic rates) and shifting resource allocation strategies along environmental clines (Urbanski et al., 2012). In populations subject to an unpredictable environment, selection will often favour early sexual maturity and rapid reproduction (i.e., bet-hedging (Mitchell, 1988) or opportunistic strategies (Roff, 1993)), which require high levels of metabolic expenditure early in life. In stable environments, selection favours significant somatic growth, increased size at maturity, and a relatively lower metabolic rate, with energetic expenditure on somatic repair mechanisms (Roff, 1993). In addition to the effects of environmental stability on somatic investment, large body size is also regularly favoured by sexual selection (Wikelski, 2005). Large individuals are often more fecund and better able to defend territories (Wikelski, 2005). Because body size is energetically costly, it cannot be faked and serves as an honest sexual signal, a primary trait used by females to assess male mate quality (Wikelski, 2005). Similarly, investment in sexual colouration is a costly reproductive investment, used to demonstrate mate quality in various species (Grafen, 1990; Servedio & Boughman, 2017). Therefore, differences in resource availability across populations can alter levels of reproductive and somatic investment through trade-offs. Thus, we assess the relative effects of adaptation and plasticity on: body size, reproductive investment, growth and metabolism in two populations of the Australian painted dragon lizard (*Ctenophorus pictus)* from opposite ends of a temperature-aridity cline acclimated to warm and cool conditions.

Ctenophorus pictus is a well-studied colour-polymorphic lizard native to a large portion of southern central Australia. Most detailed investigations of *C. pictus* have been conducted at a single population in Yathong reserve, NSW; thus, background knowledge of this species is primarily population-specific. In the Yathong population, RMR is morph-specific; individuals possessing a yellow gular patch or 'Bib" have a significantly higher metabolic rate than those without (Friesen, Johansson, & Olsson, 2017; McDiarmid, Friesen, Ballen, & Olsson, 2017). Higher metabolic rates have been linked to sexual selection, with bibbed males being more aggressive (McDiarmid et al., 2017), defending more territory (Healey & Olsson, 2009), thus requiring increased metabolic scope. As *C. pictus* is an annual lizard (roughly 80-90% die before reaching one year of age), the species, at least in the

Yathong population, takes an opportunistic strategy with a short lifespan and early maturity indicative of evolution in an unpredictable environment (Olsson et al., 2007). However, the two populations used in this study occupy vastly different abiotic environments in terms of thermal variability and seasonality, thus providing an opportunity to investigate the effects of geographic variation in thermal adaptation and life-history traits. To assess the relative effects of adaptation and plasticity in thermal physiological traits, we housed adult males from both populations in warm and cool treatments for 3 months. Subsequently, body size, growth, reproductive investment, and metabolic rate were used as indicators of adaptation (population effects), plasticity (temperature treatment effects), or the interaction between the two within this species. We predict that the warm/variable adapted population will be larger in body size, grow faster and invest more in reproduction due to increased energetics associated with warm conditions, these differences will further be enhanced in the warm treatment (M. J. Angilletta, 2009; Cano & Nicieza, 2006). Furthermore, we predict that the warm, variable-adapted population will have a higher, more plastic metabolic rate and a higher critical maximum temperature than the cooler, stable-adapted population, due to the higher evolutionary temperature variability.

2.2. Methods

2.2.1 Animal collection/housing

The Australian painted dragon lizard (*Ctenophorus pictus*) is a small, diurnal, Agamid lizard (Healey & Olsson, 2009) with a broad distribution across south-central Australia (Cogger, 2014) (*Figure 1*). Lizards were caught in October 2019 from two discrete field sites in South Australia with hypothetically zero gene flow between them due to habitat fragmentation: Muloorina station (29°14'; 137°54') to the north, and Innes National Park at the tip of the Yorke Peninsula (35°14'; 136°54') to the south (*Figure 3*). These field sites were chosen as model arid and coastal populations because of large differences in average annual rainfall with average yearly rainfall approximately three times lower at the northern site (350 mm vs 125 mm annually). Additionally, the Southern site is on average 8-10°C colder, and the average number of days over 40°C can be ~15 times less in the south during the breeding season of October to December (*Figure 2*). Furthermore, average daily temperature variation (high-low) in the north is double that of the south, and inter-season variability is considerably less in the south (*Figure 2*). As such, the northern site is classed as markedly hotter and more variable than the southern site.

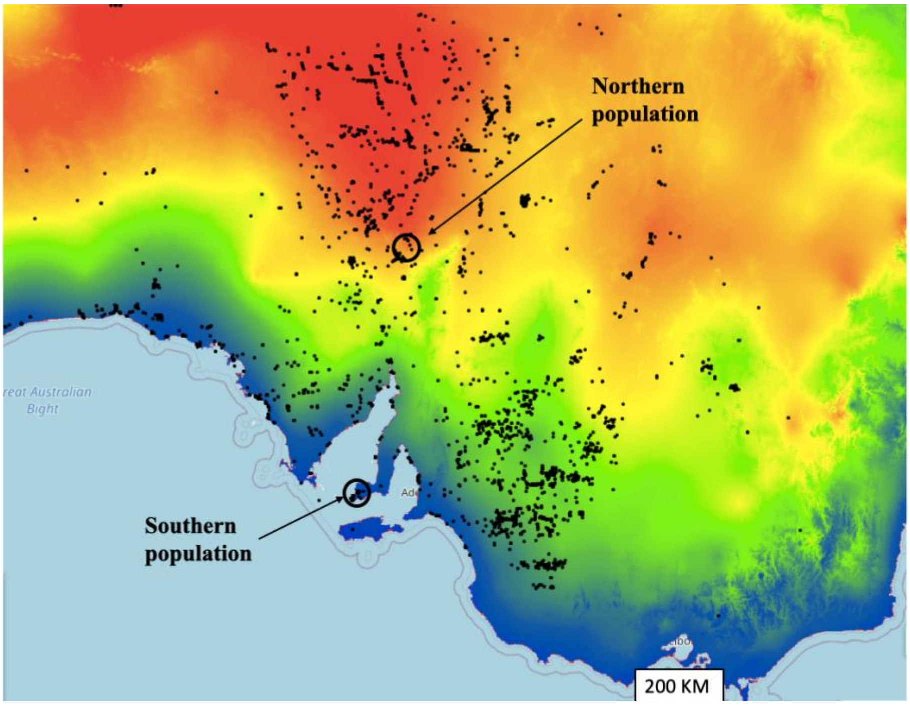

Figure 1. *Map of field sites in which populations of* C.pictus *were sourced; Muloorina station (Northern population) (29°14'; 137°54'), and Innes National Park (Southern population) (35°14'; 136°54'. Black dots indicate records of* C. pictus *from Atlas of Living Australia (https://www.ala.org.au/). Colour shading in the map*

indicates temperature/aridity (orange: high aridity, and blue: low aridity). Black dots indicate records of C. pictus *sightings*

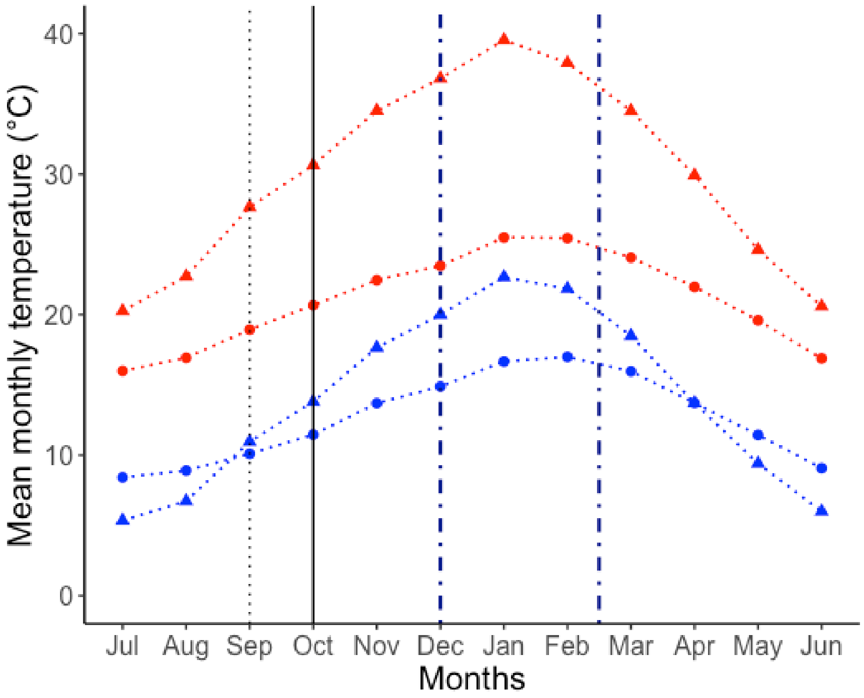

Figure 2. *Average daily High (red dotted line) and Low (blue dotted-line) temperatures for each month over the years 1996-2015 for the two locations; Northern population (Triangles) data from Marree, SA weather station. Southern population (Circles) data from Stenhouse Bay, SA weather station. Vertical lines indicate the presumed beginning of breeding season for the Northern population (dotted line) and Southern population (solid line). Climate data sourced from the Bureau of Meteorology. Vertical dot-dash lines indicate study period.*

All animals were sexed, weighed, inspected for bib presence/absence and assigned an ID upon capture via hand and lasso (total N = 40 males, North N=21, South N=19). Females were excluded due to uncertainty about the staging of their asynchronous reproductive cycles both within and between populations (Ibargüengoytía & Boretto, 2006). Additionally, we were interested in the associations of bib (sexual selection) on response variables. Lizards were transported to the University of Wollongong. The lizards were housed individually in opaque 88L enclosures (400 x 400 x 620 mm) with a heat lamp at one end to allow thermoregulation. Males from each population were randomly spread across two separate rooms (cool, N = 20; warm, N = 20). Ambient baseline and over-night temperatures in both rooms were set to 15C. To form the temperature treatments, heat lamp wattage was manipulated: One room had 40 W heat lamps whilst the other had 60W heat lamps, which generated different ambient temperatures in each room (warm mean = 24.743, ±SE = 0.093, cool mean = 20.366, ±SE = 0.055) and thus termed warm and cool treatments hereafter. Max mean temperatures under the heat lamps within the enclosures matched average maximum temperatures at both the Northern and Southern populations (Max mean tub temperature warm room = 42.5 °C, ±SE = 2.2; Max mean tub temperature cool room = 28.5 °C, ±SE =1.5, *Figure 1*). The lizards were kept in these temperature treatments for a 3-months. Each room's UV and ambient lights were set to a 10 h:14 h Light: Dark cycle, and heat lamps were set to a 7 h:17 h on: off regime. The animals were fed mealworms dusted with calcium and pre-formulated multivitamin powder and misted with water every second day.

2.2.2 Experimental procedures

2.2.2.1 Metabolism

Metabolism measurements were taken for three sets of 12 animals and one set of four animals. Each set of animals were fasted for 72-hours before measurements. The night before RMR measurements, the animals were collected from their home enclosures after the heat lamps had been turned off and ambient temperatures in the room had dropped below 18 °C. Animals were weighed, measured and placed in respirometry chambers of known volume (Mean chamber volume = 810ml, ±SE = 4 ml). The chambers were then placed in an incubator and stepwise cooled through an acclimation period of half an hour in 5 °C increments until they reached the planned starting temperature of 10 °C, at which they equilibrated for 60 minutes with valves of the metabolic chamber open. After equilibrating, the chambers were flushed with fresh air and returned to the incubator overnight (with the valve now closed to capture respired air). At the end of the measurement period, one 50 ml air sample was taken from each chamber through valves with calibrated syringe, which was immediately sealed with an air-tight cap until injecting the sample into the inlet tube of Sable Systems FMS oxygen analyser (Sable Systems, International, North Las Vegas, NV), with flow-rate set to 250ml/min to measure VO^2 (oxygen consumption as ml min^{-1}). Oxygen concentration output was recorded by Warthog LabHelperX software (build 23, October 2015, 1989–2016 Mark A. Chappell and the Regents of the University of California). Lizard mass was used as a proxy for the lizard's volume in calculations of total air volume in the chamber, which is highly correlated with volume estimated based on the water-displacement method, and is more accurate and precise than water-displacement ($R^2 = 0.957$, $n = 35$, thus 1g ≈ 1ml) (Friesen et al., 2017). After the air samples were collected from all the animals at a given temperature, the incubator temperature was raised 5 °C to the next measurement temperature. The chamber was flushed to restore O^2 levels, and the valve was left open for 30 minutes before it was closed again to start the next measurement period. This procedure was repeated every 5 °C (10-40 °C). Each measurement period was timed and recorded ± 1 s. Measurement periods were tailored to each temperature to ensure O^2 was detectable by the oxygen analyser on pilot data whilst also limiting the time lizards time spent at high temperatures (10 °C = 875 min; 15°C = 165; 20°C = 105 min; 25°C = 85 min; 30°C =70 min; 35°C = 50 min; 40°C = 40 min).

Oxygen consumption was determined by integrating the area under the curves from the output graphs, O^2 (Lighton, 2018) in Warthog LabAnalystX (build 15, October 2015, 1989–2016 Mark Chappell and the Regents of the University of California). Total O^2 consumption of the lizard was calculated as (Friesen, Powers, Copenhaver, & Mason, 2015; Lighton, 2018), $V_{\text{lizard}} = V_{\text{measured}} * (V_{\text{chamber}} / V_{\text{sample}})$, where V_{lizard} is the total volume ml O^2 consumed by the lizard during the time in the chamber; V_{measured} is the volume (ml) O^2 change in the sample calculated by integrating the peak; V_{chamber} is the chamber volume minus the lizard volume (1g ≈ 1ml), and V_{sample} is the sample volume (50 ml). The 0^2-ml min^{-1} were calculated as $\dot{V}O^2 = V_{\text{lizard}} / t$, where t is time in the enclosure in seconds (± 1s).

2.2.2.2 Basking behaviour

Basking score was tabulated roughly 2.5 times a week over the three-month acclimation period by entering the treatment rooms at various times throughout the day and noting each individual's location within their tub in a binomial fashion (e.g. 1 = basking, 0 = not basking). The time at which measurements were taken were divided into six periods of the day for ease of analysis. the categories are as such: Before heat lamps (8:30am-9:00am); = Early morning (9:00am-10:30am); Late morning (10:30am-12:00pm); = Noon (12:00pm-1:30pm); Afternoon (1:30pm-2:30pm); Late Day (2:30pm-4:30pm).

2.2.2.3 Critical temperature maximum.

In ectotherms, thermal optimum (Topt) is the ideal temperature for physiological functions, whilst critical temperatures, both high (CTmax) and low (CTmin), are the functional limits of metabolic scope (Pörtner 2002). Measuring critical maximum temperature (CTmax) as the temperature when mouth-gaping occurs avoids potential harm associated with "traditional" methods, usually determined by the point at which loss of righting response occurs (Taylor et al., 2020). Lizards were fitted with a small thermocouple ~3 mm in their cloaca, held in place by adhesive tape (3M Nexcare$^{\text{TM}}$), allowing the exact body temperature when CTmax is reached to be recorded. Lizards were heated until mouth gaping occurs, heating stopped, temperature recorded, and lizards were observed as they returned to their preferred body temperature and placed back in their home enclosure.

2.2.2.4 Dissection and tissue collection

After the 3-months, the lizards were euthanised to assess testes size alongside tissue samples required for another experiment. The lizards were heavily sedated via an injection of Zoletil (tiletamine/zolazepam); once sufficiently sedated as determined by loss of righting-response, they were euthanised with an overdose of sodium pentobarbitone and decapitated to ensure death prior to dissections. Subsequently, body size in mass (±0.001 g) and SVL (±0.5 mm) were measured alongside, testes weight (±0.001 g) were recorded. Testes size was used as a robust proxy for male reproductive investment as postcopulatory selection regularly favours large testis size relative to body mass (Lüpold, de Boer, Evans, Tomkins, & Fitzpatrick, 2020; Todd, 2008; Uller, Stuart-Fox, & Olsson, 2010). Animals were collected under a scientific research license issued by South Australia Department of Water and Environment permit (A26776-1-4), and the collection and experiments were conducted in accordance with research authority granted by University of Wollongong Animal Ethics Committee (AE1907A04).

2.2.3 Statistical methods

2.2.3.1 Morphology

All statistical analysis was conducted in R studio (RStudio Team (2019). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA). Body condition index (BCI) was calculated as the standardised residuals (mean = 0; standard deviation = 1) from a linear regression of ln(body mass) as a function of ln(SVL) per population at first time in the lab ($BCI_{initial}$) and time of euthanasia (BCI_{final}) (Labocha, Schutz, & Hayes, 2014). To model differences in BCI_{final}, mass (g), SVL (cm) and reproductive investment at time of euthanasia, separate ANOVAs were constructed with the explanatory variable of Population (Northern v Southern). Reproductive investment was evaluated by calculating the percentage of testes mass relative to body mass (testes mass/body mass x 100), termed the Gonadal somatic index (GSI) (Lüpold et al., 2020; Todd, 2008; Uller et al., 2010). To assess population, temperature treatment and bib effects on the relationship between BCI_{final} and GSI, separate ANCOVAs were run. Temperature treatment and bib showed no correlation, however population was significant. Subsequently, two linear regressions were used to determine each population's relationship between BCIe and GSI. Finally, the region of non-significance was determined using the Johnson-Neyman technique (White 2003).

2.2.3.2 Growth

Growth was calculated as the percentage change from the first measurements in the lab to time at euthanasia for both body mass (±0.001 g) and SVL (±0.5 mm). Separate ANOVA's were constructed with Population, Temperature treatment and their interaction as explanatory variables for both response variables of body mass and SVL growth. The relationship between $\text{BCI}_{\text{initial}}$ and the two response variables of growth, body mass and SVL, were modelled in separate linear regressions.

2.2.3.3 Basking behaviours

We constructed a Generalised Linear Mixed effect models (GLMM, using the R package "lme4" " (Bates, Sarkar, Bates, & Matrix, 2007) to test for significant differences in Basking score using the fixed effects: temperature treatment, Population and time of day plus the three-way interaction between these variables and Male ID as a random effect. The GLMM family used in the basking score analysis was binomial with a logit function and weighted to a value of 1 per observation. Posthoc pairwise comparisons were conducted with the package 'lsmeans" (Lenth and Lenth 2018) to attain and test which combinations of Population, temperature treatment and time of day were significantly different (Tukey adjusted p-values to account for multiple, posthoc comparisons).

2.2.3.4 Metabolism

Mass specific metabolic rate (hereafter called msRMR) was calculated as O^2 consumption rate (ml/min) divided by the lizard's mass at time of measurement (±0.001 g). Subsequently, msRMR was $\log_{10}$ transformed to improve normality and homogeneity of variance. A linear mixed model (LMM) using the R package "lme4" " (Bates et al., 2007) was constructed with Temperature (of msRMR measurement, treated as a discrete, categorical variable), Population and thermal treatment as explanatory variables, plus the interaction terms of Temperature* Population, Population* thermal treatment, Temperature* thermal treatment and ID as a random effect (to account for repeated measures at different temperatures). However, thermal treatment was not significant and replaced by Bib presence/absence, due to significant effects on msRMR in previous investigations in this species (Friesen et al., 2017), mildly improve model fit ΔAICc = -3.05 (Konishi & Kitagawa, 2008). Thus, the final LMM used Temperature, Population and Bib as explanatory variables plus the interaction terms of Temperature* Population, Population*Bib, Temperature*Bib and ID as a random. The results of the final msRMR LMM were assessed using a Type III, sums of squares (SS) ANOVA using Satterthwaite's method within the package "lmertest" (Kuznetsova, Brockhoff, & Christensen, 2017). R^2 of final msRMR LMM was calculated using the R package "MuMIn" (Barton & Barton, 2015) and posthoc pairwise comparisons were conducted using the R package "lsmeans" (Lenth & Lenth, 2018) to assess differences between groups using Tukey adjusted p-values to account for multiple comparisons. CTmax was modelled as a function of Population and temperature treatment and their interaction in an ANOVA. The significance level for all tests was $\alpha = 0.05$. Graphs were constructed using the R package "ggplot2" (Wickham, 2016). R packages "tidyverse" (Wickham 2017) and "dplyr" (Wickham et al. 2015) were used for data handling and manipulation.

2.3. Results

2.3.1 Morphology and reproductive investment

Significant differences between populations were found in Mass (g), SVL (mm) and GSI (%). The Northern population was larger in mean body mass, SVL and GSI (*Table 1*). However, $\text{BCI}_{\text{final}}$ did not differ between populations. Bib frequency differed between populations, with the Southern population being 52% bibbed and the northern population being 38% bibbed. Population significantly affected the relationship between $\text{BCI}_{\text{final}}$ and GSI ($F_{1,37} = 98.08$, $P < 0.001$), with the lower bound of the region of non-significance at 1.919, which is well above the largest BCIfinal values (*Figure 3)*. Body CI_{final} and GSI were positively correlated in the Southern population ($F_{1,\,17} = 9.937$, $P = 0.006$, $R^2 = 0.33$) whilst the Northern population had a non-significant negative relationship ($F_{1,\,19} = 3.724$, $P = 0.0687$, $R^2 = 0.12$, *Figure 3*).

***Table 1**. Per population mean and standard error of lizard mass (g), SVL (cm), GSI (%) and BCI. Significance derived from individual ANOVA's for all four variables.*

		Body Mass (g)	**SVL (mm)**	**GSI (%)**	**$\text{BCIf}_{\text{inal}}$**
Northern Population	**Mean**	13.062	71.718	1.302	0.092
	Standard error	±0.391	±0.687	±0.066	±0.211
Southern population	**Mean**	9.942	64.631	0.396	-0.102
	Standard error	±0.479	±0.852	0.062	±0.241
	Significance	**≤0.005 ***	**≤0.005 ***	**≤0.005 ***	**0.548**

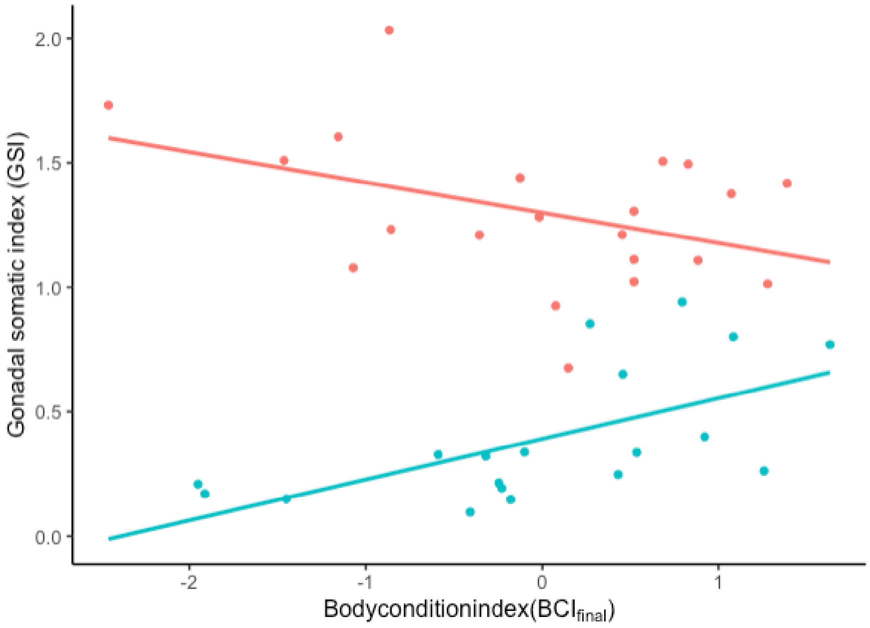

***Figure 3**. Scatterplot showing contrasting correlations of population specific body condition (BCI_{final}) and gonadal somatic index (GSI) from two populations of* C. pictus, *North (Red) and South (Blue). Coloured lines depicting population regression lines (Southern population ($F_{1,\,17} = 9.937$, $P = 0.006$, $R^2 = 0.33$), Northern*

population ($F_{1, 19} = 3.724$, $P = 0.0687$, $R^2 = 0.12$)), which significantly differed from each other across all realistic values of BCI_{final} values >1.917.

2.3.2 Growth

SVL growth as a percentage of initial SVL over the three-months differed between temperature treatments ($F_{1,35} = 7.701$, $P < 0.001$), but not Population ($F_{1,35} = 0.050$, $P = 0.824$), Bib ($F_{1,35} = 0.283$, $P = 0.598$), and did not depend on the interaction between Population and temperature treatment ($F_{1,35} = 0.146$, $P = 0.705$) (*Figure 4A*). Individuals kept in the cool treatment had significantly greater SVL growth (%) (mean = 4.723, ±SE = 0.557) than those held in the warm treatment (mean = 2.544, ±SE = 0.511) regardless of population (*Figure 4A*). SVL growth (%) was strongly positively correlated to $BCI_{initial}$ ($F_{1, 38} = 20.393$, $P < 0.001$, $R^2 = 0.334$). Mass growth as a percentage of initial mass over the three-months did not differ between Populations ($F_{1,35} = 0.572$, $P = 0.454$), temperature treatments ($F_{1,35} = 0.436$, $P = 0.513$), Bib ($F_{1,35} = 0.030$, $P = 0.846$) and did not depend on the Population* temperature treatment interaction ($F_{1,35} = 0.038$, $P = 0.846$) (*Figure 4B*). Non-significance may be attributed to small sample sizes relative to the large amount of individual variation (*Figure 4B*). There was no relationship between BCI_{inital} and mass growth ($F_{1, 38} = 1.874$, $P = 0.179$, $R^2 = 0.022$).

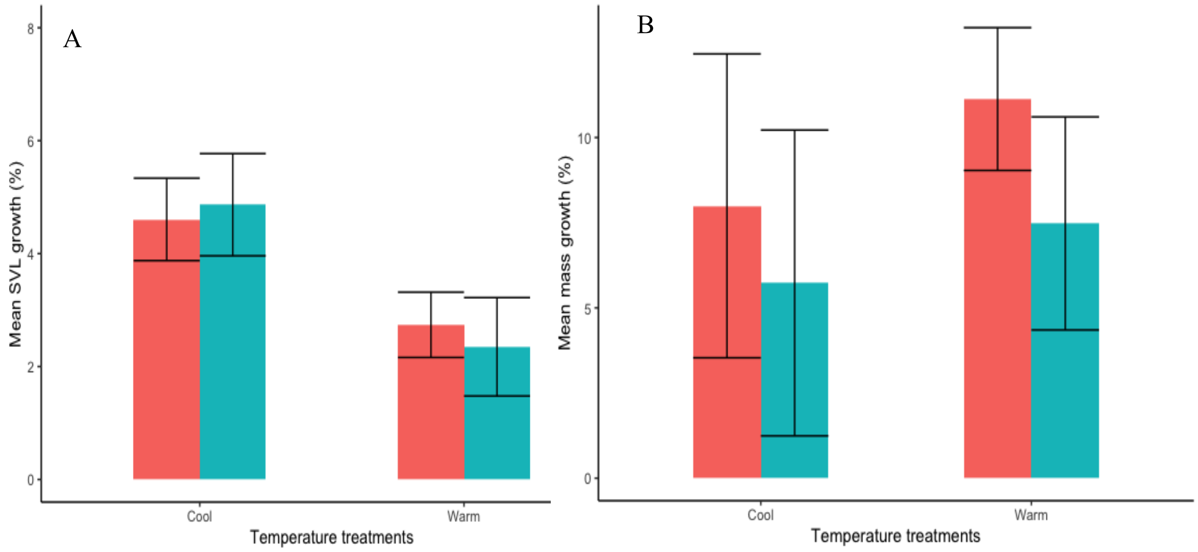

Figure 4**. Growth (%) of* C. pictus *males from Northern (Red) and Southern (Blue) populations that were kept in warm and cool treatments over a 3-month period. Error bars represent ± SE.* ***A. *mean SVL growth (%). Temperature treatment was found to be significantly different, whilst populations showed no significant differences.* ***B.*** *Mass growth (%). No significant differences between temperature treatment or population were found.*

2.3.3 Basking behaviours

In the warm treatment, significantly more males from the northern population (Mean proportion = 0.927, ±SE = 0.032) were basking only in the 'afternoon' than males from the southern population (Mean proportion = 0.4844, ±SE = 0.063) (*Figure 5*). In the cool treatment, the northern population basked considerably more (Mean proportion = 0.666, ±SE = 0.092) in the 'afternoon' than the southern population (Mean proportion = 0.069, ±SE = 0.048), and in the 'late day', with northern males (Mean proportion =0.532, ±SE = 0.042) basking more than southern males (Mean proportion = 0.310, ±SE = 0.039) (*Figure 6)*.

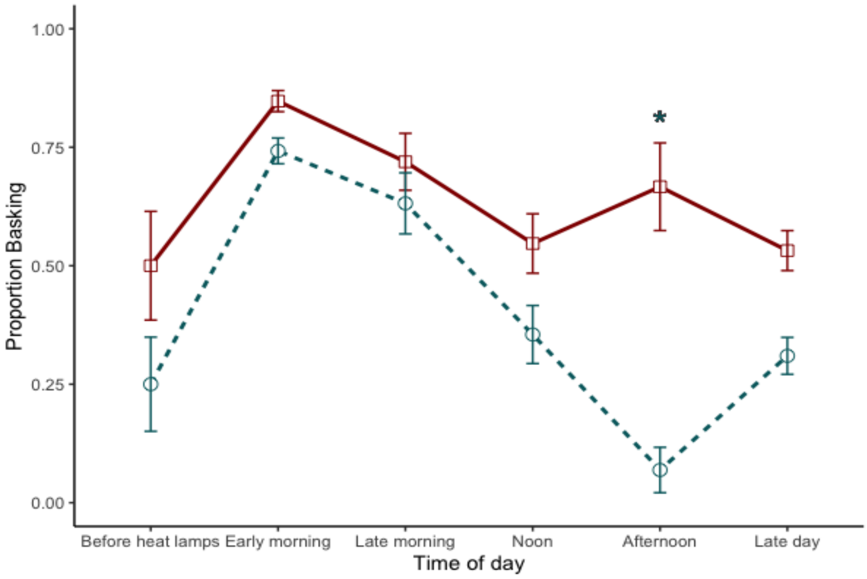

Figure 5. *The average proportion of* C. pictus *males basking throughout the day in the warm treatment. The Northern population (solid red lines), the southern population (blue dashed lines). Error bars depicting ±SE of the mean proportion in each population. Asterisk (*) depicting significance in a posthoc pairwise comparison*

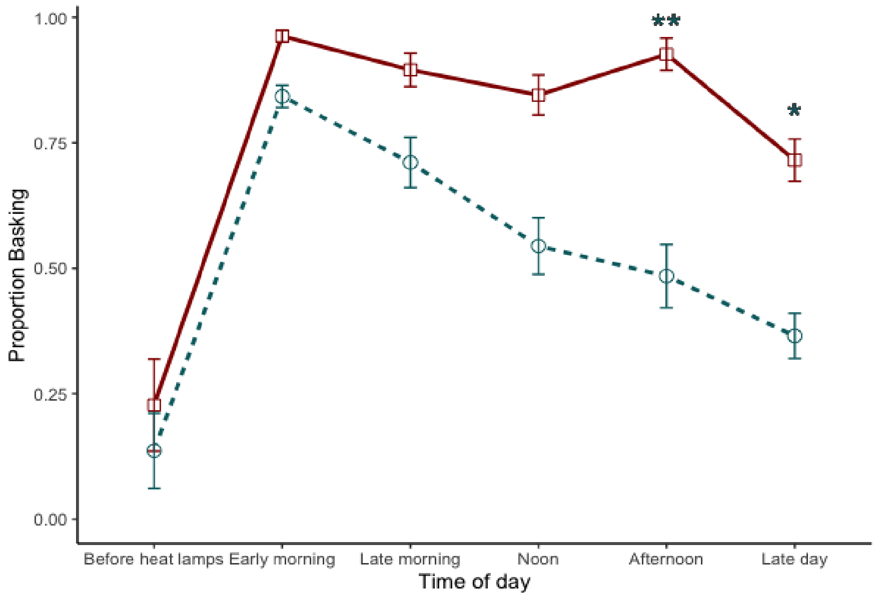

Figure 6. *Average proportion of* C.pictus *males basking throughout the day in the cool treatment. The Northern population (solid red line), the southern population (blue dashed line). Error bars depicting ±SE of the mean proportion in each population. Asterisk (*) depicting significance in a posthoc pairwise comparison*

2.3.4 Metabolism

Mass specific RMR significantly increased with temperature from 10-40 °C ($F_{6,222}$ = 916.808, P < 0.001). There was a significant Population* Temperature interaction ($F_{6,222}$ = 2.8072, P = 0.012), with the northern population having a higher RMR at most temperatures but only significantly at 40 °C (*Figure 7*). Bib was not significantly associated with msRMR ($F_{1,40}$ = 0.677, P = 0.415) nor were any interaction terms *(Table 2*). Individual rank repeatability was relatively high throughout temperatures (R^2 = 0.413, 95% CI = 0.275-0.569, P < 0.001). CTmax was statistically different between populations ($F_{1,32}$ = 26.420, P < 0.001) but not temperature treatments ($F_{1,32}$ = 0.677, P = 0.417) nor interaction term ($F_{1,32}$ = 0.658, P = 0.423), with the Northern population having a higher CTmax (mean = 41.0, 95% CI = 40.721-41.279) than the Southern population (mean = 38.9, 95% CI = 38.615-39.185, *Figure 10*).

Table 2. *msRMR. Results of type III SS ANOVA output of LMM using Satterthwaite's method. Bold text in the p-value column indicates significance at $P \leq 0.05$.*

Full model R^2	0.954					
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**P-value**
Temperature	77.962	12.9937	6	222.171	916.808	**<0.001**
Population	0.025	0.0246	1	36.401	1.735	0.196
Bib	0.010	0.0096	1	40.588	0.677	0.415
Temperature*Population	0.239	0.0398	6	222.191	2.807	**0.012**
Population*Bib	0.030	0.0304	1	40.588	2.144	0.151
Temperature*Bib	0.163	0.0272	6	222.276	1.918	0.079

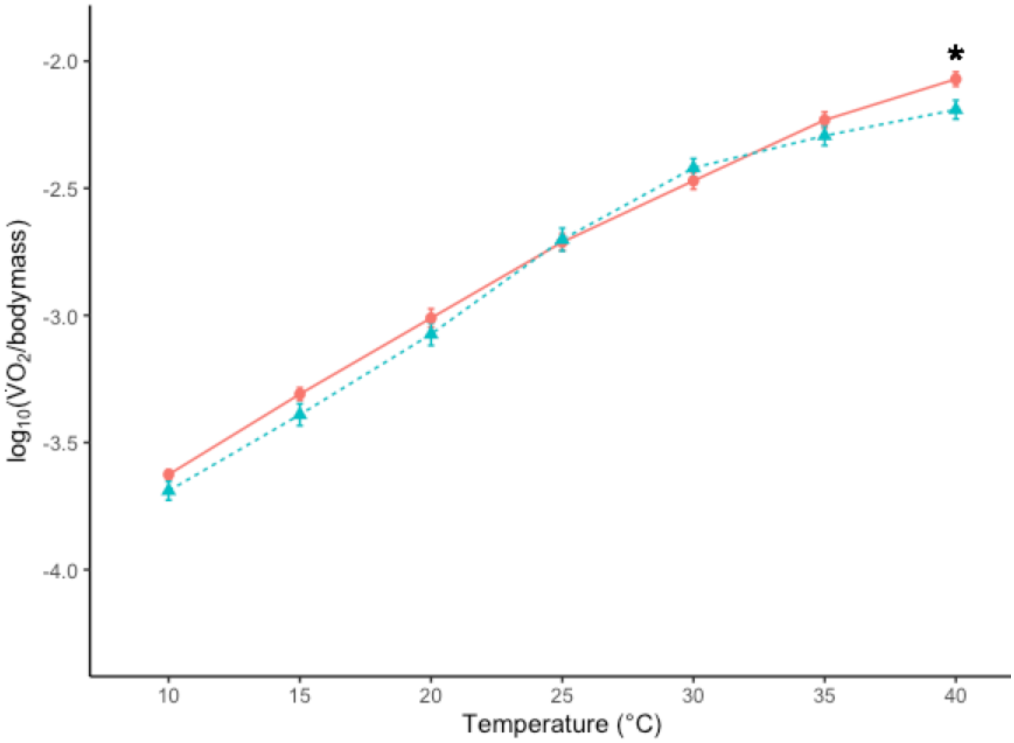

***Figure** 7. Mean values of RMR ($\log_{10}\dot{V}O2$/body mass) of adult male* C. pictus *from the Southern population (Blue triangles) and Northern population (red circles) at seven increasing temperatures (°C). The northern population has a significantly higher msRMR, particularly at 40 degrees. Error bars represent ±1 SE. Asterisk depicting significance in a posthoc pairwise comparison of an LME which used Temperature*Population as the interaction term $*\alpha \leq 0.05$.*

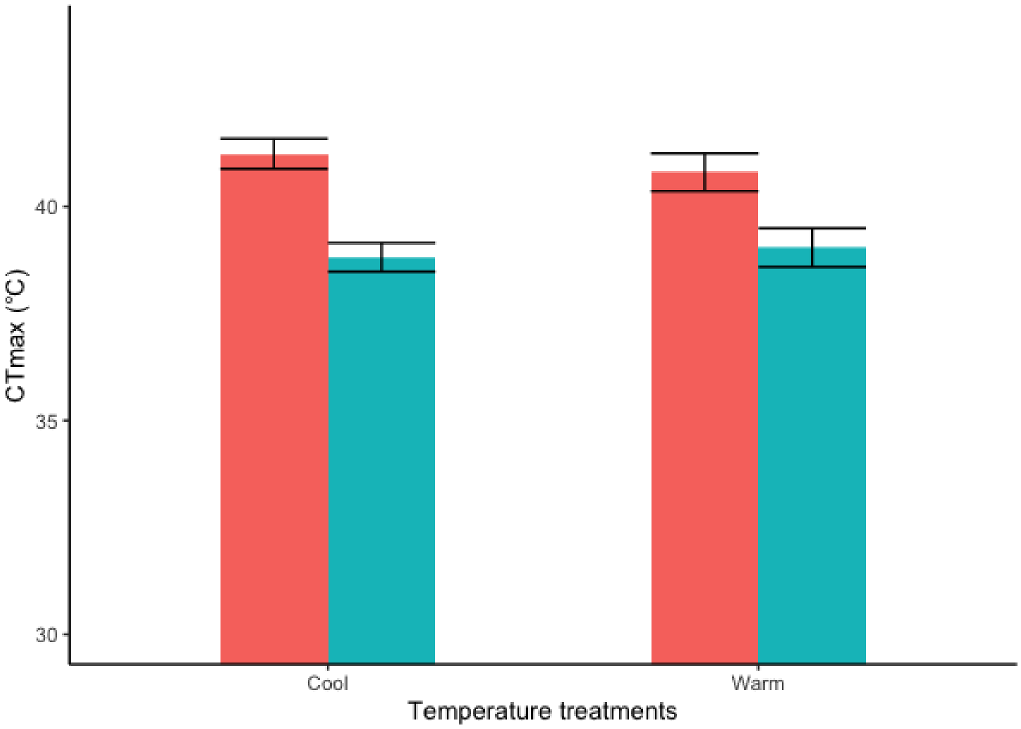

Figure 8*. Mean CTmax of* C. pictus *males from Northern (Red) and Southern (Blue) populations that were kept in warm and cool treatments over a 3-month period. Error bars represent ±1 SE. The northern population had higher Ctmax regardless of temperature treatment.*

2.4. Discussion

This study investigated metabolic and morphological differences between two populations of *C. pictus* from different thermal environments. Males from the hotter natal environment were larger in body size (mass and length), had higher metabolic rates at high temperatures, higher CTmax, basked more and invested more in reproduction (GSI) independent of body condition. The acclimation temperature treatment affected basking behaviour, and SVL growth, with lizards in the cool treatment growing in length considerably more than in the warm treatment. Thus, these results are suggestive of thermal adaptation within both populations of *C. pictus*. However, increased SVL growth (%) in the cool treatment contrasts with current evidence in ectothermic growth and instead highlighting the role of phenotypic plasticity in determining growth in the species.

2.4.1 Temperature clines in morphology

A negative temperature-body size cline is observed in many squamates (lizards and snakes) (Ashton & Feldman, 2003). Although there are various hypotheses to explain such phenomena, the maintenance of preferred body temperature is one possible explanation for negative temperature-body size clines in this species. Increases in the surface area to volume ratio of an organism allows for more rapid heating and cooling, generating selection for smaller body sizes in cooler climates for reptiles, which enables them to warm relatively quickly (Cowles 1945; Bogert 1949; Stevenson 1985). In warmer areas, where ambient heat is more readily available, selection on thermoregulatory abilities is lessened, allowing ectotherms to reach significantly larger sizes and attain the many benefits associated with it such as; advantages in intraspecific competition (Martin, Badiane, & Le Galliard, 2019), and investment in reproduction (Prado & Haddad, 2005). Temperature-body size clines may also explain the contrasting reproductive investment strategies between the populations in this study (Perrin, 1998).

Larger body size of the Northern *C. pictus* population may allow increased investment in reproduction, with GSI being three times higher than the Southern population. Additionally, GSI in the northern population was primarily independent from body condition, whilst in the Southern population, it was positively condition-dependent. Decreased testes size and condition-dependent investment in reproduction is indicative of a population experiencing a sub-optimal environment in terms of resource availability and thermal environment (M. Angilletta & Sears, 2000). A trade-off occurs between growth and reproduction and forces investment in reproduction to be a "luxury", which only individuals with high BCI may achieve effectively (Todd, 2008; Uller et al., 2010). Alternatively, this discrepancy in GSI may result from male-male competition in both populations. Higher GSI correlates to high population densities in which competition for females is fierce and larger testes and sperm competition can increase chances of competitive fertilisation success (Kahrl, Cox, & Cox, 2016). An example of this is testes size variation in the Cane toad invasion in Australia (*Rhinella marina*) (Friesen & Shine, 2019). As population densities in this species are lessened at the edge of the invasion front, males experience considerably less sperm competition and thus have smaller testes (Friesen & Shine, 2019). However, no published information on population densities and differences in resource availability is available for the two populations. Still, there is observational evidence that the northern population is less densely populated, potentially discrediting the population density GSI hypothesis (C.R. Friesen, unpublished observation). Without concrete evidence, inferences are limited and provide a future avenue of investigation. Regardless, these results demonstrate geographic variation in reproductive and somatic investment within this species.

2.4.2 Plasticity of growth

Surprisingly, in our study, SVL growth (%) was significantly higher for animals in the cool treatment, regardless of population. These results do not support our predictions that growth would increase as a function of temperature via increased metabolic activity (full review see (M. Angilletta et al., 2004)). However, several explanations could account for the temperature-dependent growth rate observed in this experiment. A strong relationship between annual growth and annual activity time is observed in a variety of lizard species (Adolph & Porter, 1993; Ferguson & Talent, 1993; Flouris & Piantoni, 2015). Therefore, during the cool treatment, lizards may bask more throughout the day and be considerably more active, as evidenced by the marked increase in basking. However, these results suggest another possible paradox: northern animals are larger, even though they originate from warmer environments. Given the positive correlation between SVL growth and $BCI_{initial}$ and the lizards' sexual maturity, it is plausible that most growth occurred before they reached the laboratory (Dumas & France, 2008). However, growth rates as a percentage are comparable between populations in captivity, so we suggest that growth rates may be significantly enhanced during late autumn or early spring due to increased temperatures and greater food availability for the Northern population (Metcalfe & Monaghan, 2003). As there were only two temperature treatments to compare, determining whether the cool treatment enhanced growth or the warm treatment suppressed growth is difficult. Therefore, for future studies, the addition of control temperature treatment, intermediate to the warm and cool treatments, would increase understanding of the relative effects of temperature on growth within this species (Atkinson, 1996). Therefore, these results suggest plastic growth in response to temperature and indicate potential underlying differences in energetics budgets between the populations.

2.4.3 Population-specific basking behaviour

A simple interpretation of the basking patterns is that the Northern population, due to increased ambient temperatures in the source population, engage in more thermophilic (Basking) behaviour than the Southern population. However, cooler conditions may force individuals to bask more (Carter et al., 2010) and, subsequently, alter growth rate (Adolph & Porter, 1993; Ferguson & Talent, 1993; Flouris & Piantoni, 2015). Underlying metabolic differences are a significant factor in driving in ectotherm basking behaviour (Stapley, 2006). Individuals and species with higher metabolic needs exhibit greater basking behaviour (Mell et al. 2016). In Ballen et al. (2012) Increased basking in *C. pictus* was associated with higher activity levels and, consequently, metabolic activity, potentially indicating that Northern populations exhibit higher metabolic activity. On the other hand, *C. pictus* individuals have also been known to seek shade in the heat of the day (Melville and Schulte II 2001), a behaviour congruent to that observed throughout the acclimation period in both the Southern population, and to a lesser extent the Northern population in our study.

2.4.5 Population-specific metabolism

Population differences in metabolic performance curves, as documented in this study, have been reported across a wide range of ectotherms (Bennett, 1982; Hu, Lu, Cheng, Luo, & Zeng, 2019; Noyola et al., 2013; Sears, 2005). In many species, population differences in msRMR are believed to be adaptive, shifting the population-specific Topt to suit the environment (Schulte et al., 2011). Shifts in Topt from thermal acclimation result in increased efficiency in utilising environmental heat, decreasing the time required to attain body temperature via thermoregulation and weakening selection on thermoregulatory traits (M. Angilletta et al., 2004). Improved efficiency results in increased energy availability, which can increase foraging time (Norberg, 1977), reproductive investment (M. Angilletta & Sears, 2000) and somatic investment (Lester, Shuter, & Abrams, 2004), all of which can significantly enhance organismal fitness. A higher metabolic rate comes at a trade-off. When food resources are scarce, an increased RMR becomes a significant burden and can lead to individuals starving and, thus, large population diebacks may occur without sufficient phenotypic flexibility in RMR (Metcalfe & Monaghan, 2003). Additionally, the physiological costs of enhanced metabolic activity, such as increasing reactive oxygen species production, may be a trade-off experienced by the Northern population and thus warrants future investigation (Smith, Nager, & Costantini, 2016). Our proposed shift of Topt is further supported via the Northern populations higher CTmax, potentially indicating the Northern population has an increased ability to deal with high temperatures (Addo-Bediako, Chown, & Gaston, 2000). Unfortunately, in the current experiment, temperatures during metabolic measurements were not raised high enough (> 40 °C) to report population changes in Topt accurately, which would be indicated by a significant drop in RMR with increased temperature (M. J. Angilletta, 2009) as we began to see in the Southern population. However, the Northern Population is likely has a higher Topt as the Southern population's thermal performance curve flattens considerably after 30 degrees whilst increases can be seen in the Northern population. Therefore, we suggest that population-specific msRMR and thermal adaptations occur in *C. pictus* in response to contrasting thermal environments and energetic requirements.

RMR is relatively understudied in polymorphic species. In polymorphic cichlids, the most dominant morph had the lowest metabolic rate, attributed to increased metabolic efficiency (Dijkstra, Pierotti, Seehausen, & Metcalfe, 2016). Morph-specific metabolism documented in another population of *C. pictus*, with temperature regimes intermediate to the two populations used in this study, was not found in this study. In Friesen et al. (2017) males with bibs had a significantly higher metabolic rate at 36 °C, suggesting underlying differences in the behavioural strategies' energetic requirements. However, in this study, Bibbed males of both populations have similar msRMR to non-Bibbed males. Bibs are much more prevalent in the Southern population, and msRMR is lower, making Bib and Population confounded, potentially leading to type II errors. Thus, for future studies, and even selection of bibbed and non-bibbed males per population and greater sample size would be beneficial to increase statistical power.

The acclimation treatment had minimal impact on msRMR in both populations. Although the measurement of msRMR can correlate with metabolic capacity, it is not entirely reflective (Gomes, Guilherme Chauí-Berlinck, Bicudo, & Navas, 2004). Resting MR is a measure of the 'cost of living' (Hulbert & Else, 2000) and thus may be unsuited to capture acclimation responses, as selection should minimise RMR and maximise metabolic capacity. Hence, although informative, it may be inaccurate to use RMR as a measure of acclimation or adaptive response to environmental change (Rogers, Thompson, & Seebacher, 2007). Alternatively, combining mitochondrial oxygen metabolism measures with the measurement of metabolic enzymes such as lactate dehydrogenase, cytochrome c, and citrate synthase across a range of temperatures may provide a deeper understanding of the thermal effects of acclimation on metabolism (Rogers et al., 2007). Enhanced population-specific acclimation responses would allow *C. pictus* to achieve greater reproductive success in thermally variable environments, thereby expanding its ecological niche (Kearney & Porter, 2004). Based on our results, *C. pictus* may have limited phenotypic plasticity to alter msRMR post-maturity; therefore, msRMR may be determined by ontogeny or be a heritable trait. However, a common garden experiment is required to fully test this hypothesis.

The Northern population, sourced in significantly warmer conditions, demonstrated increased metabolic function compared to the cool-adapted southern population, indicative of thermal adaptation. These thermal adaptations may provide increase metabolic scope for early developmental somatic growth and enable the Northern population to attain significantly larger sizes and invest more in reproduction. However, post-maturity growth measured in the lab did not differ between populations and was instead plastic to temperature treatments with individuals growing considerably more in the cool treatment, possibly due to increased activity. Therefore, natal thermal environment, thermal adaptation and phenotypic plasticity all play a critical role in determining variation of life-history traits within *C. pictus*.

References

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 267*(1445), 739-745.

Adolph, S. C., & Porter, W. P. (1993). Temperature, activity, and lizard life histories. *The American Naturalist, 142*(2), 273-295.

Angilletta, M., & Sears, M. (2000). The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology, 14*(1), 39-45.

Angilletta, M., Steury, T. D., & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and comparative biology, 44*(6), 498-509.

Angilletta, M. J. (2009). *Thermal adaptation: a theoretical and empirical synthesis*: Oxford University Press.

Ashton, K. G., & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution, 57*(5), 1151-1163.

Atkinson, D. (1996). Ectotherm life-history responses to developmental temperature. *Animals and temperature: phenotypic and evolutionary adaptation, 1996*, 183-204.

Ballen, C., Healey, M., Wilson, M., Tobler, M., Wapstra, E., & Olsson, M. (2012). Net superoxide levels: steeper increase with activity in cooler female and hotter male lizards. *Journal of Experimental Biology, 215*(5), 731-735. doi: 10.1242/jeb.062257

Barton, K., & Barton, M. K. (2015). Package 'MuMIn'. *Version, 1*, 18.

Bates, D., Sarkar, D., Bates, M. D., & Matrix, L. (2007). The lme4 package. *R package version, 2*(1), 74.

Bennett, A. F. (1982). The energetics of reptilian activity. *Biology of the Reptilia, 13*, 155-199.

Burton, T., Killen, S., Armstrong, J., & Metcalfe, N. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences, 278*(1724), 3465-3473.

Cano, J. M., & Nicieza, A. (2006). Temperature, metabolic rate, and constraints on locomotor performance in ectotherm vertebrates. *Functional Ecology*, 464-470.

Chandan, P., Bhattacharya, T., Rajkumar, U., Prince, L., & Chatterjee, R. (2019). Estimation of genetic parameters of growth and egg production traits by animal model in IWK layer strain. *Indian Journal of Animal Research, 53*(9), 1252-1257.

Chiari, Y., Glaberman, S., Tarroso, P., Caccone, A., & Claude, J. (2016). Ecological and evolutionary influences on body size and shape in the Galápagos marine iguana (Amblyrhynchus cristatus). *Oecologia, 181*(3), 885-894.

Cogger, H. (2014). *Reptiles and amphibians of Australia*: Csiro Publishing.

Cox, R. M., Parker, E. U., Cheney, D. M., Liebl, A. L., Martin, L. B., & Calsbeek, R. (2010). Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. *Functional Ecology, 24*(6), 1262-1269.

Dijkstra, P. D., Pierotti, M. E., Seehausen, O., & Metcalfe, N. B. (2016). Metabolism, oxidative stress and territorial behaviour in a female colour polymorphic cichlid fish. *Behavioral ecology and sociobiology, 70*(1), 99-109.

Dumas, A., & France, J. (2008). Modelling the ontogeny of ectotherms exhibiting indeterminate growth. *Journal of theoretical biology, 254*(1), 76-81.

Ferguson, G. W., & Talent, L. G. (1993). Life-history traits of the lizard Sceloporus undulatus from two populations raised in a common laboratory environment. *Oecologia, 93*(1), 88-94.

Flouris, A. D., & Piantoni, C. (2015). Links between thermoregulation and aging in endotherms and ectotherms. *Temperature, 2*(1), 73-85.

Flukes, E. B., Wright, J. T., & Johnson, C. R. (2015). Phenotypic plasticity and biogeographic variation in physiology of habitat-forming seaweed: response to temperature and nitrate. *Journal of phycology, 51*(5), 896-909.

Friesen, C. R., Johansson, R., & Olsson, M. (2017). Morph-specific metabolic rate and the timing of reproductive senescence in a color polymorphic dragon. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 327*(7), 433-443. doi: 10.1002/jez.2118

Friesen, C. R., Powers, D. R., Copenhaver, P. E., & Mason, R. T. (2015). Size dependence in non-sperm ejaculate production is reflected in daily energy expenditure and resting metabolic rate. *Journal of Experimental Biology, 218*(9), 1410-1418.

Friesen, C. R., & Shine, R. (2019). At the invasion front, male cane toads (Rhinella marina) have smaller testes. *Biology letters, 15*(7), 20190339.

Gomes, F. R., Guilherme Chauí-Berlinck, J., Bicudo, J. E. P., & Navas, C. A. (2004). Intraspecific relationships between resting and activity metabolism in anuran amphibians: influence of ecology and behavior. *Physiological and Biochemical Zoology, 77*(2), 197-208.

Grafen, A. (1990). Biological signals as handicaps. *Journal of theoretical biology, 144*(4), 517-546.

Healey, M., & Olsson, M. (2009). Too big for his boots: Are social costs keeping condition-dependent status signalling honest in an Australian lizard? *Austral Ecology, 34*(6), 636-640. doi: 10.1111/j.1442-9993.2009.01968.x

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 483-492.

Hu, Y.-C., Lu, H.-L., Cheng, K.-M., Luo, L.-G., & Zeng, Z.-G. (2019). Thermal dependence of feeding performance and resting metabolic expenditure in different altitudinal populations of toad-headed lizards. *Journal of thermal biology, 80*, 16-20.

Huey, R. B., & Kingsolver, J. G. (2019). Climate warming, resource availability, and the metabolic meltdown of ectotherms. *The American Naturalist, 194*(6), E140-E150.

Hulbert, A., & Else, P. L. (2000). Mechanisms underlying the cost of living in animals. *Annual review of physiology, 62*(1), 207-235.

Ibargüengoytía, N., & Boretto, J. (2006). Asynchronous spermatogenesis and biennial female cycle of the viviparous lizard Phymaturus antofagastensis (Liolaemidae): reproductive responses to high altitudes and temperate climate of Catamarca, Argentina. *Amphibia-Reptilia, 27*(1), 25-36.

Johnsson, J., Nöbbelin, F., & Bohlin, T. (1999). Territorial competition among wild brown trout fry: effects of ownership and body size. *Journal of Fish Biology, 54*(2), 469-472.

Kahrl, A. F., Cox, C. L., & Cox, R. M. (2016). Correlated evolution between targets of pre- and postcopulatory sexual selection across squamate reptiles. *Ecology and evolution, 6*(18), 6452-6459.

Kearney, M., & Porter, W. P. (2004). Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology, 85*(11), 3119-3131.

Konishi, S., & Kitagawa, G. (2008). *Information criteria and statistical modeling*: Springer Science & Business Media.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software, 82*(13), 1-26.

Labocha, M. K., Schutz, H., & Hayes, J. P. (2014). Which body condition index is best? *Oikos, 123*(1), 111-119.

Lenth, R., & Lenth, M. R. (2018). Package 'lsmeans'. *The American Statistician, 34*(4), 216-221.

Lester, N., Shuter, B., & Abrams, P. (2004). Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(1548), 1625-1631.

Lighton, J. R. (2018). *Measuring metabolic rates: a manual for scientists*: Oxford University Press.

Lüpold, S., de Boer, R. A., Evans, J. P., Tomkins, J. L., & Fitzpatrick, J. L. (2020). How sperm competition shapes the evolution of testes and sperm: a meta-analysis. *Philosophical Transactions of the Royal Society B, 375*(1813), 20200064.

Marshall, D. J., & McQuaid, C. D. (2011). Warming reduces metabolic rate in marine snails: adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. *Proceedings of the Royal Society B: Biological Sciences, 278*(1703), 281-288.

Martin, M., Badiane, A., & Le Galliard, J.-F. (2019). The relative importance of body size and UV coloration in influencing male-male competition in a lacertid lizard. *Behavioral Ecology and Sociobiology, 73*(7), 98.

McDiarmid, C. S., Friesen, C. R., Ballen, C., & Olsson, M. (2017). Sexual coloration and sperm performance in the Australian painted dragon lizard, *Ctenophorus pictus*. *Journal of Evolutionary Biology, 30*(7), 1303-1312. doi: 10.1111/jeb.13092

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in ecology & evolution, 21*(4), 178-185.

Metcalfe, N. B., & Monaghan, P. (2003). Growth versus lifespan: perspectives from evolutionary ecology. *Experimental gerontology, 38*(9), 935-940.

Mitchell, J. C. (1988). Population ecology and life histories of the freshwater turtles Chrysemys picta and Sternotherus odoratus in an urban lake. *Herpetological Monographs*, 40-61.

Norberg, R. A. (1977). An ecological theory on foraging time and energetics and choice of optimal food-searching method. *The Journal of Animal Ecology*, 511-529.

Noyola, J., Caamal-Monsreal, C., Díaz, F., Re, D., Sanchez, A., & Rosas, C. (2013). Thermopreference, tolerance and metabolic rate of early stages juvenile Octopus maya acclimated to different temperatures. *Journal of thermal biology, 38*(1), 14-19.

Olsson, M., Healey, M., Wapstra, E., Schwartz, T., Lebas, N., & Uller, T. (2007). Mating system variation and morph fluctuations in a polymorphic lizard. *Molecular Ecology, 16*(24), 5307-5315.

Perrin, N. (1998). On Body Size, and Energy and Fitness: JSTOR.

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, 132*(4), 739-761. doi: 10.1016/s1095-6433(02)00045-4

Prado, C., & Haddad, C. F. (2005). Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, south-western Brazil. *The Herpetological Journal, 15*(3), 181-189.

Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in Ecology & Evolution, 17*(10), 462-468.

Roff, D. (1993). *Evolution of life histories: theory and analysis*: Springer Science & Business Media.

Rogers, K. D., Thompson, M. B., & Seebacher, F. (2007). Beneficial acclimation: sex specific thermal acclimation of metabolic capacity in the striped marsh frog (Limnodynastes peronii). *Journal of Experimental Biology, 210*(16), 2932-2938.

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and comparative biology, 51*(5), 691-702.

Sears, M. W. (2005). Resting metabolic expenditure as a potential source of variation in growth rates of the sagebrush lizard. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140*(2), 171-177.

Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *Journal of Comparative Physiology B, 175*(7), 453-461. doi: 10.1007/s00360-005-0010-6

Seebacher, F., & Franklin, C. E. (2011). Physiology of invasion: cane toads are constrained by thermal effects on physiological mechanisms that support locomotor performance. *Journal of Experimental Biology, 214*(9), 1437-1444.

Servedio, M. R., & Boughman, J. W. (2017). The role of sexual selection in local adaptation and speciation. *Annual Review of Ecology, Evolution, and Systematics, 48*, 85-109.

Sibly, R., & Atkinson, D. (1994). How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology*, 486-493.

Smith, S. M., Nager, R. G., & Costantini, D. (2016). Meta-analysis indicates that oxidative stress is both a constraint on and a cost of growth. *Ecology and Evolution, 6*(9), 2833-2842.

Stapley, J. (2006). Individual variation in preferred body temperature covaries with social behaviours and colour in male lizards. *Journal of Thermal Biology, 31*(4), 362-369.

Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., . . . Sun, B. j. (2020). The thermal ecology and physiology of reptiles and amphibians: A user's guide. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*.

Todd, A. C. (2008). Using testis size to predict the mating systems of New Zealand geckos. *New Zealand Journal of Zoology, 35*(2), 103-114.

Uller, T., Stuart-Fox, D., & Olsson, M. (2010). Evolution of primary sexual characters in reptiles. In J. L. Leonard & A. Córdoba-Aguilar (Eds.), *Evolution of Primary Sexual Characters in Animals* (pp. 425-452). Oxford: Oxford University Press.

Urbanski, J., Mogi, M., O'Donnell, D., DeCotiis, M., Toma, T., & Armbruster, P. (2012). Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. *The American Naturalist, 179*(4), 490-500.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*: Springer.

Wikelski, M. (2005). Evolution of body size in Galapagos marine iguanas. *Proceedings of the Royal Society B: Biological Sciences, 272*(1576), 1985-1993.

Wikelski, M., & Thom, C. (2000). Marine iguanas shrink to survive El Niño. *Nature, 403*(6765), 37-38.

Winwood-Smith, H. S., Alton, L. A., Franklin, C. E., & White, C. R. (2015). Does greater thermal plasticity facilitate range expansion of an invasive terrestrial anuran into higher latitudes? *Conservation physiology, 3*(1).