

Thermal variability induces sex-specific morphometric changes in zebrafish (*Danio rerio*)

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Abstract

In nature, organisms are exposed to variable environmental conditions that impact their performance and fitness. Despite the ubiquity of environmental variability, substantial knowledge gaps in our understanding of organismal responses to nonconstant thermal regimes remain. In the present study, using zebrafish (*Danio rerio*) as a model organism, we applied geometric morphometric methods to examine how challenging but ecologically realistic diel thermal fluctuations experienced during different life stages influence adult body shape, size, and condition. Zebrafish were exposed to either thermal fluctuations (22–32°C) or a static optimal temperature (27°C) sharing the same thermal mean during an early period spanning embryonic and larval ontogeny (days 0–30), a later period spanning juvenile and adult ontogeny (days 31–210), or a combination of both. We found that body shape, size, and condition were affected by thermal variability, but these plasticity-mediated changes were dependent on the timing of ontogenetic exposure. Notably, after experiencing fluctuating temperatures during early ontogeny, females displayed a deeper abdomen while males displayed an elongated caudal peduncle region. Moreover, males displayed beneficial acclimation of body condition under lifelong fluctuating temperature exposure, whereas females did not. The present study, using ecologically realistic thermal regimes, provides insight into the timing of environmental experiences that generate phenotypic variation in zebrafish.

KEYWORDS

acclimation, development, morphometric, plasticity, sex-specific, thermal variability

1 | INTRODUCTION

Genotype–environment interactions are ubiquitous among living organisms and ultimately affect organismal form and function. Of the various environmental factors that can affect phenotype, temperature is one of the most influential (Angilletta & Michael, 2009; Johnston &

Bennett, 2008). Temperature has profound within-lifetime effects on ectothermic organisms and can alter the physiology and life history of individuals (Gunderson & Leal, 2016; Hutchings, 2021; Johnston et al., 2006; Paaijmans et al., 2013). The mechanism by which these changes occur is known as phenotypic plasticity, defined as the ability of an organism to alter its phenotype in response to stimuli from its environment (West-Eberhard, 2008). Phenotypic plasticity is a particularly important driver of phenotypic variation in teleost fish, which are highly plastic in their morphology, life history, and physiology

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(Beaman et al., 2016; Jayasundara & Somero, 2013; Johnston et al., 2006; West-Eberhard, 2008).

The size and shape of organisms represent important metrics for understanding the phenotypic effects of plasticity to temperature in ectotherms, given their role as fitness correlates (Cargnelli & Gross, 1996; Dickerson et al., 2002; Hassell et al., 2012; Koops et al., 2004). Numerous studies exposing developing fishes to constant temperatures have illustrated plastic morphological responses. For example, warm temperatures experienced during incubation have been shown to decrease both vertebral number and body size (Garside, 1966; Reyes, 2015). Indeed, warm constant temperatures often lead to decreases in body size across ectothermic taxa, establishing the temperature–size rule whereby organisms mature at smaller sizes under warmer thermal conditions (Atkinson, 1994; Forster & Hirst, 2012). In contrast, cool temperatures experienced during incubation have been shown to increase body size and number of vertebrae, and can also decrease the number of muscle fibers (Galloway et al., 1998; Hubbs, 1922; Johnston et al., 2006). However, the nature of morphological changes varies depending on the species, temperature regime applied, and ontogenetic timing of exposure (Chown & Terblanche, 2006; Scharf et al., 2015). Indeed, studies have occasionally found opposing effects of early vs. later ontogenetic thermal exposure to the same conditions, suggesting we must address the timing-dependent effects of temperature on phenotypic variation (Ivy et al., 2021; Kingsolver & Woods, 2016).

Notably, the vast majority of studies on plastically induced morphological changes in ectotherms have been done under constant thermal conditions (Massey & Hutchings, 2021; Noble et al., 2018). This is not realistic, nor ecologically relevant, as thermal variability is the norm in nature (Shine et al., 1997). In turn, this means that our knowledge of the effects of temperature on organisms is difficult to apply to realistic scenarios (Massey & Hutchings, 2021; Morash et al., 2018). Moreover, as anthropogenic climate change continues, much of the world will experience greater thermal variability in addition to higher mean temperatures (Hansen et al., 2006). Although its effects are largely underappreciated, thermal variability has the potential to significantly impact organismal performance in ways that we currently cannot anticipate (Marshall et al., 2021; Schulte et al., 2011; Slein et al., 2023).

In the present experiment, we quantify variation in body shape, body length, and body condition in response to thermal variability imposed during different life stages, in comparison with a constant temperature regime sharing the same thermal mean. Our factorial experimental design is based on the “strong inference approach” laid out by Huey et al. (1999), in which the effects of early life exposure, later life exposure, and their interaction in response to thermal regimes are compared. Possible interactions indicate the existence of beneficial or detrimental acclimation to early developmental conditions (Huey et al., 1999). We expect to see differences in body size and morphometry between the thermal treatment groups depending on the timing of exposure. However, given the remarkable morphological variation in response to temperature demonstrated in fish taxa thus far (Hubbs, 1922; Noble et al., 2018; Ramler et al., 2014; Vasseur

et al., 2014) and the paucity of existing morphological studies using thermal variability (Massey & Hutchings, 2021), we make no predictions regarding the direction or magnitude of these effects.

Here, we also examine sex-specific effects of thermally variable conditions, as many ectotherms, including zebrafish, display different acclimation capacities and physiological requirements that interact with sex (dos Santos et al., 2021; Hoey et al., 2007; Pottier et al., 2021; Vossen et al., 2022). Furthermore, zebrafish have an environmental sex determination (ESD) system, in which temperatures experienced during larval development influence the outcome of sex, such that warmer incubation temperatures increase the proportion of males in a clutch (Ospina-Álvarez & Piferrer, 2008; Uchida et al., 2002). Because thermal ESD is associated with sex-specific phenotypes and life-history patterns, we assumed that thermal variability may differentially affect male and female body shape and size.

With this information, we empirically address a crucial knowledge gap in our understanding of the fundamental morphological changes that occur under ecologically realistic temperature regimes.

2 | METHODS AND MATERIALS

2.1 | Natural history and experimental design

Zebrafish were chosen for this experiment for several reasons. Notably, zebrafish are a model species in biology and are well-suited to studies of thermal variability, as they experience large daily temperature fluctuations of at least $\sim 5^{\circ}\text{C}$ and have been known to survive temperatures as low as 6°C and as high as 38°C (Spence et al., 2008). Thus, because they experience thermal variability in their natural habitats, they may have adapted the capacity to plastically respond to highly variable temperature regimes (Johnston et al., 2006). Zebrafish are also short-lived organisms, with lifespans in the wild rarely reaching 2 years (Spence et al., 2008); it therefore follows that their thermal environment during early development may serve as a reliable cue for later conditions, a requisite for adaptive plasticity (Taborsky, 2017).

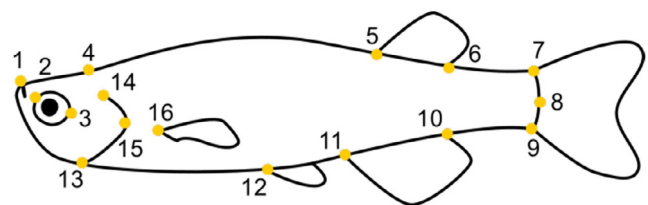


FIGURE 1 Position of 16 landmarks placed on zebrafish (*Danio rerio*) for use in geometric morphometric analysis: 1, anterior tip of the jaw; 2, anterior margin of the eye; 3, posterior margin of the eye; 4, top of the cranium anterior to the dorsal hump; 5, anterior base of the dorsal fin; 6, posterior base of the dorsal fin; 7, upper base of the caudal fin; 8, base of the central caudal ray; 9, lower base of the caudal fin; 10, posterior base of the anal fin; 11, anterior base of the anal fin; 12, base of the pelvic fin; 13, distal tip of the angular articular; 14, ventral tip of the gill cover; 15, posterior tip of the gill cover; 16, upper base of the left pectoral fin.

In the present experiment, we selected two thermal treatments: constant (C; 27°C, static) and fluctuating (F; 22–32°C, sinusoidal diel fluctuating). The diel fluctuating range of temperatures was selected to represent the maximum range of temperatures under which zebrafish can normally develop, and are thus considered a challenging but ecologically relevant level of thermal variability (Spence et al., 2008).

We used a factorial experimental design to contrast the contributions of “early” fluctuating temperature exposure, “later” fluctuating temperature exposure, and their interaction towards variation in body

shape and size (Huey et al., 1999; Massey et al., 2022). Briefly, we split clutches of fish during embryonic and larval stages, placing them in either C or F early temperature treatments until the approximate juvenile stage was reached (30 days post-fertilization [dpf]). At 30 dpf, each group was split once more into C or F for the remainder of their juvenile and adult ontogeny (until ~180 dpf) to isolate later temperature effects.

We used 30 dpf to define the early ontogenetic period because it is largely recognized as the onset of the juvenile period, at which adult characteristics are defined in the absence of sexual maturity, reflecting a natural widely used timepoint for investigating early developmental effects (Bradford et al., 2022; Parichy & Kaplan, 1995). Because it is a recognized standard, this timepoint allows for comparisons with other experimental studies (e.g., Massey et al., 2022).

Although there appears to be natural variation in timing of zebrafish juvenile maturity between individuals (Singleman & Holtzman, 2014), in this study it was not logistically feasible to test every individual such that they entered their later treatment at the same maturity stage. However, given that the latency period between the end of our early treatment period and measurement time was a significant portion of the experiment (5 months, >80%) and zebrafish lifespan, we expect that slight differences in maturity on treatment-switching will not have significantly biased our results.

TABLE 1 Anatomical descriptions of the positions of 15 whole-body landmarks placed on adult zebrafish (*Danio rerio*) for use in geometric morphometric analysis

Landmark	Description
1	Anterior tip of the jaw
2	Anterior margin of the eye
3	Posterior margin of the eye
4	Top of the cranium anterior to the dorsal hump
5	Anterior base of the dorsal fin
6	Posterior base of the dorsal fin
7	Upper base of the caudal fin
8	Base of the central caudal ray
9	Lower base of the caudal fin
10	Posterior base of the anal fin
11	Anterior base of the anal fin
12	Base of the pelvic fin
13	Distal tip of the angular articular
14	Ventral tip of the gill cover
15	Posterior tip of the gill cover
16	Upper base of the left pectoral fin

2.2 | Fish rearing

In February 2021, four clutches of 0–1 h post-fertilization (hpf) wildtype-AB zebrafish eggs from different non-sibling fish pairings (“families”) were collected from the Dalhousie Zebrafish Core Facility. Within 4 h of oviposition, eggs from each clutch were randomly and equally divided between the early C and F temperature treatments at a density of 40 individuals/2.8-L tank, distributed into six replicate

TABLE 2 Multiple analysis of variance describing the effects of predictors of body shape coordinates across 16 landmarks in zebrafish (*Danio rerio*) exposed to either constant (27°C) or fluctuating (22–32°C, diel) thermal regimes during early temperature exposure (0–30 days post-fertilization) and later temperature exposure (30–210 days post-fertilization)

	Df	SS	MS	Rsq	F	Z	P
Log (centroid size)	1	0.00749	0.007487	0.01172	3.2623	2.5863	0.004
Early temperature	1	0.00313	0.003128	0.0049	1.3628	0.8667	0.204
Late temperature	1	0.0129	0.012901	0.0202	5.6215	3.6391	0.001
Sex	1	0.07723	0.077225	0.1209	33.6504	7.2112	0.001
Family-level effects	3	0.05388	0.017961	0.08435	7.8264	6.5329	0.001
Early: later temperature	1	0.00276	0.002757	0.00432	1.2012	0.6094	0.267
Early temperature: sex	1	0.0038	0.0038	0.00595	1.656	1.2756	0.095
Later temperature: sex	1	0.00292	0.00292	0.00457	1.2722	0.749	0.231
Early:later temperature: sex	1	0.00214	0.002143	0.00336	0.9338	0.0731	0.477
Residuals	191	0.43833	0.002295	0.68621			

Note: Numbers in bold indicate significant shape variation associated with each predictor, and centroid size can be interpreted as a measure of overall body size ($N = 203$).

Abbreviations: Df, degrees of freedom; F, *f*-score; MS, mean squares; P, *p*-value; Rsq, *r*-squared; SS, sum of squares; Z, *z*-score.

tanks per treatment (total = 24 tanks). Fish were fed twice daily with commercial feed (Gemma Micro 150; Skretting).

The eggs and larvae were reared in these treatments until they entered the juvenile period (30 dpf). After this point, the two treatment groups were once again divided into later C or F temperature treatments and placed in tanks at a density of 12–15 per 2.8-L tank. Ultimately, each tank experienced a combination of early temperature treatment (C or F) and later temperature treatment (C or F).

2.3 | Data collection

In August 2021, when the fish were approximately 6 months old, up to 10 fish per tank were randomly selected from each of the six replicate tanks (49–53 fish per factorial combination) and anesthetized using tricaine methanesulfonate (MS-222) at a concentration of 80 mg/L to assess morphology. The fish were weighed, measured, and photographed with a digital camera (OMD-Mark III; Olympus) using best-practice photography methods after Muir et al. (2012). Briefly, the left side of each fish was photographed under soft light with a scale bar in each image and straightened along their axis of alignment prior to photographing. All images used a fixed focal length of 45 mm and an aperture of f/8, and care was taken to ensure the focal plane of the camera was horizontal with respect to the fish. In cases where a large body depth caused the head and/or tail to bend downwards, a thin wedge of foam was placed underneath the anterior and/or posterior end of the fish to reduce distortion (Muir et al., 2012). Fish were sexed at the time of photographing by experienced zebrafish researchers (M. Massey and M. K. Fredericks) based on established dimorphic external characteristics commonly used in zebrafish laboratories (Schilling, 2002; Spence et al., 2008). Fish were classified as male if they presented yellow coloration on the tail fin and/or abdominal underside, presented relatively dark blue stripes, lacked obvious gravidity, and lacked visible genital papillae (Schilling, 2002; Spence et al., 2008; Yossa et al., 2013). We measured standard length using Image J software with a transparent standard ruler for calibration (Schneider et al., 2012).

2.4 | Statistical analysis

Photographs were used to perform a geometric morphometric analysis of body shape variation among treatment groups. The “geomorph” package (Adams et al., 2020) in the R Statistical Environment (v4.2.3) was implemented to perform analyses. A total of 16 anatomical fixed landmarks were selected based on those used in previous morphometric studies of zebrafish (Figure 1 and Table 1; Geoga & Koumoundouros, 2010). We also estimated body condition using Fulton’s K , which is based on the body length (anterior tip of the jaw to the tail fork) to weight ratio ($K = 100 \times \text{mass} \times \text{length}^{-3}$; Nash et al., 2006).

After cataloguing anatomical landmarks on each specimen, we used a generalized Procrustes analysis (GPA) to standardize shape

data (Dryden & Mardia, 2016). GPA is a standardization method that uses the first image in the data set as the reference image on which all others will be optimally superimposed to facilitate direct comparison. This requires a process that translates each specimen to the central origin, scales each to a unit centroid size, and optimally rotates them until corresponding landmark points are as closely aligned as possible (Mitteroecker et al., 2013; Zelditch et al., 2004).

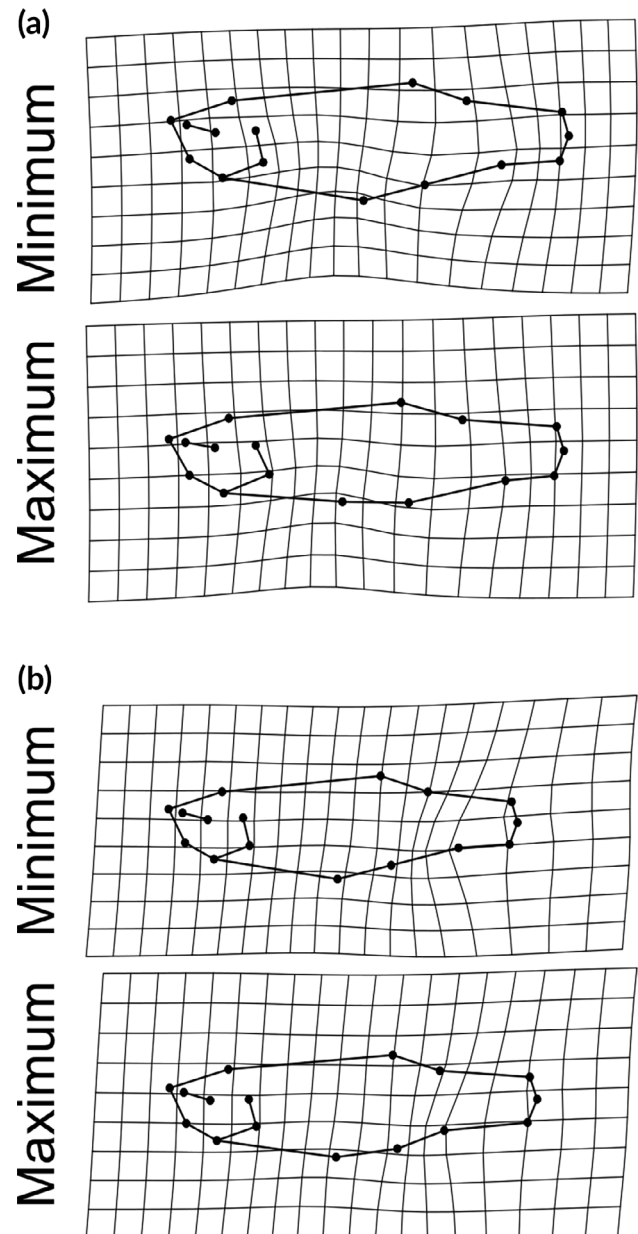


FIGURE 2 Warp meshes depicting the minimum and maximum landmark values detected in zebrafish (*Danio rerio*) exposed to either constant (27°C) or fluctuating (22–32°C on a diel basis) thermal treatments during early ontogeny (0–30 days post-fertilization) or later ontogeny (30+ days post-fertilization). (a) Minimum and maximum values for principal component (PC) 1, summarized by reductions in abdominal depth. (b) Minimum and maximum values for PC2, summarized by lengthening of the caudal peduncle.

We performed a principal components analysis (PCA) on shape (Procrustes coordinates) to visualize the major aspects of body shape variation, using the “gm.pcomp” function in geomorph (Adams et al., 2020). A multivariate analysis of variance (MANOVA) was used to examine the influence of treatment (early temperature treatment, later temperature treatment, and their interaction), centroid size, sex, and family on multivariate shape variation using the “procD.lm” function in the “geomorph” package, implementing the type II (hierarchical) sum of squares (Adams & Collyer, 2018). Body size was represented by the centroid size of each specimen, calculated as the square root of the sum of squared distances of each landmark from the origin, or centroid, position (Zelditch et al., 2004). Family was included in MANOVA analyses to account for variation between different lineages of fish, assumed to be due to genetic differences and/or parental effects from stock fish, as we started the experiment with four clutches of fish from different nonsibling fish pairings.

We implemented Bayesian univariate generalized linear mixed models using the “brms” package in the R Statistical Environment

(v4.2.3) to estimate the effects of early temperature, later temperature, sex, and their interactions on principal components (PCs) 1–5, as well as standard length and body condition. To account for correlation between tankmates within treatment groups (pseudoreplication), we included “tank” as a random intercept in all univariate models (Harrison et al., 2018). All models used Gaussian distributions with identity link functions. Weakly informative default priors were used to provide moderate regularization.

2.5 | Animal use

The fish photographed in this experiment were originally part of a larger study using the long-term experimental treatments described herein (Massey et al., 2022) in order to ethically increase the scientific value of their use. All 2021 studies on this colony were approved by the Dalhousie University Committee on Laboratory Animals (Protocol 19-105).

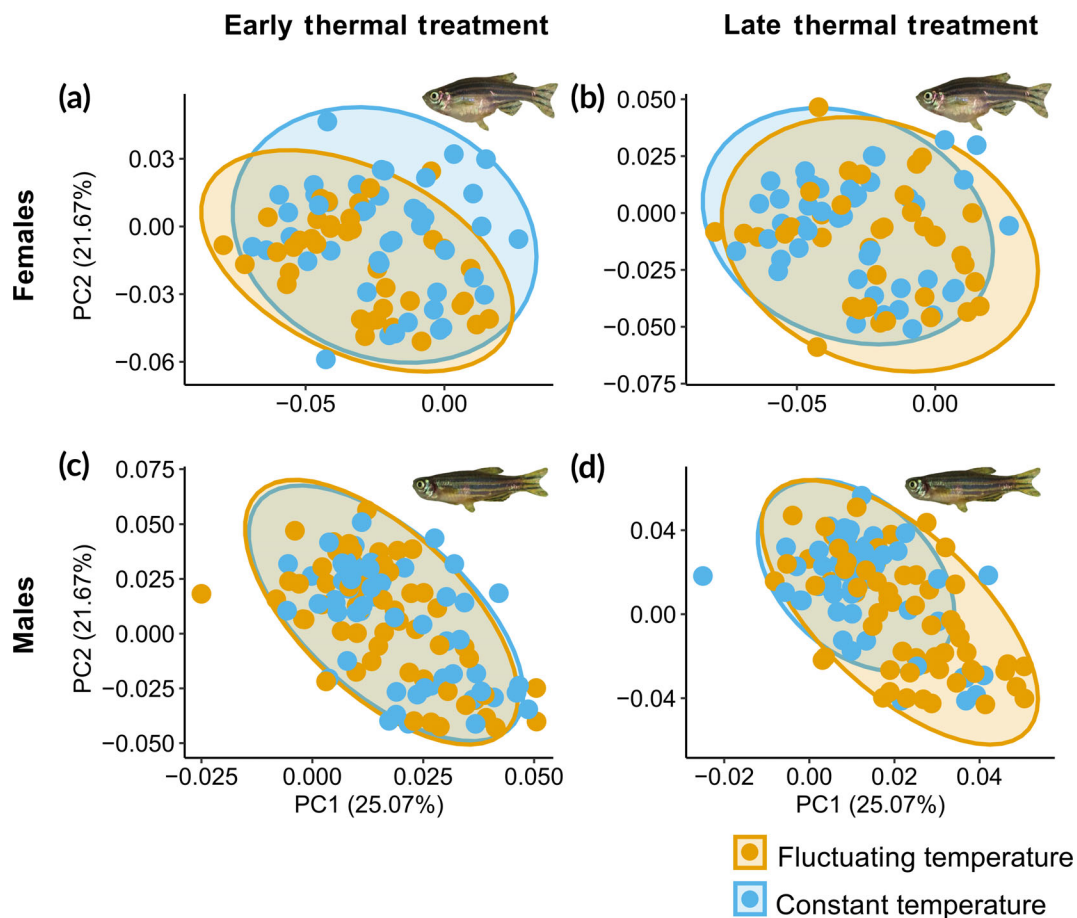


FIGURE 3 Principal component analysis of the first two principal components (PCs) of body shape variation in zebrafish (*Danio rerio*) exposed to either constant (27°C) or fluctuating (22–32°C on a diel basis) thermal treatments during early ontogeny (0–30 days post-fertilization) or later ontogeny (30+ days post-fertilization). PC1 is associated with abdominal depth, explaining 25.07% of variation in overall body shape. An increasing PC1 score is indicative of decreasing abdominal depth. PC2 is associated with caudal peduncle length, explaining 21.67% of variation in body shape. An increasing PC2 score is indicative of decreasing caudal peduncle length. Data points are separated by sex (females: a, b; males: c, d) and exposure period (early temperature: a, c; later temperature: b, d). Ellipses denote clusters of individuals that experienced the constant (blue) or fluctuating (orange) temperature regime during early or later exposure periods.

3 | RESULTS

3.1 | Plasticity in overall body shape and individual PCs of shape

We detected significant differences in overall body shape between groups (Table 2) caused by the later temperature treatment ($F_{(1,202)} = 5.62$, $p = 0.001$), centroid size ($F_{(1,202)} = 3.26$, $p = 0.004$), sex ($F_{(1,202)} = 33.65$, $p = 0.001$), family-level effects ($F_{(3,202)} = 7.83$, $p = 0.001$), and the interaction between early temperature and sex (marginally significant; $F_{(3,202)} = 1.66$, $p = 0.095$). There were no significant differences in any other interactions or predictor variables (Table 2).

Our PCA revealed 46.74% of overall variance in body shape could be explained by the first two PCs, which we focus on here (additional PCs are detailed in Figures S1 and S2, and Tables S3 and S4). Positive scores along PC1 were largely associated with decreased abdominal depth (Figure 2a), whereas positive PC2 scores were largely associated with increased caudal peduncle length (Figure 2b). Variation along PC1 and PC2, separated by sex, early temperature, and later temperature is illustrated in Figure 3.

We detected complex effects of early temperature, later temperature, sex, and their interactions on variation in abdominal depth (PC1) using a

Bayesian generalized linear mixed model (Table 3). Because Bayesian analyses make no claims of “significance,” for ease of interpretation we describe effects as “significant” if 90% uncertainty intervals (UIs) do not overlap zero, and “marginally significant” if 50% UIs do not overlap zero.

First, the early fluctuating temperature treatment had a significant, positive effect on abdominal depth (negative effect on PC1 scores) in females (Table 3; $\beta = -0.0150$, 90% UIs $[-0.0277, -0.0019]$), but the opposite effect in males (Figure 3c and Table 3; $\beta = 0.00117$, 90% UIs $[0.0010, 0.0230]$). There was a further, marginally significant positive interaction between early temperature and later temperature in response to fluctuating temperatures, positively affecting PC1 scores (shallower abdominal depth) in both sexes (Table 3; $\beta = 0.0125$, 50% UIs $[-0.0190, -0.0059]$). Sex also significantly influenced PC1 scores, with males having shallower abdominal regions (Table 3; $\beta = 0.0346$, 90% UIs $[0.0270, 0.0425]$); this was the strongest fixed effect of all predictors. We also detected a small, positive effect of later fluctuating temperature on abdominal depth, but there was low certainty on this estimate (Table 3; $\beta = 0.0012$, 50% UIs $[-0.0040, 0.0063]$).

We detected complex effects of early temperature, later temperature, sex, and their interactions with caudal peduncle length (PC2; Table 3). Early fluctuating temperature had a marginally significant, negative effect on caudal peduncle length in females (Table 3;

TABLE 3 Fixed-effect posterior mean estimates, and 50% and 90% uncertainty intervals (UIs) from Bayesian generalized linear mixed models explaining effects of predictors on abdominal depth decreases (principal component 1) and increases in the length of the caudal peduncle (principal component 2) in zebrafish (*Danio rerio*)

Predictor	Posterior mean effect size estimate	5% UI	95% UI	25% UI	75% UI
Principal component 1 (decrease in abdominal depth)					
Intercept	-0.021	-0.0302	-0.0123	-0.0245	-0.0174
Early fluctuating temperature ^a	-0.015	-0.0277	-0.0019	-0.0202	-0.0101
Later fluctuating temperature	0.0012	-0.0112	0.0142	-0.0040	0.0063
Male sex ^a	0.0346	0.0270	0.0425	0.0314	0.0378
Early:later fluctuating temperature ^b	0.0138	-0.0050	0.0330	0.0064	0.0210
Early fluctuating temperature: male ^a	0.0117	0.0010	0.0230	0.0072	0.0161
Later fluctuating temperature: male ^b	0.0068	-0.0039	0.0173	0.0023	0.0113
Early:later fluctuating temperature: male ^b	-0.0125	-0.0290	0.0035	-0.0190	-0.0059
Principal component 2 (increase in length of caudal peduncle)					
Intercept	-0.0011	-0.0151	0.0129	-0.0069	0.0048
Early fluctuating temperature ^b	-0.0137	-0.0333	0.0061	-0.0217	-0.0056
Later fluctuating temperature ^b	-0.0125	-0.0321	0.0080	-0.0207	-0.0043
Male ^a	0.0151	0.0053	0.0245	0.0112	0.0190
Early:later fluctuating temperature ^b	0.0133	-0.0157	0.0412	0.0022	0.0245
Early fluctuating temperature: male ^a	0.0166	0.0031	0.0304	0.0112	0.0219
Later fluctuating temperature: male	-0.0021	-0.0160	0.0111	-0.0076	0.0036
Early:later fluctuating temperature: male ^b	-0.016	-0.0354	0.0038	-0.0242	-0.0078

Note: Fixed predictors are early fluctuating temperature, later fluctuating temperature, male, and their interaction. Reference categories are constant temperature and female. Posterior mean effect size estimates can be interpreted analogously to frequentist coefficient estimates. UIs can be interpreted analogously to frequentist confidence intervals; nonoverlap of zero indicates higher confidence in effect size estimates ($N = 203$).

^aImplies certainty at the 90% level.

^bImplies certainty at the 50% level.

$\beta = -0.0137$, 50% UIs $[-0.0217, -0.0056]$), but this effect was reversed in males (Table 3; $\beta = 0.0166$, 90% UIs $[0.0031; 0.0304]$). Likewise, later fluctuating temperature also had a marginally significant negative effect on caudal peduncle length in both sexes (Table 3; $\beta = -0.0125$, 50% UIs $[-0.0207, -0.0043]$). Sex overall significantly affected caudal peduncle length, such that males had longer caudal peduncles (Table 3; $\beta = 0.0151$, 90% UIs $[0.0053, 0.0245]$). No other covariates or interactions explained significant variation in caudal peduncle length (Table 3).

3.2 | Plastic effects on standard length and body condition

Early fluctuating temperature had a significant and positive effect on standard length on females (Figure 4a and Table 4; $\beta = 1.1159$, 90%

UIs $[0.0272, 2.2466]$), but both early and later fluctuating temperature treatments negatively interacted with male sex (Table 4; $\beta = -0.5654$ and -0.6317 , 50% UIs $[-0.9959, -0.1113]$, $[-1.0625, -0.2055]$, respectively). Later fluctuating temperature significantly and negatively impacted standard length (Table 4; $\beta = -1.2289$, 90% UIs $[-2.3016, -0.1028]$). Likewise, males had lower standard length (marginally significant; Table 4; $\beta = -0.3169$, 90% UIs $[-0.6189, -0.0155]$). Females that experienced both early and later fluctuating temperatures had lower standard length (Table 4; $\beta = -1.2975$, 50% UIs $[-1.9409, -0.6475]$), but this pattern was reversed for males (Table 4; $\beta = 1.4117$, 50% UIs $[0.7755, 2.0735]$).

We also detected sex-specific effects of early temperature treatment, later temperature treatment, and their interactions on body condition, measured as Fulton's K (Figure 4b and Table 5). For females, both early and later fluctuating temperatures had a

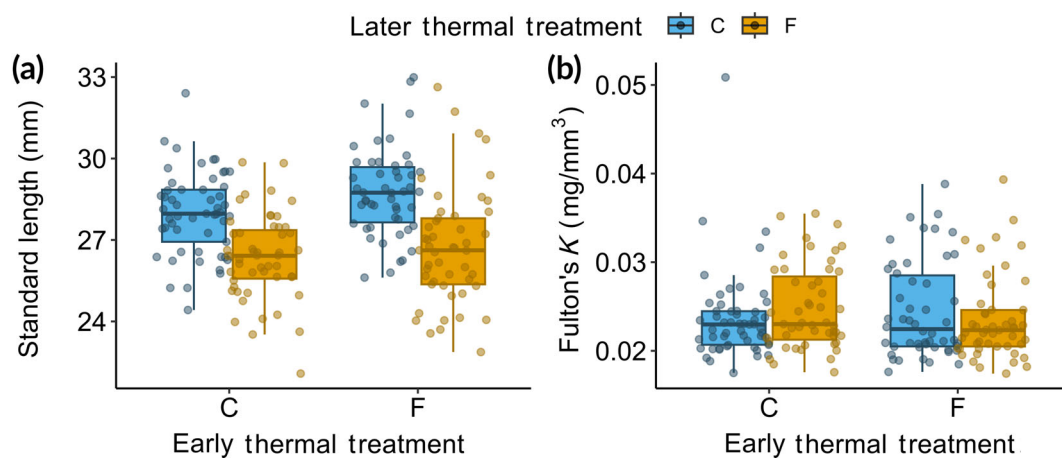


FIGURE 4 Box plots depicting variation in standard length (a) and body condition (Fulton's K ; b) in zebrafish (*Danio rerio*) exposed to constant (C, 27°C) or fluctuating (F, 22–32°C on a diel basis) thermal treatments during early ontogeny (0–30 days post-fertilization) or later ontogeny (30+ days post-fertilization). Data are categorized by early temperature treatment (x axis) and further split into later temperature treatments, where blue represents constant and orange represents fluctuating. Each data point represents one individual sampled, $N = 203$.

TABLE 4 Fixed-effect coefficient estimates, and 50% and 90% uncertainty intervals (UIs) from Bayesian generalized linear mixed models explaining effects of predictors on standard length in zebrafish (*Danio rerio*)

Predictor	Posterior mean effect size estimate	5% UI	95% UI	25% UI	75% UI
Intercept ^a	28.1726	27.3427	28.9861	27.8519	28.5151
Early fluctuating temperature ^a	1.1159	0.0272	2.2466	0.6657	1.5763
Later fluctuating temperature ^a	-1.2289	-2.3016	-0.1028	-1.6715	-0.7915
Male ^b	-0.3169	-1.0715	0.4155	-0.6189	-0.0155
Early:later fluctuating temperature ^b	-1.2975	-2.9512	0.3316	-1.9409	-0.6475
Early fluctuating temperature: male ^b	-0.5654	-1.6551	0.4776	-0.9959	-0.1113
Later fluctuating temperature: male ^b	-0.6317	-1.6939	0.4180	-1.0625	-0.2055
Early:later fluctuating temperature: male ^b	1.4117	-0.1975	2.9942	0.7755	2.0735

Note: Fixed predictors are early fluctuating temperature, later fluctuating temperature, male, and their interaction. Reference categories are constant temperature and female. Posterior mean effect size estimates can be interpreted analogously to frequentist coefficient estimates. UIs can be interpreted analogously to frequentist confidence intervals; nonoverlap of zero indicates higher confidence in effect size estimates ($N = 203$).

^aImplies certainty at the 90% level.

^bImplies certainty at the 50% level.

TABLE 5 Fixed-effect coefficient estimates, and 50% and 90% uncertainty intervals (UIs) from Bayesian generalized linear mixed models explaining effects of predictors on body condition (Fulton's *K*) in zebrafish (*Danio rerio*)

Predictor	Posterior mean effect size estimate	5% UI	95% UI	25% UI	75% UI
Intercept ^a	0.0267	0.0254	0.0279	0.0262	0.0272
Early fluctuating temperature ^a	0.0026	0.0008	0.0044	0.0019	0.0033
Later fluctuating temperature ^a	0.0026	0.0008	0.0044	0.0018	0.0033
Male ^a	-0.0052	-0.0069	-0.0035	-0.0059	-0.0045
Early:later fluctuating temperature ^a	-0.0040	-0.0067	-0.0013	-0.0051	-0.0029
Early fluctuating temperature: male ^a	-0.0034	-0.0058	-0.0009	-0.0043	-0.0024
Later fluctuating temperature: male ^a	-0.0024	-0.0047	0.0000	-0.0034	-0.0014
Early:later fluctuating temperature: male ^a	0.0045	0.0010	0.0079	0.0032	0.0059

Note: Fixed predictors are early fluctuating temperature, later fluctuating temperature, male, and their interaction. Reference categories are constant temperature and female. Posterior mean effect size estimates can be interpreted analogously to frequentist coefficient estimates. UIs can be interpreted analogously to frequentist confidence intervals; nonoverlap of zero indicates higher confidence in effect size estimates ($N = 203$).

^aImplies certainty at the 90% level.

significant, positive effect on body condition (Table 5; $\beta = 0.0026$, 90% UIs [0.0008, 0.0044] for both predictors), but the positive effect disappeared if females were reared in fluctuating temperature conditions for both ontogenetic periods (Table 5; $\beta = -0.0040$, 90% UIs [-0.0040, -0.0067]). This trend was reversed for males, who experienced benefits to body condition when reared in fluctuating temperatures throughout ontogeny (Table 5; $\beta = 0.0045$, 90% UIs [0.0010, 0.0079]). Males had overall lower average body condition compared to females (Table 5; $\beta = -0.0052$, 90% UIs [-0.0069, -0.0035]).

4 | DISCUSSION

Thermal variability is pervasive in nature and can have important consequences for organisms (Massey & Hutchings, 2021), but its plastic effects on fish morphology are largely unknown. In this study, we examined the relationship between overall body morphology and thermal variability during different periods of ontogeny, allowing us to isolate the effects of early (embryonic and larval), later (juvenile and adult), and lifelong exposure to fluctuating temperatures. Although our constant and fluctuating thermal regimes shared the same mean temperature, we found several differences in body shape and size owing to thermal variability that were driven by early thermal treatments, later thermal treatments, or a combination thereof.

A notable result of our study is that differences in body shape and size owing to early thermal exposures were largely sex-specific in nature. Here, both abdominal depth and caudal peduncle length were influenced through early life exposure to fluctuating temperatures, such that females had deeper abdomens and shorter caudal peduncles, while males displayed shallower abdomens and longer caudal peduncles. Interestingly, these two traits are associated with the reproductive biology of zebrafish. For example, the abdominal girth of female fish is often positively associated with fecundity (Ghaflemarammazi et al., 1998; Narejo et al., 1998). Indeed, in a recent

sister study using the same experimental rearing protocol, Massey et al. (2022) found early developmental exposure to thermal variability had an enhancing effect on fecundity. Given these data, it is likely that under thermal variability, female abdominal depth increased as a result of preferentially reallocating resources towards gonadal development and reproduction (Guderley & Pörtner, 2010; Jasper & Evenson, 2006). Previous studies have also found that male fish have longer caudal peduncles on average, and consequently faster swimming speeds (Ackerly & Ward, 2016; Conradsen & McGuigan, 2015). These findings suggest that, for both sexes, thermal variability experienced during early life may enhance traits associated with reproductive success. It is possible that any benefits to reproduction may represent a life-history trade-off, especially given that long-term rearing in fluctuating thermal conditions can impose constraints on body size (Massey et al., 2022).

Sex-specific effects were also notable when the interactive effects between ontogenetic treatments were compared. We found that females who experience fluctuating thermal treatments during both early and later ontogeny had a shallower abdomen and lower body condition score than those who experienced only one fluctuating thermal treatment throughout their lifetime. Males, on the other hand, benefited in terms of their body condition when reared in the same conditions throughout their lifetime, regardless of the thermal regime experienced. These results suggest that under long-term thermal variability, there may be sexually dimorphic metabolic costs associated with coping, and in this species, males appear to benefit from environmental stability, regardless of the environmental conditions (Missionário et al., 2022; Øverli et al., 2006; Whitney et al., 2016). Furthermore, these sex-specific effects represent a case of sexual dimorphism in “beneficial acclimation” (Leroi et al., 1994), or the capacity of an organism to perform better in conditions previously experienced. Empirical examples of beneficial acclimation are rare in the literature (Angilletta & Michael, 2009; Wilson & Franklin, 2002), but to our knowledge, this phenomenon has not

yet been addressed through the lens of sex-specificity. Thus, incorporating treatment interactions with sex in future studies examining beneficial acclimation is warranted, especially in species displaying sexual dimorphism.

There also appeared to be constraints imposed by long-term later life exposure to fluctuating temperatures in later ontogeny. We found that exposure during laterontogeny to thermal variability led to decreases in both the length of the caudal peduncle and standard length, and that these effects were not sex-specific. One explanation for these changes may be the “temperature-size rule (TSR)” (Atkinson, 1994), which suggests that experiencing warmer temperatures during early ontogeny generally decreases adult body size in ectotherms. This process is believed to occur due to differential temperature-dependent rates of somatic growth and differentiation (i.e., maturity), such that differentiation occurs more rapidly at high temperatures (Huey & Kingsolver, 1989; Van der Have & De Jong, 1996). Consequently, maturity in our later fluctuating temperature group, which repeatedly experienced warm temperatures, may have been achieved at smaller body sizes (Van der Have & De Jong, 1996), although a metric of age at maturity would be needed to unequivocally confirm this. Although the TSR typically refers to thermal conditions experienced during early life (Atkinson, 1994), in the present experiment we found that these reductions in body size were associated significantly with thermal experience during the juvenile and adult life stages, emphasizing the importance of long-term experiments that extend beyond embryonic and larval conditions (Massey & Hutchings, 2021). Ultimately these changes may have ecological ramifications, as body size is correlated with both swimming speed and predator avoidance (Conradsen & McGuigan, 2015; James & Johnston, 1998).

There were several limitations to our study that open doors for future work. First, the morphological changes we saw implied that reproductive success, life history, and locomotion may also be impacted. However, it was beyond the scope of this study to investigate these consequences directly. Future studies on factors such as mating behaviors and sexual competition, age at maturity, and physiology of movement would address these remaining questions. Second, in this study we compared two of an infinite number of possible thermal scenarios. In nature, temperatures change on daily, seasonal, and stochastic bases, in elaboration of the predictable diurnal cycle we simulated. Continued empirical exploration of ecologically informed temperature ranges, including seasonal variation and thermal stochasticity, will benefit future studies, especially those investigating wild organisms (Chevin et al., 2010).

Overall, we found that thermal variability has significant influence on the morphology of zebrafish through early ontogenetic exposure, later ontogenetic exposure, and their interaction, and these changes appear to be sex-specific. Moreover, differences emerged even when mean temperatures were shared between treatment groups, suggesting that our current reliance on empirical data from constant temperature studies can bias our understanding of organismal responses to realistic temperatures (Massey &

Hutchings, 2021). The morphological differences detected herein have significant fitness implications related to reproduction, predator avoidance, locomotion, and life history (Ackerly & Ward, 2016; Hassell et al., 2012; Slein et al., 2023). Adapting life-history patterns through plasticity may represent avenues for zebrafish and physiologically similar species to contend with the changing climatic conditions observed worldwide, although these responses will be dependent on when climate change stressors are experienced, relative to organismal ontogeny (Chevin et al., 2010; Seebacher et al., 2015; Shama et al., 2014).

AUTHOR CONTRIBUTIONS

Ideas for the project were developed by M.D.M. and J.A.H. Data were generated by K.M.F., A.G.G., and M.D.M. Data analyses were conducted by A.G.G., T.L.M., and M.D.M. Manuscript preparation was done by A.G.G., T.L.M., and M.D.M. Funding was acquired by J.A.H. and M.D.M. All authors reviewed drafts of this manuscript.

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DATA AVAILABILITY STATEMENT

Raw data and R code used for analyses are available upon request from the authors.

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