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Transcriptomics at the thermal limits of an urban introduced lizard

Samantha S. Fontaine *, Brian K. Trevelline

Department of Biological Sciences, Kent State University, 800 E. Summit St, Kent, OH, 44242, USA

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A B S T R A C T

Rapid changes in gene expression can result in physiological plasticity that assists animals in coping with environmental stressors. Increased capacity for physiological plasticity may then facilitate adaptation to stressful habitats like urban heat islands or invasion into novel ranges. Currently, temperature stress is a leading threat to organisms, especially ectotherms. While exposure to changing temperatures is known to shift gene expression patterns in ectothermic animals, many studies are conducted after lengthy acclimation times. However, exposure to thermal stress in nature can occur rapidly. We assessed the capacity for gene expression plasticity in response to a brief exposure to extreme thermal stress in an urban, introduced species, the common wall lizard (*Podarcis muralis*). Lizards were ramped to their critical thermal maximum (CT_{max}) or minimum (CT_{min}) followed by rapid recovery. We used RNA-sequencing to compare the transcriptomes of lizards exposed to CT_{max}, CT_{min}, or control conditions using heart, liver, and large intestine tissue. Exposure to heat stress induced a much stronger gene expression response across tissues than cold exposure. In response to heat, there was systemic upregulation of heat shock proteins and stress response pathways. Heat also induced changes in transcription, translation, and metabolic processes but these effects were more tissue specific. Although fewer gene expression changes were observed in response to cold, some genes were upregulated that could be beneficial under cooling stress. Our data suggests gene expression plasticity could facilitate range expansion in this species, but more work is needed to assess the transcriptomic response to temperature stress in nature.

1. Introduction

Gene expression change is a fundamental mechanism by which species acclimate and adapt to changing environmental conditions (Schulte, 2004; López-Maury et al., 2008). Changes in gene expression patterns often underlie organismal physiological plasticity—which occurs when different physiological phenotypes are expressed under different environments—and can facilitate tolerance to environmental stressors (Rivera et al., 2021). The capacity for plasticity in gene expression can therefore allow species to exploit otherwise extreme environments, such as urban heat islands (Campbell-Staton et al., 2020), toxic habitats (Greenway et al., 2020), or drought conditions (Wehner et al., 2016). Plasticity in gene expression may be particularly important for the ability to colonize new locations (Morris et al., 2014) and drive the range expansion of invasive species (Zerebecki and Sorte, 2011; Yagound et al., 2022).

Due to climate change, temperature stress is one of the most challenging environmental variables animals currently face, especially ectothermic species which cannot regulate their body temperature physiologically (Paaijmans et al., 2013). Climate change has resulted in the decline of ectothermic species worldwide (Sinervo et al., 2010; Wagner, 2020; Luedtke et al., 2023). These losses are expected to escalate dramatically this century due to the increasing intensity and frequency of heatwaves and daily temperature fluctuations (Jørgensen et al., 2022). Physiological plasticity underlain by changes in gene expression can allow animals to cope with fluctuating temperatures over short time scales (Seebacher et al., 2015), and may facilitate evolutionary adaptation of populations in response to thermal stress (Snell-Rood et al., 2018; Campbell-Staton et al., 2020; Vinton et al., 2022). Furthermore, if organisms can respond to thermal stress physiologically, they may be more successful in the face of climate change (Somero, 2010) because other mechanisms to cope with temperature stress like behavioral thermoregulation can come at severe costs, such as limiting the time available for critical activities like foraging and reproduction (Kearney et al., 2009; Gunderson and Leal, 2016; Doucette et al., 2023; Perry et al., 2025). When testing the impact of temperature change on ectotherm gene expression across the whole transcriptome, most experimental designs expose organisms to thermal stress for

* Corresponding author.
E-mail address: sfontai1@kent.edu (S.S. Fontaine).

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periods of time ranging from hours (Hu et al., 2016; Jesus et al., 2016; Etges et al., 2017; Fontaine and Kohl, 2023; Rosso et al., 2024; Wuthrich et al., 2025), to days (Narum and Campbell, 2015; Akashi et al., 2016; Lim et al., 2016; Zhou et al., 2023; Li et al., 2024), weeks (Smith et al., 2013; Coughlin et al., 2019; Wellenreuther et al., 2019), and even months (Kim et al., 2017). Very few studies have tested the response of ectotherm transcriptomes to extremely rapid exposures (e.g., seconds to minutes) to acutely stressful temperatures, such as the critical thermal limits (Lancaster et al., 2016; Sørensen et al., 2016; Pimsler et al., 2020). However, understanding physiological responses to these critical temperatures is important because exposure to temperatures beyond the thermal limits is becoming more common with climate change (Dufour et al., 2024), and even when rare, exposure to thermal extremes can drive the evolution of thermal physiology traits in ectotherms (Buckley and Huey, 2016).

Previous studies demonstrate some consistent gene expression responses to temperature change. Exposure to temperature stress nearly ubiquitously activates the expression of heat shock proteins (HSPs) in ectotherms (Fangue et al., 2006; Clark et al., 2008; Sørensen et al., 2019; Logan and Cox, 2020; Azambuja et al., 2021; Wuthrich et al., 2025). HSPs are highly conserved and act as molecular chaperones to prevent or repair protein denaturation after exposure to a variety of acute stressors including toxins, dehydration, and high or low temperatures (Feder and Hofmann, 1999; Kregel, 2002). In addition to widespread induction of the HSP response, temperature change often impacts the expression of genes associated with transcription, translation, and metabolic processes (Logan and Cox, 2020). Further, the gene expression response to temperature may differ across organs and tissues in both the magnitude of change and in the functions affected. For example, more genes are differentially expressed in the brain compared to other tissues in lizards exposed to warming (Akashi et al., 2016; Rosso et al., 2024; Wuthrich et al., 2025). Reflecting organ function, heat stress impacts processes related to oxygen demand in gills (Zhang et al., 2023), and nutrient metabolism in the gut (Fontaine and Kohl, 2023). Lastly, the transcriptomic response to heat in ectotherms tends to be more pronounced than the shifts that occur in response to cold (Lancaster et al., 2016; Mallard et al., 2020; Pimsler et al., 2020; Rosso et al., 2024). These plastic responses may provide protection to species under further thermal stress. For example, previous exposure to heat stress experimentally increases survival under additional heat shock (Arias et al., 2012; Moghadam et al., 2019), and incorporating physiological plasticity into modeling approaches reduces the predicted risk of overheating for a variety of organisms (Gunderson et al., 2017; Morley et al., 2019). Interestingly, even the physiological plasticity that occurs in response to cold can protect organisms under future heat stress, largely through the induction of HSPs (Sejerkilde et al., 2003). However, save for few studies (Lancaster et al., 2016; Sørensen et al., 2016; Pimsler et al., 2020) it is not clear how very brief exposures to thermal extremes impact gene expression in ectothermic animals, especially vertebrates (but see (Dammark et al., 2018)).

Here, we tested the sensitivity of the whole transcriptome to a brief exposure to highly stressful temperatures (the critical thermal maximum, CT_{max} or critical thermal minimum, CT_{min}) in an urban, introduced population of the common wall lizard (*Podarcis muralis*) in Cincinnati, OH. Common wall lizards are heliothermic, lacertid lizards native to central and southern Europe (Michaelides et al., 2015). This species is also a successful invasive with introduced populations in the United Kingdom (Williams et al., 2021), Germany (Heym et al., 2013), British Columbia, Canada (Engelstoft et al., 2020), and Cincinnati, Ohio in the US. The Cincinnati population was established in the 1950s after a very small number of individuals (~10) were released (Brown et al., 1995). The population is now thriving with estimates in the hundreds of thousands of individuals (Amer et al., 2024). Common wall lizards inhabit stone walls throughout the city (Mackey, 2010) which are similar to those found in their native habitats (Heym et al., 2013). Common wall lizards of Cincinnati are an ideal model to test questions related to the capacity for rapid plasticity in gene expression after exposure to the thermal limits. Cincinnati experiences an urban heat island effect and extreme heat occurrences are increasing in magnitude and frequency with thermal patterns that differ from surrounding areas (Morgan, 2021), suggesting lizard populations will be challenged by thermal variation as they continue to expand. Theory predicts that this type of variation at range edges can promote the evolution of plasticity during range expansion (Usui et al., 2023). As common wall lizard populations continue to expand their range in the Cincinnati area (Bode, 2025), they have demonstrated a remarkable ability to rapidly adapt in response to local conditions in several morphological phenotypes (Wyatt, 2013; Vaughn et al., 2021; Gangloff et al., 2025). Additionally, in response to the thermal environment, populations further from the introduction point demonstrate wider thermal tolerance breadths (Litmer and Murray, 2019), suggesting thermal flexibility may be a key factor facilitating population spread.

2. Methods

2.1. Lizard field collections and laboratory husbandry

We captured adult, gravid, female common wall lizards (N = 26) from urban stone wall habitats in Cincinnati, Ohio from April–June 2024 using a thread lasso attached to an extendable fishing pole. We focused on gravid females to obtain eggs for a separate experiment. We placed lizards individually into breathable, nylon cloth sacks which we then placed into a cooler for transport to the laboratory. In the laboratory, lizards were housed individually in plastic rat cages (18″ L x 9.25″ W x 6.5” H) affixed with a mesh lid. We placed a 40W incandescent heat bulb in a 5.5-inch dome fixture above one end of the cage to maintain a temperature gradient from ~25 to 40 °C within cages to allow for behavioral thermoregulation. Additionally, we placed a 39W 10.0 UVB light tube horizontally across cages to provide a source of UV light. UV and ambient light were provided on a 14hr: 10hr daily light: dark cycle. Heat bulbs were turned on for a total of 6 h per day in alternating 1 h on/1 h off intervals during the diurnal light cycle. Inside cages, we provided lizards with a basking platform doubling as a shelter underneath the warm end of the cage, a Petri dish filled with distilled water, and a nest box for egg laying. For nest boxes, we used the bottom of a pipette tip box filled with sterilized play sand kept moist with distilled water. We fed lizards every other day a diet of crickets and mealworms dusted with a reptile vitamin supplement *ad libitum*. Every day, we replenished lizard water dishes, misted their cage with a spray bottle, and checked their nest boxes for egg laying.

2.2. Measurement of lizard thermal tolerance limits and dissections

After lizards laid eggs (although of 26 animals, 6 did not ever lay eggs), we randomly assigned them to one of three treatment groups for the measurement of critical thermal limits: critical thermal maximum (CT_{max}, N = 10), critical thermal minimum (CT_{min}, N = 10), or a control treatment (N = 6). All lizards were fasted for 24 h before measurements to ensure digestion would not interfere with thermal physiology (Tosini et al., 1994).

For a general overview of our experimental design, see Fig. 1A. We measured lizard critical thermal limits using Hutchison's dynamic method (Lutterschmidt and Hutchison, 1997). To measure CT_{max}, lizards were placed into a plastic 64 oz Tupperware container (8.64″ L x 6.44″ W x 3.94″ H) without a lid and acclimated to the container at room temperature for 15 min. We then turned on a 100W incandescent heat bulb in a 5.5-inch dome fixture suspended 30 cm above the container. The bulb increased temperature in the container at 1 °C/min intervals and temperature was monitored throughout the trial using a wire thermocouple probe taped to the bottom of the container attached to a Traceable Type K thermometer (Fisher). Slight adjustments were made to the bulb height to maintain an accurate heating rate if needed. Beginning

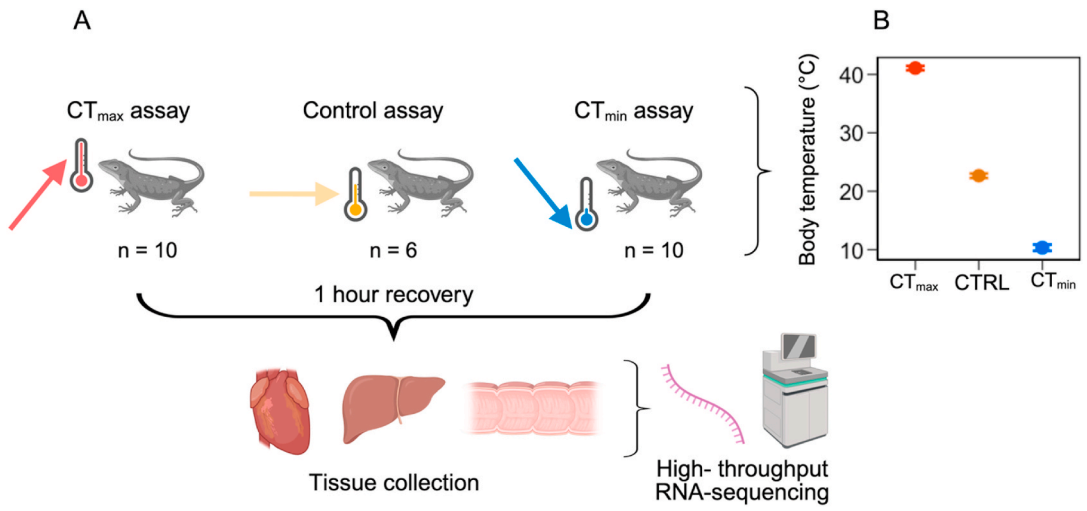

Fig. 1. (A) Schematic of experimental design and (B) lizard body temperature at the end of the thermal tolerance assay in CT_{max}, control, and CT_{min} groups (mean ± s.e.m.). Figure created with BioRender.com.

when the temperature in the container reached 30 °C, we flipped lizards onto their backs once per minute to assess their righting response. When lizards could not right themselves within 5 s and did not respond to light prodding, we immediately removed them from the trial and measured their body temperature as CT_{max} by inserting a wire thermocouple probe attached to a second Traceable Type K thermometer into their cloaca. We then dipped the animal up to their neck in room temperature water to allow their body temperature to cool rapidly (Phillips et al., 2016). The lizard was then placed into a dry empty Tupperware container and held at room temperature for recovery.

We measured CT_{min} in a similar manner. Lizards were placed into a plastic 64 oz Tupperware container (8.64″ L x 6.44″ W x 3.94” H) that was partially submerged in a water bath. One edge of the container was clamped to the side of the water bath, and the other edge was secured in a horizontal position to the outside of the water bath using duct tape such that the bottom section of the container was held below the surface of the water, but no water was inside the container. We affixed the lid, which contained air holes, to the top of the container using tape. A wire thermocouple probe attached to a Traceable Type K thermometer was threaded through an airhole and taped to the bottom of the container to monitor temperature in the container throughout the trial. We allowed lizards to acclimate to the container at room temperature for 15 min. Once the trial began, ice was added to the water bath using a beaker every minute to maintain a constant cooling rate inside the container of 1 °C/min. We varied the amount of ice added as needed to maintain the proper cooling rate, and bailed water out of the bath using a beaker to maintain a constant water level. Once the temperature in the container reached 15 °C, we flipped lizards onto their backs once per minute to assess their righting response. When lizards could not right themselves within 5 s and did not respond to light prodding, we immediately removed them from the trial and measured their body temperature as CT_{min} by inserting a wire thermocouple probe attached to a second Traceable Type K thermometer into their cloaca. The lizard was then placed into a dry empty Tupperware container and held at room temperature for recovery.

In control lizards, we simulated a thermal tolerance assay to account for handling stress, but these animals were not exposed to thermal variation. These lizards were placed in a plastic 64 oz Tupperware container (8.64″ L x 6.44″ W x 3.94″ H) with a wire thermocouple probe attached to a Traceable Type K thermometer taped to the bottom of the container. They were acclimated to the container for 15 min and then observed for an additional 15 min without handling to mimic the amount of time CT_{max} or CT_{min} lizards were maintained before assessment of the righting response. Then, lizards were flipped onto their backs once per minute for the next 15 min. We inserted a wire thermocouple probe attached to a second Traceable Type K thermometer into their cloaca to measure body temperature at the conclusion of the assay. We then placed the lizard into a dry empty Tupperware container at room temperature for recovery. Across all lizards in the experiment, we were able to reliably insert the temperature probe and measure body temperature within 10 s of the conclusion of the assay. We expect any changes to the lizard’s body temperature due to handling to be minimal in that timeframe.

Regardless of trial type, because $CT_{min/max}$ assays are necessarily non-lethal, all lizards were allowed to recover at room temperature for 1 h to assess survival (Raby et al., 2025). All animals successfully recovered. After recovery, we measured each lizard’s mass (g) and snout-vent length (SVL; mm). We then euthanized lizards via rapid decapitation followed by double pithing and dissected them to remove tissue from the heart, liver, and large intestine. These organs were selected for analysis because they are energetically expensive (Aiello and Wheeler, 1995) and have roles in ectotherm thermal tolerance (Pörtner et al., 2017; Fontaine and Kohl, 2023; Zhang et al., 2024). Thus, we hypothesized lizards may strongly regulate gene expression in these organs under thermal stress to protect their functions. The entire heart was removed and weighed (mg) and a 30 mg sample of the proximal portion of the liver and emptied large intestine were removed. These samples were placed in 600 μl of DNA/RNA shield (Zymo Research), frozen immediately on dry ice, and stored long term at −80 °C. We also noted during dissection if lizards were gravid and if so, the broad stage of egg development (early, mid, or late). Because lizards were in varying stages of gravidity and had been in the laboratory for differing amounts of days since field capture and egg laying, we tested for impacts of these variables on CT_{max} and CT_{min} using ANOVAs or linear regression in R (version 3.6.3). We additionally tested for effects of body mass or SVL on CT_{max} and CT_{min} using linear regression in R. For each variable, we verified normality using Shapiro-Wilk tests.

2.3. RNA extraction, sequencing, and transcriptomic analysis

We extracted RNA from heart, liver, and large intestine tissue samples using a QIAGEN RNeasy Mini Kit following the manufacturer’s protocol for purification of total RNA from animal tissues omitting the optional on-column DNase digestion step. At step 3, we homogenized the tissue in the 600 μl of DNA/RNA shield that the sample was stored in (to prevent losses of RNA) with 350 μl buffer RLT using an Omni International Bead Ruptor Elite at 6.00 m/s for 30 s. Extracted RNA was quantified using a BioTek Microplate reader and stored at −80 °C. We

sent samples of total RNA to Novogene (Sacramento, CA) for library preparation and transcriptomic sequencing. Briefly, mRNA was purified using poly A enrichment followed by cDNA library synthesis and adapter ligation. Purified and quantified libraries were then sequenced on the Illumina NovaSeq X Plus platform to generate paired-end 150 bp reads.

We used TrimGalore version 0.6.6 (Krueger, 2015) to remove adaptor sequences and filter raw reads for quality. We mapped reads to an available *Podarcis muralis* reference genome (Andrade et al., 2019) using bwa (Li and Durbin, 2009). For all samples, >97 % of reads were successfully mapped to the reference genome. We used StringTie version 3.0.0 for transcript assembly and to create a matrix of read counts per gene across samples (Pertea et al., 2015). We subset the matrix by tissue type and for each tissue we removed any genes from analysis that had an abundance of less than 2 counts per million or were present in less than 3 samples. In R, we used edgeR (Robinson et al., 2010) to identify genes for each tissue type that were differentially expressed between samples in three treatment comparisons: CT_{max} vs. CT_{min}, CT_{max} vs. control, and CT_{min} vs. control. P-values were corrected using the Benjamini–Hochberg false discovery rate (BH-FDR).

Next, we performed a gene ontology enrichment analysis to identify biological processes that were enriched in either up or downregulated gene sets across the three treatment group comparisons for each tissue. We compared the differentially expressed genes to a background set of all expressed genes for the respective tissue type. Prior to the analysis, we assigned gene names to any genes in the full dataset that were previously unannotated, if possible. Specifically, we used the blastx function in DIAMOND version 2.0.9 (Buchfink et al., 2021) to blast unannotated sequences in our dataset against NCBI's swissprot protein database. We set k to 1 to extract only the single best result per sequence with the e value set to the default of 0.001. Overall, we were able to assign 82 % of unannotated genes to a protein ID using this method. We then used the UniProt online ID mapping tool to assign a gene name to the protein IDs which was successful for 97 % of IDs. If an unannotated gene was successfully matched to a gene ID, we used this ID for enrichment analysis but retained any unmatched genes in the analysis. To perform the enrichment analysis, we used GOrilla (Eden et al., 2009) with BH-FDR corrected p-values.

Lastly, we performed a weighted gene co-expression network analysis using the R package WGCNA (Langfelder and Horvath, 2008) to identify modules of co-expressed genes within each tissue type and to identify modules with expression patterns correlated with treatment. Prior to analysis, we normalized read count data using DESeq (Love et al., 2014). After identifying co-expression modules for each tissue, we identified correlations between any modules and treatment by using the 'module.trait.correlation' function in WGCNA based on body temperature at the end of the thermal tolerance or control assay (which captures treatment effects). We used GOrilla as previously described to identify biological processes enriched in any modules associated with treatment for each tissue type. We used the 'chooseTopHubInEachModule' function to identify a top hub gene for modules associated with treatment. This gene is the one in each module with the highest intramodular connectivity (associations with other genes in the module).

3. Results

3.1. Thermal tolerance assays

Mean body temperature (±s.e.m.) at the conclusion of the thermal tolerance assay was 41.08 ± 0.37 °C for CT_{max} lizards, 22.67 ± 0.39 °C for control lizards, and 10.34 ± 0.54 °C for CT_{min} lizards (Fig. 1B). CT_{max} and CT_{min} were not impacted by body mass ($p = 0.27$ for CT_{max} and 0.93 for CT_{min} lizards), SVL ($p = 0.39$ for CT_{max} and 0.97 for CT_{min} lizards), gravidity stage ($p = 0.97$ for CT_{max} and 0.36 for CT_{min} lizards), number of days since egg laying ($p = 0.97$ for CT_{max} and 0.85 for CT_{min} lizards), or number of days since field capture ($p = 0.26$ for CT_{max} and 0.32 for CT_{min} lizards).

3.2. Transcriptomics

After filtering, we retained an average of 73,053,276 reads per sample across 13,952 transcripts in the heart, 75,937,851 reads per sample across 13, 473 transcripts in the liver, and 77,982,668 reads per sample across 15,728 transcripts in the large intestine.

In the heart, there were 85 genes upregulated and 25 genes downregulated in CT_{max} compared to CT_{min} lizards (Fig. 2A, Table S1). Of the upregulated genes, 8 % were HSPs (Table 1). There were 35 genes upregulated and 17 genes downregulated in CT_{max} lizards compared to control lizards (Table S1). There were no differentially expressed genes between CT_{min} and control lizards in this tissue. In the liver, in CT_{max} lizards compared to CT_{min} lizards, there were 17 genes upregulated (41 % were HSPs, Table 1) and 1 gene downregulated (Fig. 2B–Table S1). There were 16 genes upregulated and 3 genes downregulated in CT_{max} lizards compared to control lizards (Table S1). There were 4 genes downregulated and no genes upregulated in CT_{min} lizards compared to control lizards (Table S1). In the large intestine, there were 49 genes upregulated (20 % were HSPs, Table 1) and 8 genes downregulated in CT_{max} lizards compared to CT_{min} lizards (Fig. 2C–Table S1). There were 44 genes upregulated and 5 genes downregulated in CT_{max} lizards compared to control lizards, and 1 gene upregulated and no genes downregulated in CT_{min} lizards compared to control lizards (Table S1).

In terms of functional enrichment, in the heart and large intestine, there were 155 and 24 GO terms (respectively) enriched in the genes that were upregulated in CT_{max} animals compared to CT_{min} animals (Fig. 3A,C, Table S1). Also in these tissues, there were 79 and 107 GO terms enriched in the genes that were upregulated in CT_{max} animals compared to control animals (Table S1). The biological processes enriched in these groups were largely related to the HSP response, protein folding, the response to stress and stimulus, transcription, and biosynthetic and metabolic processes (Fig. 3, Table S1). Fewer GO terms were enriched in response to heat in the liver with 4 GO terms enriched in the genes upregulated in CT_{max} animals compared to CT_{min} animals (Fig. 3B, protein folding, FDR-$p < 0.001$; response to unfolded protein, FDR-$p < 0.01$; response to topologically incorrect protein, FDR-$p < 0.01$; and regulation of cellular response to heat, FDR-$p < 0.01$; Table S1), and 1 enriched term in the genes upregulated in CT_{max} animals compared to control animals (protein folding, FDR-$p = 0.02$; Table S1). The only GO term that was enriched in CT_{min} animals compared to any other treatment group was sphingomyelin catabolic process in the large intestine as compared to CT_{max} animals (FDR-$p = 0.046$; Table S1).

In the heart, we identified six distinct modules of genes with co-expression patterns using WGCNA. One of these modules (turquoise), was associated with thermal tolerance treatment ($p < 0.001$). There were 200 GO terms enriched in this module including biological processes related to metabolism, response to stimulus and stress, protein folding, and the cell cycle (Table S1). The top hub gene of this module was *PPP1R15A* (Fig. 4A), protein phosphatase 1 regulatory subunit 15A, an important component of the integrated stress response (Magg et al., 2024). This gene was upregulated in CT_{max} animals as compared to both CT_{min} and control animals in all tissues (Table S1). In the liver, there were four distinct gene modules overall and one module (yellow) was associated with treatment ($p = 0.04$). There were 151 biological processes enriched in this module composed of similar processes described above for the heart as well as processes associated with transcription and immune function (Table S1). The hub gene of this module was *NR4A1* (Fig. 4B), a nuclear receptor protein and regulator of the cell cycle and apoptosis (Yousefi et al., 2022). This gene was upregulated in CT_{max} lizards compared to CT_{min} and control lizards in the heart only (Table S1). In the large intestine, we identified five distinct gene modules, and one of these modules (yellow) was associated with treatment ($p < 0.001$). There were 152 GO terms enriched in this module representing biological processes similar to those as described for the heart (stress response, cell cycle, metabolism; Table S1). The hub gene of this module was *BAG3* (Fig. 4C), a molecular chaperone regulator which is

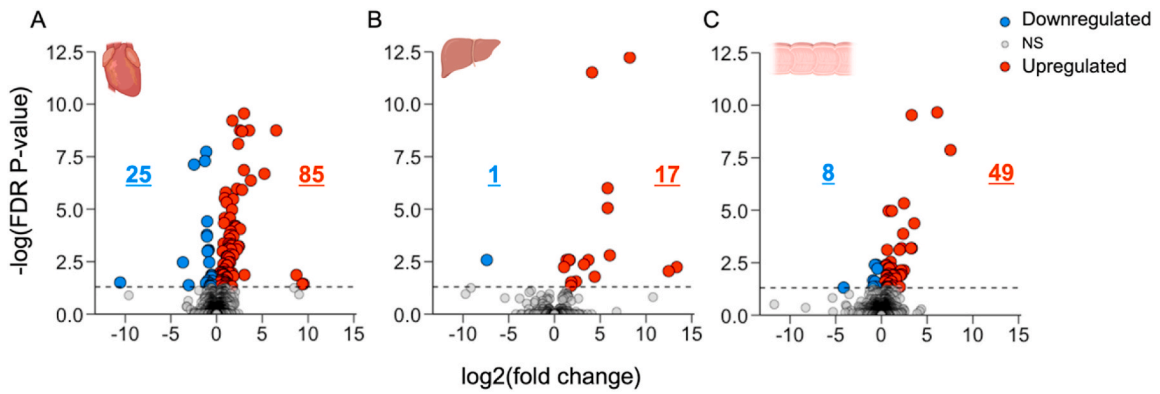

Fig. 2. Volcano plots displaying the number of genes in the heart (A), liver (B), and large intestine (C) that were differentially expressed between CT_{max} lizards and CT_{min} lizards. Genes shown in red were upregulated in CT_{max} lizards (downregulated in CT_{min} lizards). Genes shown in blue were downregulated in CT_{max} lizards (upregulated in CT_{min} lizards). Genes in gray were not significantly differentially expressed between groups. Y-axes show the P-value (negative log scale) of the differential expression test for each gene with the threshold for statistical significance shown with a dotted line, and the x-axes show the direction and magnitude of each gene's change in expression (log2 scale). On each plot, the total number of genes that were upregulated (in red) and downregulated (in blue) in CT_{max} lizards compared to CT_{min} lizards is written in text. Figure created with BioRender.com.

Table 1
Differentially expressed heat shock protein genes in the heart, liver, and large intestine between CT_{max} and CT_{min} lizards. For any genes that were unannotated (LOC), the closest match assigned using blast is listed in parentheses. logFC = log fold change between treatment groups. FDR P = P-value after FDR correction.

Organ	Gene	HSP family	logFC	FDR P
Heart	*HSPA2*	HSP70	6.52	<0.0001
	LOC114588554 (hsp30)	sHSP	5.23	<0.0001
	DNAJB1	HSP40	3.00	<0.0001
	HSP90AA1	HSP90	2.45	0.0006
	HSPB8	sHSP	1.39	0.0006
	DNAJB4	HSP40	1.37	0.0008
	HSPA8	HSP70	1.10	0.0307
Liver	*HSPA2*	HSP70	8.23	<0.0001
	LOC114590273 (CRYAB)	sHSP	6.05	0.0016
	HSPB6	sHSP	5.81	<0.0001
	LOC114598380 (CRYAB)	sHSP	5.81	<0.0001
	LOC114588554 (hsp30)	sHSP	4.39	0.0164
	DNAJB1	HSP40	4.10	<0.0001
	HSP90AA1	HSP90	3.22	0.0043
Large Intestine	*LOC114588554 (hsp30)*	sHSP	7.56	<0.0001
	HSPA2	HSP70	6.10	<0.0001
	HSP90AA1	HSP90	3.32	0.0006
	DNAJB1	HSP40	3.31	<0.0001
	LOC114590273 (CRYAB)	sHSP	2.46	0.0073
	HSPB6	sHSP	2.20	0.0116
	LOC114598380 (CRYAB)	sHSP	2.15	0.0073
	LOC114598379 (CRYAB)	sHSP	2.13	0.0116
	DNAJA4	HSP40	2.05	0.0128
	DNAJB4	HSP40	0.63	0.0452

induced by stressful stimuli (Rosati et al., 2011). This gene was upregulated in CT_{max} lizards compared to CT_{min} and control lizards in the large intestine, and in CT_{max} lizards compared to CT_{min} lizards only in the heart and liver (Table S1).

4. Discussion

Common wall lizards demonstrated rapid plasticity, measured through changes in gene expression, after a brief exposure to their critical thermal limits. Gene expression change in response to heat stress was more pronounced than changes observed in response to cold stress. There were between 3.5 and 17 times more genes upregulated across tissues in CT_{max} animals compared to genes upregulated in CT_{min} animals when these groups were compared to one another (Fig. 2). Further, there were substantial shifts in the gene expression profiles of CT_{max} lizards compared to control lizards, but very few changes in CT_{min} lizards compared to control lizards. A dampened response to cold stress compared to heat stress is consistent with studies in other invertebrate and vertebrate ectotherms that quantified gene expression responses to thermal stressors over short time scales (Lancaster et al., 2016; Mallard et al., 2020; Pimsler et al., 2020; Rosso et al., 2024). It is commonly observed that the lower thermal limits vary more across species and latitudes, while the upper thermal limits are more conserved across species (Sunday et al., 2011; Araújo et al., 2013; Hoffmann et al., 2013; Guiterrez-Pesquera et al., 2016; Bishop et al., 2017; Campbell-Staton et al., 2018). These differences can reflect more variable minimum environmental temperatures compared to maximum temperatures across latitudes (Guiterrez-Pesquera et al., 2016; Campbell-Staton et al., 2018), but are also likely linked to hard physiological boundaries imposed upon animals by the impacts of heat on membrane and protein structure (Araújo et al., 2013). Metabolic costs increase faster with heating than they do with cooling (Harding et al., 2023) which can ultimately lead to sublethal fitness deficits (Shine et al., 2003) or mortality (Jørgensen et al., 2022). Ectotherms typically prefer body temperatures below their physiological optimum to minimize the risk of overheating and the severe performance declines it induces (Martin and Huey, 2008; Buckley et al., 2022). The stronger fitness costs associated with overheating as opposed to cooling likely explain the more rapid gene expression response to heat stress compared to cold stress.

We observed large shifts in gene expression in response to a brief exposure to the thermal maximum. Specifically, there were many genes differentially expressed between lizards exposed to CT_{max} and those exposed to CT_{min} or control lizards (Table S1). Numerous biological processes were also enriched in CT_{max} lizards (Table S1). In gene co-expression networks, modules associated with treatment appeared to be driven largely by genes associated with heat (Fig. 4, Table S1). A main driver of the response to heat was the induction of HSPs (Table 1). The differential expression of these genes resulted in the enrichment of biological processes related to protein folding and the response to stress and stimulus in CT_{max} animals (Fig. 3, Table S1). The HSPs induced in response to heat were similar across all tissue types, indicating a systemic effect that included members of the HSP70, HSP40, HSP90, and sHSP (small heat shock protein) families (Table 1). The HSP70 family is highly inducible and particularly important in the response to stress and heat by maintaining protein integrity and translation (Kregel, 2002). The HSP40 (or DnaJ) family, known as co-chaperones, stabilize interactions between HSP70s and substrates through ATP hydrolysis

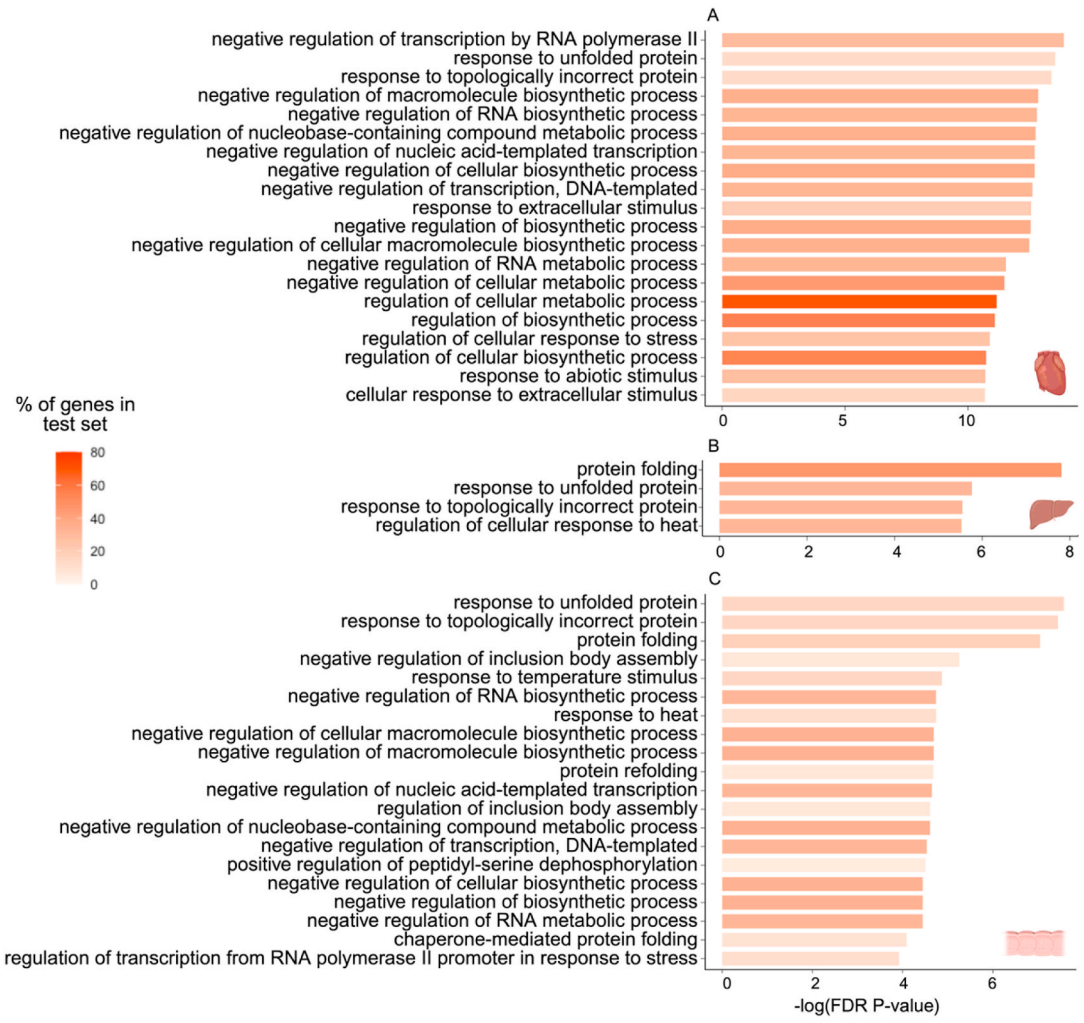

Fig. 3. Enriched GO term biological processes in genes upregulated in the heart (A), liver (B), and large intestine (C), by CT_{max} animals compared to CT_{min} animals. For the heart and large intestine, the top 20 terms are shown. For the liver, only 4 significantly enriched terms were identified which are each shown. In each plot, terms are ordered by their p-value (negative log scale) and bars are colored by the percentage of genes in the gene test set (upregulated genes) that were associated with that biological process. Figure created with BioRender.com.

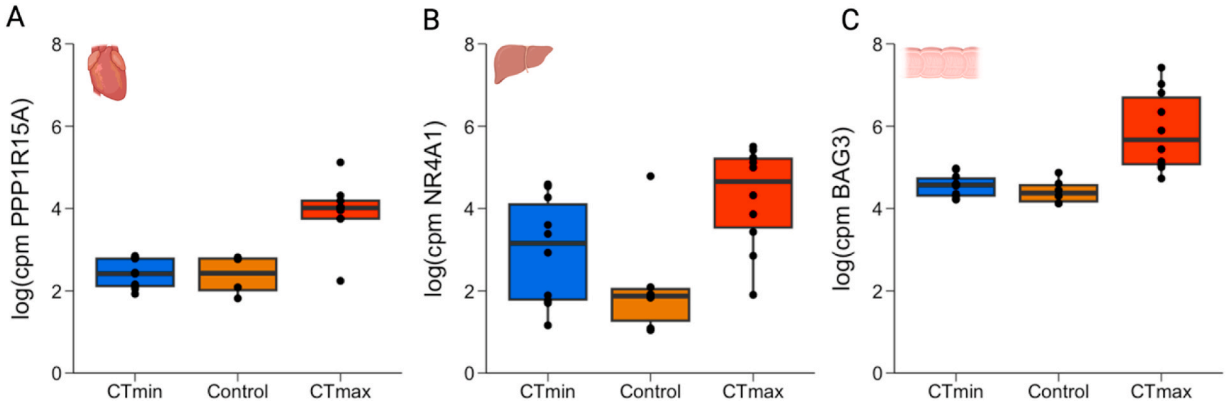

Fig. 4. Counts per million (log scale) across treatment groups of the top hub gene for the gene co-expression module associated with treatment in the heart (A), liver (B), and large intestine (C). Figure created with BioRender.com.

which is crucial for HSP70s to function (Qiu et al., 2006). The BAG family of proteins also interact with HSP70s and notably, *BAG3*, the only member of the family induced by stress (Rosati et al., 2011), was upregulated in CT_{max} animals consistently (Table S1), and was the hub gene in the gene co-expression network associated with treatment in the large intestine (Fig. 4). The HSP90 family is also composed of important molecular chaperones that assist in protein folding, with inducible and non-inducible isoforms (Chen et al., 2005). *HSP90AA1*, which was consistently upregulated in CT_{max} animals in our study (Table 1), is an inducible isoform (Krone and Sass, 1994). sHSPs are less conserved

molecular chaperones that can bind multiple misfolded proteins simultaneously creating large substrate-chaperone complexes. Similar repertoires of HSPs to those we report were induced after a 2 h heat stress exposure in lizards (Wuthrich et al., 2025) and after exposure to CT_{max} in invertebrates (Lancaster et al., 2016; Pimsler et al., 2020) and fish (Dammark et al., 2018). Interestingly, studies that examine the induction of HSPs after heat stress for longer time periods may underestimate the plasticity of the HSP response, as exposure to CT_{max} results in higher expression of several HSPs than exposure to a chronic heat stressor for 24 or 48 h (Dammark et al., 2018).

The induction of the HSP response after heat stress could have a protective effect under warming. Typically, animals exposed to a heat stressor are more tolerant to future exposures (Arias et al., 2012; Moghadam et al., 2019). If lizards can increase their heat tolerance through physiological plasticity, they could reduce their need for behavioral thermoregulation and the subsequent loss of activity time (Kearney et al., 2009; Gunderson and Leal, 2016; Huey and Kingsolver, 2019; Doucette et al., 2023). Over time it is even possible that HSP expression could become fixed evolutionarily in response to high temperature exposure. For example, desert lizards show higher constitutive expression of HSPs than lizards from less thermally-extreme habitats (Ulmasov et al., 1992; Zatsepina et al., 2000). Although plasticity and adaptation in gene expression responses have the potential to protect lizards from warming climates, it is not fully clear how laboratory observations are associated with actual expression of HSPs in nature after stressful events (Sørensen, 2010). Thus, future experiments that quantify variables such as gene expression and HSP production, activity time, and natural thermal exposures in wild lizard populations will be important next steps in understanding the role of rapid thermal plasticity in ectotherm ecology and evolution.

Although the HSP response was consistent across all tissues, we noted additional trends in the gene expression response to heat that were more tissue specific. Specifically, there was a stronger shift in gene expression in the heart and large intestine in response to heat than there was in the liver. For example, there were fewer differentially expressed genes in CT_{max} animals compared to other groups in the liver (Fig. 2) and there were fewer enriched biological processes in response to heat in the liver which were related only to protein folding and the heat shock response (Table S1). In contrast, in the heart and large intestine, there were many enriched biological processes related to transcription, translation, and biosynthetic and metabolic processes in CT_{max} lizards (Fig. 3, Table S1). These results are in alignment with numerous previous studies assessing the transcriptomic response of ectotherms to heat stress (Logan and Cox, 2020). In the large intestine specifically, there was a pronounced shift in various metabolic processes after exposure to heat stress (Fig. 3). For example, the most significantly enriched GO term in CT_{max} animals compared to control animals was the regulation of nitrogen compound metabolic processes (Table S1). This result could be related to the increased use of amino acids as an energy source by ectotherms under warming (Tripp-Valdez et al., 2017; Fontaine and Kohl, 2023). Indeed, ectotherms often prefer more protein-rich food sources at warmer temperatures (DeVries and Appel, 2014; Schmitz et al., 2016; Rowe et al., 2018). The regulation of nitrogen compound metabolic processes was also enriched in CT_{max} animals in the heart (Table S1) and interestingly, consumption of amino acids and protein increases cardiac performance and acclimation capacity under warming (Hardison et al. 2021, 2023; Dixon et al., 2023). It is possible similar processes could impact the liver. For example, the livers of salamanders are protein-deficient under warming which contributes to reduced body sizes, but these effects can be alleviated with a high-protein diet (Zhu et al., 2023). However, we did not observe changes in gene expression related to nutrient metabolism in the liver, suggesting these effects may only be apparent after exposure to warming for longer time periods.

Although the response to heat stress was more pronounced, we did observe some gene expression changes in lizards exposed to their CT_{min} which may have relevance for cold tolerance. In the heart, the gene that was most strongly upregulated in CT_{min} lizards compared to CT_{max} lizards was the glycoprotein-coding gene *AMBP* (Table S1; log fold change = 10.6). Many glycoproteins are ice-binding proteins (Duman, 2015) that act as an anti-freeze and play critical roles in species' ability to avoid freezing, such as in extreme cold-water fishes (Raymond et al., 1975; Van Voorhies et al., 1978; Cheng and Chen, 1999). Increasing cell membrane permeability is another crucial mechanism organisms use to protect from cold (Angilletta Jr, 2009). In the large intestine, the one gene upregulated in CT_{min} animals compared to control animals was *SMIM10L3*, which codes for an integral membrane protein (Table S1). Integral membrane proteins can increase membrane fluidity by reducing the packing of lipids (Fowler et al., 2016). Further, the one enriched biological process in the large intestine of CT_{min} lizards was the catabolism of sphingomyelin (Table S1), a type of sphingolipid found in cell membranes. Reductions in sphingomyelin in cell membranes can act to increase their permeability (Gensure et al., 2006).

In the UK, northward range expansion of common wall lizards is limited by cold winter temperatures, however, this effect may be mitigated in urban areas due to heat island effects (Williams et al., 2021). Cincinnati also experiences an urban heat island effect (Morgan, 2021), which can keep temperatures in the city warmer than surrounding areas year round, including in winter. Thus, cooler winter temperatures in surrounding areas could limit the spread of common wall lizards beyond urban habitats. Our results suggest that lizards begin to alter their physiology in response to cold even after very brief exposures in ways that may improve cell function in colder temperatures, which could allow for rapid adaptation to local environmental conditions. These plastic effects may become even more pronounced after longer-term seasonal acclimation allowing for further spread of invasive lizards (Haro et al., 2023).

We have demonstrated that gene expression of common wall lizards is plastic in response to temperature within a very short time period. A major future direction will be to understand how this capacity for rapid plasticity may influence the evolution of these invasive lizards as they continue to expand their range. For example, thermal plasticity could be lost through genetic assimilation if the thermal environment changes in a consistent manner (Pigliucci et al., 2006). However, Cincinnati wall lizards are expanding in a thermally heterogenous environment (i.e., an urban heat island with cooler surrounding areas), which may be more likely to promote genetic accommodation in which selection leads to increased plasticity in thermal physiology (Jones and Robinson, 2018). Specifically, evolution of increased plasticity is likely when populations are expanding their ranges in highly variable environments (Usui et al., 2023). Consistent with this idea, the thermal tolerance breadth of wall lizard populations does increase with distance from the site of introduction (Litmer and Murray, 2019). However, future studies will need to explicitly test the variation in gene expression plasticity across populations and the heritability of this trait to further understand the evolutionary implications of thermal plasticity in this system.

5. Conclusion

In general, we observed rapid plasticity in gene expression after exposure to the critical thermal maximum and minimum in common wall lizards. In response to heat, HSPs were induced systemically across tissues and metabolic changes were observed in the heart and large intestine. While the response to cold was less extreme, several genes were upregulated in CT_{min} animals that could be adaptive in response to cold, including those related to cryoprotective glycoproteins and plasma membrane components. These physiological adjustments in response to thermal stress could facilitate range expansion in introduced common wall lizards and protection from climate change. However, more data is needed from wild lizards that can link thermal stress to gene expression change in nature. Moving forward, it will be important to understand how this plasticity in response to thermal stress impacts the ecology and evolution of common wall lizards under climate change and continued

range expansion in invaded habitats (Bode, 2025).

CRediT authorship contribution statement

Samantha S. Fontaine: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Brian K. Trevelline:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition.

Ethical approval

All animal experiments were conducted in accordance with the Kent State University IACUC (Protocol 560 BT 24-02). Animals were collected with permission from the Ohio Division of Wildlife (Scientific Collection License SC230080) and Cincinnati Parks Division of Natural Resources (Permit 24-02).

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Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2025.104305.

Data availability

Raw sequencing data and metadata are available from NCBI's Sequence Read Archive under BioProject ID PRJNA1302484.

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