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Functional consequences of phenotypic variation between locally adapted populations: Swimming performance and ventilation in extremophile fish

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Abstract

Natural selection drives the evolution of traits to optimize organismal performance, but optimization of one aspect of performance can influence other aspects of performance. Here, we asked how phenotypic variation between locally adapted fish populations affects locomotion and ventilation, testing for functional trade-offs and trait–performance correlations. Specifically, we investigated two populations of livebearing fish (*Poecilia mexicana*) that inhabit distinct habitat types (hydrogen-sulphide-rich springs and adjacent nonsulphidic streams). For each individual, we quantified different metrics of burst swimming during simulated predator attacks, steady swimming and gill ventilation. Coinciding with predictions, we documented significant population differences in all aspects of performance, with fish from sulphidic habitats exhibiting higher steady swimming performance and higher ventilation capacity, but slower burst swimming. There was a significant functional trade-off between steady and burst swimming, but not between different aspects of locomotion and ventilation. Although our findings about population differences in locomotion performance largely parallel the results from previous studies, we provide novel insights about how morphological variation might impact ventilation and ultimately oxygen acquisition. Overall, our analyses provided insights into the functional consequences of previously documented phenotypic variation, which will help to disentangle the effects of different sources of selection that may coincide along complex environmental gradients.

KEYWORDS

burst swimming, gill ventilation, hydrogen sulphide spring, local adaptation, locomotion, *Poecilia mexicana* (Poeciliidae), steady swimming, trade-offs

1 | INTRODUCTION

Divergent selection along environmental gradients is a primary driver of phenotypic evolution and local adaptation (Kawecki & Ebert, 2004). Establishing the origins of phenotypic diversity requires an understanding of how variation in selection favours different traits that mediate organismal performance and how trade-offs potentially prevent the simultaneous optimization of multiple organismal

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functions and the evolution of general-purpose phenotypes. Trade-offs in the expression of traits frequently arise as a consequence of constraints associated with the genetic architecture underlying phenotypic traits (Scarcelli, Cheverud, Schaal, & Kover, 2007), developmental mechanisms (Jeffery,2009) or energy availability (Emlen, 2001). In addition, there may be functional constraints where optimizing a particular trait for one function impairs the utility of that trait for other functions (Ghalambor, Walker, & Reznick, 2003; Johnson, Burt, & DeWitt, 2008). In the context of complex environmental gradients with coinciding sources of selection, establishing how trait variation impacts different metrics of organismal performance facilitates a better understanding of the causal drivers of phenotypic evolution and functional trade-offs that may limit evolutionary outcomes.

Fishes provide an excellent example of staggering morphological variation within and among species (Domenici, 2003a; Farina, Near, & Bemis, 2015; Hulsey & Garcia De Leon, 2005; Langerhans, Layman, & DeWitt, 2005; Webb, 1982), making them conducive to the investigation of adaptive evolution at micro- and macro-evolutionary scales (Jacquemin & Pyron, 2016). The ultimate causes underlying the variation in fish morphology are tied to diverse functions that include locomotion (Domenici, 2003a), feeding (Hulsey & Garcia De Leon, 2005; Webb, Kostecki, & Stevens, 1984), oxygen acquisition (Farina et al., 2015) and reproduction (Basolo, 1990; Bisazza, 1993). A key question in understanding patterns of diversification is how different sources of selection are related to morphological variation within and among species, and how morphological variation in turn influences different functions contributing to organismal performance across environmental contexts (Arnold, 1983). Linking selection, phenotypic variation and organismal performance are perhaps best investigated in the context of body shape variation and associated trade-offs between steady and burst swimming performance (Blake, 2004; Domenici, 2003a; Langerhans & Reznick, 2010; Videler, 1993; Webb, 1984). Streamlined body shapes are optimal for energy-efficient steady swimming, which is favoured in high flow environments, open and structure-poor habitats, and habitats with high levels of competition (Domenici, 2003b; Langerhans & Reznick, 2010; Plaut, 2001; Rice & Hale, 2010). In contrast, body shapes with an enlarged caudal area are optimal for burst swimming that mediate quick bursts of acceleration, which are favoured in high-predation environments that require rapid acceleration during predator attacks (Blake, 2004; Langerhans, Layman, Shokrollahi, & DeWitt, 2004; Walker, 1997; Webb & Weihs, 1983). There are also trade-offs associated with different modes of feeding, including one documented between the generation of suction pressure and the volumetric expansion of the buccal cavity that impacts foraging habits in centrarchid fishes (Carroll, Wainwright, Huskey, Collar, & Turingan, 2004). Although we have a thorough understanding of trade-offs between different modes of swimming or foraging, there are fewer examples of how phenotypic diversification is potentially impacted by trade-offs among different types of organismal function (locomotion vs. foraging, locomotion vs. reproduction, etc.) (Billerbeck, Lankford, & Conover, 2007; Higham, Hulsey, Rican, & Carroll, 2007; Webb, 1984). In this study, we were particularly interested in investigating the functional implications of morphological variation in the context of locomotion and respiration using a well-established study system with local adaptation to divergent environmental conditions.

Poecilia mexicana is a widespread livebearing fish occurring in various freshwater habitats of Mexico and Central America (Palacios, Voelker, Arias Rodriguez, Mateos, & Tobler, 2016). In addition, this species has independently colonized multiple toxic, hydrogen-sulphide (H_2S)-rich springs in southern Mexico (Tobler et al., 2011). Populations in adjacent nonsulphidic and sulphidic habitats face strong and multifarious divergent selection that includes variation in abiotic and biotic environmental conditions (Tobler, Kelley, Plath, & Riesch, 2018). Fish inhabiting sulphide springs are exposed to high levels of toxicity associated with H_2S, hypoxia, high densities of intraspecific competitors and reduced species-richness with few aquatic predators and inter-specific competitors (Culumber et al., 2016; Greenway, Arias-Rodriguez, Diaz, & Tobler, 2014; Plath et al., 2007; Riesch, Schlupp, Tobler, & Plath, 2006). In contrast, fish inhabiting normal freshwater streams experience higher oxygen concentrations and more complex communities with higher levels of inter-specific competition and predation. Divergent selection across habitat types has led to locally adapted *P. mexicana* populations that are phenotypically distinct and are undergoing ecological speciation (Tobler et al., 2018).

Given the complexity of selective regimes and phenotypic variation, it remains unclear what selective pressures drove the evolution of specific traits. For example, previous studies have demonstrated morphological variation between fish from the two habitat types, with sulphide spring fish exhibiting significantly enlarged heads, different caudal peduncle proportions and lower body depths compared to populations in nonsulphidic environments (Tobler et al., 2011). Although H_2S is a strong source of selection that can impact a variety of traits (Tobler, Passow, Greenway, Kelley, & Shaw, 2016), it is unlikely that H_2S per se caused the observed morphological divergence (Tobler et al., 2018). Two alternative scenarios have been proposed: (1) Body shape differences may have arisen through selection on locomotion. Fish in the sulphidic habitats face constraints in organismal energy budgets (Passow, Arias-Rodriguez, & Tobler, 2017a), and energy limitation—in conjunction with high levels of intraspecific competition (Culumber et al., 2016)—should select for energy-efficient steady swimming. In contrast, higher levels of predation in nonsulphidic habitats (Riesch et al., 2010; Tobler et al., 2006) should select for a higher burst swimming performance to evade predator attacks. (2) Body shape differences may have arisen through selection on oxygen acquisition. Respiratory adaptations have previously been shown to facilitate survival in the toxic and hypoxic sulphide spring environments. Sulphide spring fish spend a substantial amount of time engaging in aquatic surface respiration (Plath et al., 2007), exhibit increased gill surface areas (Tobler et al., 2011) and have physiological adaptations that impact oxygen transport and consumption (Barts et al., 2018; Passow, Arias-Rodriguez, et al., 2017a). Therefore, hypoxia in sulphidic habitats might select for a higher ventilation capacity, allowing fish to maximize the

amount of water they can pump over their gills. Naturally, these two hypotheses are not mutually exclusive; fish from sulphidic environments may simultaneously exhibit more efficient steady swimming and higher ventilation capacity. However, functional trade-offs may cause discrepancies between predicted and observed performance differences between fish from different habitats (Ghalambor et al., 2003). Based on previously established trade-offs between steady and burst swimming, we expect that neither sulphidic nor nonsulphidic fish can optimize energy-efficient swimming and fast-start performance simultaneously. Similarly, selection for higher ventilation capacity may adversely impact aspects of swimming performance, or vice versa.

To understand how morphological variation between fish from sulphidic and nonsulphidic environments impacts performance, we quantified multiple metrics of organismal performance related to locomotion and ventilation. Performance metrics were used to test hypotheses about performance differences between populations, identify potential functional trade-offs and relate individual variation in performance to variation in morphology.

2 | MATERIALS AND METHODS

2.1 | Study organisms and general experimental design

Several sulphide springs inhabited by *P. mexicana* occur in the states of Tabasco and Chiapas, Mexico (Palacios et al., 2016). For the present study, we collected young adult fish (standard length: 28–38 mm) from a sulphidic (El Azufre I: N 17.442°/W 92.775°) and a nonsulphidic population (Río Tacotalpa: N 17.275°/W 92.462°) near Tapijulapa, Tabasco. Fish were then transported them to Kansas State University (KSU), where they were housed in 80-litre tanks and given two months to acclimate to standardized laboratory conditions, irrespective of their habitat of origin. Fish were kept under nonsulphidic and normoxic conditions, a constant temperature of 25°C and a 12:12 hr-light:dark photoperiod. Fish were fed ad libitum and had access to flake food and frozen brine shrimp twice daily. We chose to acclimate all individuals to one standardized environment, because maintaining stable H_2S-rich water is difficult in the laboratory. In addition, the toxic effects of H_2S lead to high mortality of fish from nonsulphidic populations, preventing factorial experimental designs where the performance of fish from different habitats is measured under multiple environmental conditions. Consequently, our experiments only quantify the functional repercussions of traits that vary between sulphidic and nonsulphidic populations, and we cannot capture potential trait–environment interactions that might impact performance of fish in their natural habitats.

After acclimation, fish were separated into groups of 3–4 individuals and housed in 40-litre tanks, facilitating the tracking of individual fish across different portions of the experiment. For each fish, we quantified three aspects of performance: (a) burst swimming upon a simulated predator attack, (b) steady swimming and (c) gill ventilation. The order of performance trials was randomized across individuals, and individuals were allowed to recover for at least one week between trials. Fish were fasted for 24 hr prior to each performance trial to ensure they were in a post-absorptive state (Kieffer, 2000; Niimi & Beamish, 1974). After the completion of all performance trials, individuals were euthanized using MS-222 (500 mg/L buffered to pH 7.5 with sodium bicarbonate) for morphological analyses. A total of N = 71 fish were tested (Table S1). Unless stated otherwise, all statistical analyses were conducted in R version 3.2.2 (R Core Team, 2019).

2.2 | Burst swimming performance

We quantified burst swimming performance of fish during their reflexive escape response (c-start) to simulated predator attacks (Domenici & Blake, 1997; Eaton, Bombardieri, & Meyer, 1977; Harper & Blake, 1990; Howland, 1974; Weihs, 1973). Fish from high-predation environments have previously been documented to perform faster c-starts than fish from low-predation environments (Langerhans, Gifford, & Joseph, 2007; Langerhans et al., 2004; Walker, 1997), and several studies have linked burst swimming performance with survival in the presence of predators (e.g. Ingley & Johnson, 2016; Walker, Ghalambor, Griset, Mckenny, & Reznick, 2005).

Methods for the quantification of fast-start responses were adapted from previous studies (Ingley, Camarillo, Willis, & Johnson, 2016; Langerhans et al., 2004). For each trial, we placed a fish into a clear acclimation cylinder (5.5 cm in diameter) within a larger test arena (circular tank with a 40 cm diameter). To minimize vertical displacement and approximate two-dimensional escape responses, the water level was maintained at a depth of ~3 cm. After a 10-min acclimation period, the cylinder was removed, and we struck the arena with a probe (6 mm in diameter and 90 cm long) within ~1 body length of the fish's caudal region to evoke an escape response. After the first trial, fish were placed back into the cylinder, given the same acclimation period, and tested twice more with the same procedures, yielding three burst swimming performance trials per fish. Each trial was filmed from above with a Sony NXCAM NEX-FS700 high-speed camera (Sony Corporation, Tokyo, Japan) at 120 frames per second (fps).

Videos were analysed frame by frame using the DLTdv6 tracking software (Hedrick, 2008) in MATLAB 2016a (MathWorks Inc.) to quantify four metrics of burst swimming performance (Walker et al., 2005) following methods established by Langerhans (2009b): (a) total distance travelled (d_{net} [cm]) is the net distance a fish travelled within 1/12 of a second after bending into the c-shape; (b) rotational velocity (ω_S [°/s]) is the average rotational velocity of the head from the moment the fish begins bending into the c-shape until it has completely bent (rotational angle of bend divided by duration); (c) maximum velocity (v_{max} [cm/s]) is the greatest change in distance between two consecutive frames (1/120 of a second); and (d) maximum acceleration (a_{max} [cm/s^2]) is the greatest positive change in velocity

between two consecutive frames. For each individual, we calculated the average of each metric across the three trials.

To reduce data dimensionality, we conducted a principal component analysis (PCA) on the different metrics of burst swimming performance using a correlation matrix, and we retained a single PC axis that accounted for 57% of variation for subsequent analyses. Variables met all assumptions of PCA. PC axis 1 was positively correlated with maximum velocity, maximum acceleration and distance travelled (Table S2). To compare burst swimming performance between populations, we used PC scores as the dependent variable in a multivariate generalized linear model (GLM) using the *car* package (Fox & Weisberg, 2011) in R. Habitat of origin (sulphidic vs. nonsulphidic) and sex were used as factors and body mass ($\log_{10}$-transformed) as a covariate. Note that interactions between predictor variables were not significant ($F < 0.045$, $p > .833$) and excluded from the final model to allow interpretation of main effects.

2.3 | Steady swimming performance

We quantified metrics for steady swimming performance, an energy-efficient mode of locomotion that fish use during place-holding against water flow, foraging and mate searching (Blake, 2004; Domenici, 2003b; Langerhans, 2008). To quantify steady swimming performance, we measured the critical swimming speed and swimming kinematics at different swimming speeds for each individual.

Critical swimming speed is defined as the maximum speed at which a fish can maintain steady swimming (Blake, 2004; Domenici, 2003a; Plaut, 2001) and can be quantified by incrementally increasing the swimming speed until an individual fatigues (Brett, 1964). Critical swimming speed can then be calculated as $U_{crit} = U_f + U_s \times (t_f/t_s)$, where U_f is the highest flow velocity maintained for a full time interval, U_s is the velocity increment, t_f is the time to fatigue at the last flow speed and t_s is the time interval at which increases in speed occur (Brett, 1964). To quantify U_{crit}, individual fish were placed in a 5-L swim tunnel (Loligo Systems ApS), in which they were exposed to laminar flow with adjustable speed (Ellerby & Herskin, 2013). Trials started with a 20-min acclimation period, including 10 min without flow and 10 min at a flow speed of one body length per second (BLs^{-1}). After acclimation, fish were incrementally exposed to higher flow speeds (U_s = 1 BLs^{-1}) every 10 min (t_s = 600 s). Acclimation times and speed increments were adopted from previous studies (Hammill, Wilson, & Johnston, 2004; Oufiero & Garland, 2009; Sfakianakis, Leris, & Kentouri, 2011). Trials were immediately terminated once a fish reached fatigue.

During each steady swimming trial, fish were filmed at 120 fps using a high-speed camera twice during each interval (once immediately after the speed was increased and a second time halfway into a time interval). Four videos were selected at speeds ~25%, 50%, 75% and 100% of each individual fish's U_{crit} (Oufiero & Garland, 2009). At each speed, we quantified five kinematic variables relevant for the hydrodynamics of steady swimming (see Langerhans, 2009b; McHenry, Pell, & Long, 1995): (a) tail-beat frequency (f [Hz]) was measured as the inverse average period of ten complete tail-beat cycles; (b) rostral amplitude (R [mm]) was measured as half the distance between right and left excursions of the anterior tip of the rostrum; (c) tail-beat amplitude (H [mm]) was measured as half the distance between right and left excursions of the caudal fin; (d) propulsive wavelength (λ [mm]) was quantified as double the posterior half-wavelength; and (e) propulsive wave speed (c [mm/s]) was calculated by multiplying the propulsive wavelength with the tail-beat frequency ($c = \lambda * f$). For R, H and λ, measurements were taken by averaging the values of each across three complete tail beats.

Variation in U_{crit} (in BLs^{-1}) was analysed using a GLM with habitat of origin and sex as factors and body mass ($\log_{10}$-transformed) as a covariate. Note that interactions between predictor variables were not significant ($F < 0.049$, $p > .826$) and excluded from the final model. To analyse swimming kinematics, we conducted a PCA on the different metrics using a correlation matrix, and we retained a single PC axis accounting for 45% of variation for subsequent analyses. PC axis 1 was positively correlated with all five kinematic variables (Table S3). Since we had multiple measurements of swimming kinematics per individual at different swimming speeds, we used PC1 score as a dependent variable in mixed models as implemented in R's lme package (Bates et al., 2018). We contrasted alternative models to illuminate the effects of swimming speed, sex, habitat of origin and their interactions. The null model included body mass ($\log_{10}$-transformed) and an individual ID (random factor; to account for the nonindependence of measurements at different speeds). Models were evaluated based on Akaike information criterion for small sample sizes (AIC_c) (Johnson & Omland, 2004); models with $\Delta AIC_c < 2$ were assumed to be well supported (Burnham & Anderson, 2004).

2.4 | Gill ventilation capacity

Gill ventilation in teleost fishes has been described as a two-pump system, in which water is taken through the mouth into the buccal cavity, pumped into opercular cavity and out through the opercular openings, resulting in a unidirectional flow across the respiratory surfaces (Hughes, 1958). The rate at which water is pumped over the gills is consequently limited by the capacity of the first pump (i.e. the frequency of ventilation and the buccal volume). We quantified these variables for each individual to estimate the maximum gill ventilation capacity.

To measure the maximum ventilation frequency, fish were driven to maximum aerobic performance using chase trials (see Brennan, Hwang, Tse, Fangue, & Whitehead, 2016). Fish were placed in a circular arena (28 cm in diameter), given a 10-min acclimation period, and then chased along the edge of the arena for 6 min. After chasing, individuals were immediately transferred into a photo tank (18 × 10.5 × 10 cm) with aerated water and filmed at 60 fps. Fish were recorded eight times for 3 s each, 15 s apart. We counted the number of respiratory cycles for each 3-s interval (in pumps/s), retaining the highest value for subsequent analyses.

To estimate the volume of the buccal cavity for each fish, we performed a sagittal cut from the centre of the body towards the mouth and a second cut in a planar fashion just behind the opercula, yielding in two separate halves of the head. Digital images were then taken of each sagittal plane using a Canon Rebel T5i camera (Canon USA Inc.), allowing for the measurement of the length of the buccal cavity. Additional images were taken of the planar planes at the anterior and posterior edge of the buccal cavity, allowing for the measurement of the corresponding radii of the buccal cavity. Measurements on the two halves were averaged. To approximate the buccal volume, we treated the shape of the cavity as a circular truncated cone, for which the volume can be calculated as $V_b = (1/3) * h * \pi * (r_1^2 + r_1 r_2 + r_2^2)$, where h is the length of the buccal cavity, and r_1 and r_2 are the radii of the anterior and posterior ends, respectively.

Maximum gill ventilation frequency (in s^{-1}; square-root-transformed) was analysed using a GLM with habitat of origin and sex as factors and body mass (log_{10}-transformed) as a covariate. Note that interactions between predictor variables were not significant ($F < 0.005$, $p > .943$) and excluded from the final model. Buccal cavity volume (in mm^3) was log_{10}-transformed and also analysed with GLM using the same predictor variables. Interactions between predictor variables were not significant ($F < 1.488$, $p > .227$) and excluded from the final model. Finally, we approximated the volume of water an individual can pump per unit time (V_W in $mm^3 s^{-1}$) by multiplying the maximum ventilation frequency with the buccal volume for each individual. Log_{10}-transformed values of V_W were analysed using GLM with the same models described above; interactions between predictor variables were not significant ($F < 0.908$, $p > .344$) and excluded from the final model.

2.5 | Testing for functional trade-offs and linking performance to morphology

To identify potential functional trade-offs, we tested for correlations between performance metrics (burst swimming performance, U_{crit} and maximum ventilation frequency) using Pearson correlation implemented in the *Hmsci* package in R (Harrell Jr, 2018). To relate variation in performance to variation in morphology, we quantified the body shape based on lateral photographs of each individual using geometric morphometrics. We digitized 11 landmarks using the software program tpsDig version 2.10 (Rohlf, 2006): (a) tip of the upper jaw; (b) anterior and (c) posterior insertions of the dorsal fin; the (d) dorsal and (e) ventral insertions of the caudal fin; (f) the anterior junction of the anal fin; (g) the bottom of the head where the operculum breaks away from the body outline; (h) the dorsal endpoint of the opercular bone; (i) the dorsal and (j) ventral insertions of the pectoral fin; and (k) the centre of the orbit. We then conducted a two-block partial least-squares analysis (PLS) to test for covariation among performance metrics and body shape as implemented in the program tpsPLS (Rohlf, Corti, & Olmstead, 2000). PLS reduces data dimensionality by creating new linear combinations between the two different sets of data (*i.e.* body shape and performance), and singular axes are generated to maximize the covariation between these two sets of variables (Rohlf et al., 2000). Different performance metrics (PC axes describing fast-start performance, U_{crit} and maximum ventilation frequency; all *z*-transformed prior to PLS) were designated as the first variable block, landmark coordinates describing body shape as the second variable block.

3 | RESULTS

3.1 | Variation in performance

Analysis of PC1 describing variation in burst swimming revealed significant differences between *P. mexicana* from sulphidic and nonsulphidic habitats, but no effects of sex or body mass (Table 1A). Compared to fish from sulphidic habitats, those from nonsulphidic habitats reached higher velocities, accelerations and longer distances travelled (Figure 1, Table S2).

TABLE 1 Results of GLM on different response variables quantified in this study: (A) burst swimming performance (PC score based on multiple metrics; Table S2), (B) critical swimming speed (U_{crit}), (C) maximum gill ventilation frequency, (D) buccal cavity size (V_B) and (E) the total volume of water an individual can pump per unit time (V_W)

	F	*df*	*P*	Effect size
(A) Burst swimming (PC1)				
Sex	0.1587	1	.692	0.003
Habitat	20.520	1	<.001	0.268
Body mass (log_{10}-transformed)	1.773	1	.187	0.033
(B) U_{crit}				
Sex	0.195	1	.660	0.003
Habitat	5.393	1	.023	0.075
Body mass (log_{10}-transformed)	1.038	1	.312	0.015
(C) Maximum gill ventilation frequency (sqrt-transformed)				
Sex	0.029	1	.866	<0.001
Habitat	46.645	1	<.001	0.411
Body mass (log_{10}-transformed)	0.940	1	.362	0.012
(D) V_B (log_{10}-transformed)				
Sex	1.009	1	.319	0.015
Habitat	37.391	1	<.001	0.358
Body mass (log_{10}-transformed)	28.052	1	<.001	0.295
(E) V_W (log_{10}-transformed)				
Sex	1.284	1	.261	0.019
Habitat	19.105	1	<.001	0.222
Body mass (log_{10}-transformed)	28.137	1	<.001	0.296

Note: Effect sizes were quantified with partial eta squared.

Poecilia mexicana from the sulphidic habitat reached significantly higher U_{crit} than those from nonsulphidic habitats (Figure 2a). Neither sex nor body mass were significant predictors of U_{crit} (Table 1B). Population differences in steady swimming were also reflected in swimming kinematics; the sole supported model ($\Delta AIC_c < 2$) indicated effects of swimming speed and habitat of origin (Table 2). PC scores increased with increasing swimming speeds in both populations, and fish from the sulphidic population exhibited lower PC scores than those from the nonsulphidic populations (Figure 2b). That is, for any given swimming speed, fish from the sulphidic habitat exhibited lower propulsive wavelength, tail-beat frequency, rostral amplitude, tail-beat amplitude and propulsive wave speed.

Analysis of maximum ventilation rates revealed no effects of size or sex, but fish from nonsulphidic habitats were able to go through more respiratory cycles than those from sulphidic habitats (Table 1C, Figure 3a). Buccal volumes were significantly correlated with body mass, and sulphidic fish exhibited significantly larger buccal volumes than nonsulphidic ones (Table 1D, Figure 3b). The larger buccal volumes in sulphidic fish offset lower maximum ventilation rates; the estimated volume of water an individual can pump per unit time correlated with body size and was significantly higher in fish from the sulphidic than the nonsulphidic population (Table 1E, Figure 3c).

3.2 | Functional trade-offs and morphology–performance relationships

The Pearson correlations provided evidence for a trade-off (negative correlation) between burst and steady swimming ($r_p = -0.29$, $p = .016$). In addition, there was a positive correlation between burst swimming and maximum ventilation frequency ($r_p = 0.28$, $p = .018$). Correlations among all other performance metrics were not significant ($|r_p| < 0.16$, $p > .184$; see Table S4 for details). The PLS analysis produced four dimensions of covariation between body shape and performance variables, revealing clear correlations between body shape and performance (Table 3, Figure 4). The first pair of singular axes explained 92.3% of covariation between body shape and performance ($p = .038$), and correlation between the two variable blocks was 0.539 ($p = .001$). The latent body shape vector of the first PLS dimension was associated with variation in head size and caudal peduncle proportions (Figure 4) and closely resembled body shape variation between sulphidic and nonsulphidic populations (Figure S1). Individuals with larger heads and longer caudal peduncles tended to exhibit high U_{crit}, and those with smaller heads and higher caudal peduncles tended to exhibit high burst speed and high ventilation frequencies (Figure 4).

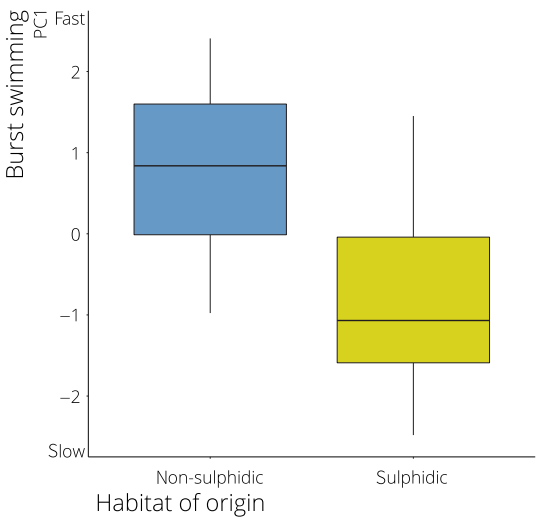

FIGURE 1 Box plot showing differences in burst swimming performance (PC1, see Table S2) between individuals from the sulphidic and nonsulphidic population. Positive scores indicate faster burst swimming responses and negative scores slower ones. Boxes cover the first through third quartile of the data; horizontal black lines indicate the median

4 | DISCUSSION

We investigated populations of *Poecilia mexicana* that are locally adapted to divergent environmental conditions to test how phenotypic variation impacts different aspects of organismal performance and to identify potential functional trade-offs. Consistent with our predictions, we found correlations between body shape and performance. Fish from sulphidic habitats exhibited a higher steady swimming performance and a higher ventilation capacity, whereas those from nonsulphidic habitats had faster escape responses to simulated predator attacks. Functional trade-offs were evident between different modes of swimming, but not between aspects of swimming and ventilation. Overall, our analyses provided insights into the functional consequences of previously documented phenotypic variation.

4.1 | Swimming in sulphidic and nonsulphidic habitats

Quantifying different aspects of swimming performance in fish from sulphidic and nonsulphidic populations revealed significant differences, both in metrics associated with burst and steady swimming. During simulated predator attacks, fish from nonsulphidic habitats exhibited faster escape responses, a performance metric that predicts survival in the presence of predators in other fish species (Langerhans, 2009a; Langerhans et al., 2007, 2004; Walker, 1997). In contrast, fish from sulphidic habitats appeared to be more energy-efficient swimmers. They exhibited higher critical swimming speeds and had to exert less effort (*i.e.* lower trail-beat frequency, rostral amplitude, trail-beat amplitude, propulsive wave length and speed) to maintain any given speed. These population differences in burst and steady swimming were evident in a functional trade-off, where individuals with high fast-start performance exhibited lower critical swimming speeds and *vice versa*. Functional trade-offs between burst and steady swimming

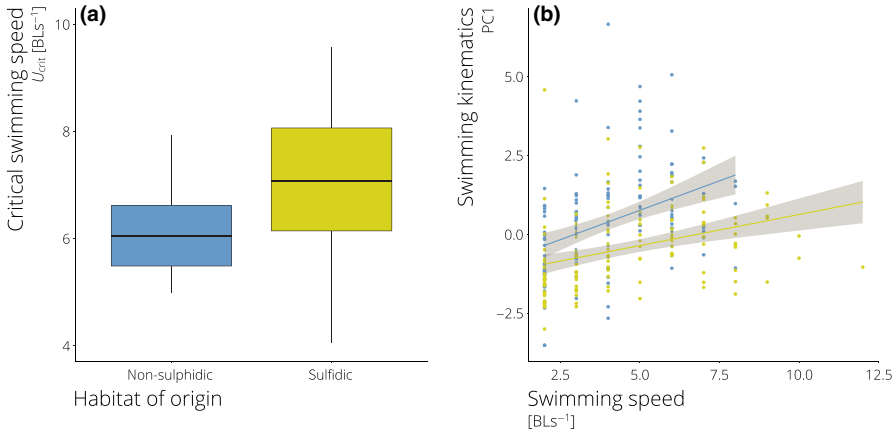

FIGURE 2 Comparison of steady swimming performance between individuals from the sulphidic and nonsulphidic population. (a) Box plot showing the difference in critical swimming speed (U_{crit}); boxes cover the first through third quartile of the data; horizontal black lines indicate the median. (b) Scatter plot visualizing the best fitting model explaining variation in steady swimming kinematics (PC1, see Table S3). Data points for fish from the nonsulphidic population are shown in blue, and those for fish from the sulphidic population are shown in yellow. Grey shaded areas represent the 95% confidence intervals for the population-specific regressions

have been documented in poeciliids (Ingley et al., 2016; Langerhans, 2009b) and other fishes (Ellerby & Gerry, 2011; Yan, He, Cao, & Fu, 2012) and underlie population differences in morphology when divergent selection favours different aspects of locomotion performance (Langerhans, Layman, Langerhans, & Dewitt, 2003). Fundamental constraints in combination with replicated environmental gradients may therefore be important contributors to convergent morphological evolution, both across independent lineages that have colonized H_2S-rich environments (Tobler et al., 2011) and in other systems (Ingley et al., 2016; Langerhans, 2009b).

Differences in swimming performance between fish from sulphidic and nonsulphidic environments match a priori predictions based on the prevailing ecological conditions in each habitat type. Flow regimes, which can have a profound impact on fish morphology and swimming (Langerhans, 2008; Meyers & Belk, 2014), are unlikely to drive population differences, because fish from nonsulphidic habitats had a lower steady swimming performance even though they are usually exposed to higher flows. Sulphidic habitats are also characterized by toxic levels of H_2S and hypoxia (Bagarinao, 1992; Tobler et al., 2016), and they exhibit species-poor communities with few inter-specific competitors and aquatic predators (Greenway et al., 2014). Despite the lack of predatory fishes, it remains unclear whether predation pressure is actually relaxed in sulphidic habitats because of aerial predation by birds (Riesch et al., 2010) and insects (Tobler, 2009). Thus, we hypothesize that increases in steady swimming at the cost of burst swimming performance is primarily driven by energetic constraints. Energy limitation in sulphidic environments arises because the presence of H_2S and hypoxia requires fish to trade off time between benthic feeding and aquatic surface respiration (Tobler, Riesch, Tobler, & Plath, 2009), which mediates short-term survival in the extreme environment (Plath et al., 2007). In addition, the maintenance of

TABLE 2 Results of mixed models analysing steady swimming kinematics (PC1; Table S3)

	−2 × log-likelihood	*N*	AIC_C	ΔAIC_C	AIC_C weight
Speed × habitat + body mass + (1|ID)	−452.56	280	931.40	0.0	0.8388
Speed + body mass + (1|ID)	−458.79	280	931.00	3.6	0.1415
Speed × sex × habitat + body mass + (1|ID)	−449.33	280	940.30	8.8	0.0101
Speed × sex + body mass + (1|ID)	−457.09	280	940.40	8.9	0.0096
Body mass + (1|ID) (null model)	−486.03	280	982.50	51.1	<0.001
Habitat + body mass + (1|ID)	−484.62	280	982.90	51.5	<0.001
Sex × Habitat + body mass + (1|ID)	−482.07	280	983.20	51.8	<0.001
Sex + body mass + (1|ID)	−485.69	280	985.00	53.5	<0.001

Note: Models are ordered based on ΔAIC_C values. The null model included body mass (log_{10}-transformed) and the individual identification number (1|ID) as a random factor.

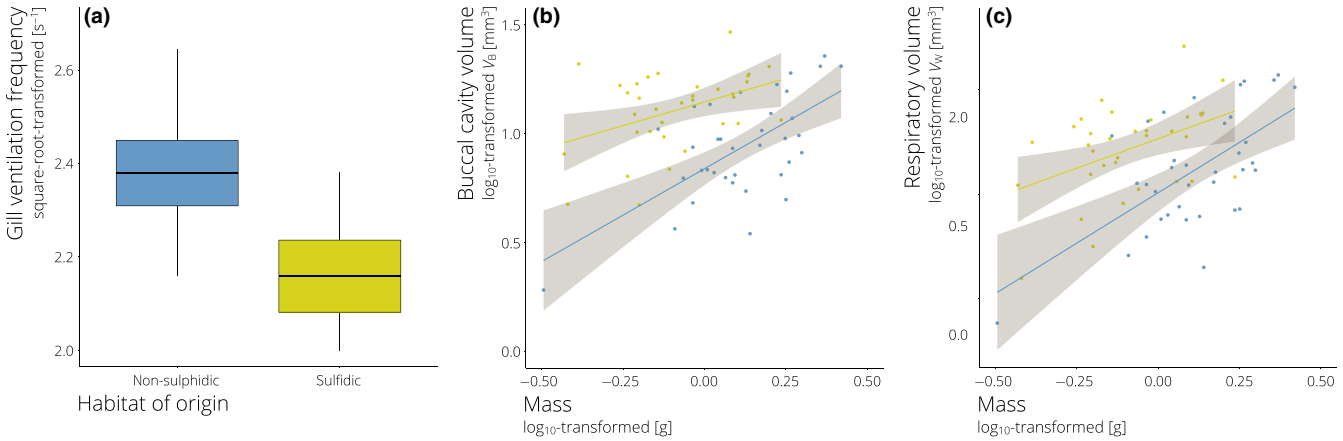

FIGURE 3 Comparison of metrics contributing to ventilation efficiency between individuals from the sulphidic and nonsulphidic population. (a) Box plot comparing differences in gill ventilation frequency (sqrt-transformed) between populations; boxes cover the first through third quartile of the data; horizontal black lines indicate the median. (b) Scatter plot illustrating differences in buccal cavity volume (V_B, $\log_{10}$-transformed) as a function of body mass ($\log_{10}$-transformed) in fish from sulphidic (yellow) and nonsulphidic (blue) populations. (c) Scatter plot illustrating differences in the total volume of water an individual can pump per unit time (V_W, $\log_{10}$-transformed) as a function of body mass ($\log_{10}$-transformed) in fish from sulphidic and nonsulphidic populations. Grey shaded areas in scatter plots represent the 95% confidence intervals for the population-specific regressions

TABLE 3 Results of partial least-squares analysis (PLS) examining covariation between performance variables and body shape for (A) males and (B) females

	Dim 1	Dim 2	Dim 3	Dim 4
Burst swimming PC1	0.613	0.015	−0.280	0.739
Burst swimming PC2	0.239	−0.291	0.913	0.154
U_{crit}	−0.569	0.565	0.236	0.549
Gill ventilation frequency	0.494	0.772	0.177	−0.358
Singular value	0.923	0.061	0.013	0.003
$P_{singular\ value}$	0.038	0.910	0.980	0.998
Correlation	0.539	0.271	0.379	0.288
$P_{correlation}$	0.001	0.589	0.039	0.154

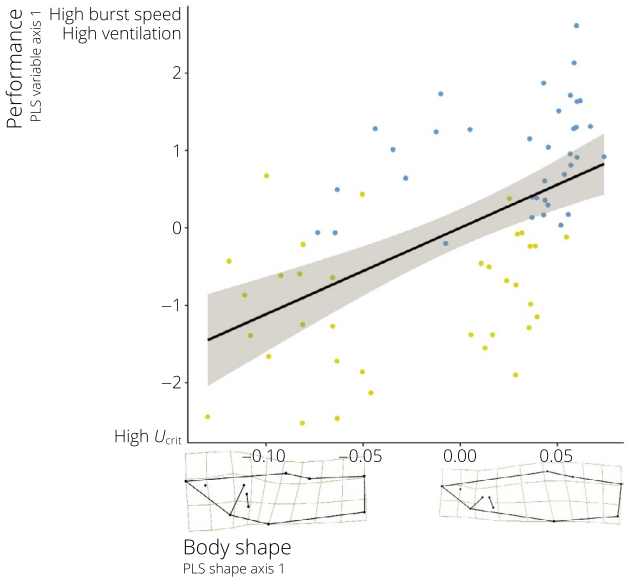

FIGURE 4 Correlation of partial least-squares latent vectors of body shape and performance (r = .539, p = .001). Deformation grids illustrate the body shapes at either end of the latent body shape vector. Detailed correlation of different performance metrics along the y-axis can be found in Table 3. Data points in blue represent individuals from nonsulphidic habitats, those in yellow from sulphidic ones. The grey shaded area in the plot represents the 95% confidence intervals for the regression

homeostasis in sulphide springs is energetically costly (Tobler et al., 2018). Constraints in energy acquisition and increased maintenance costs are reflected in fish from sulphidic habitats having comparatively low body condition (Tobler, 2008). Since fish used in our experiments were acclimated to the same environmental conditions, including ad libitum access to food, energy limitation is unlikely to be a proximate cause for differences in steady swimming performances between populations; rather, we suggest that exposure to energetic constraints in the natural habitats has caused evolutionary shifts in traits underlying steady swimming. Energy limitation has also been hypothesized to be the key driver underlying the evolution of other phenotypic traits in *P. mexicana* that have colonized sulphidic habitats, including changes in body size and routine metabolic rates (Passow, Greenway, Arias-Rodriguez, Jeyasingh, & Tobler, 2015), the expression of energetically costly behaviours (Plath, 2008) and organs (Schulz-Mirbach et al., 2016), as well as reproductive life-history traits (Riesch, Plath, Schlupp, Tobler, & Langerhans, 2014). Higher burst swimming performance in fish from nonsulphidic habitats may therefore simply indicate that escaping predators has more substantial fitness impacts than energetic constraints associated with steady swimming. Ultimately, our analyses of swimming performance in sulphidic and

nonsulphidic populations of *P. mexicana* largely match the findings in other study systems, even if the relative importance of different sources of selection that push populations along the burst-to-steady swimming continuum may differ. However, even though our findings match a priori predictions, we cannot exclude the possibility that there are nonadaptive explanations for the patterns we observed. Future studies will consequently need to investigate how performance variation links to fitness under natural conditions and whether patterns of convergent evolution in body shape across replicated populations of *P. mexicana* inhabiting H_2S-rich habitats have the same functional repercussions as documented here for a single population pair. Directly linking performance variation to fitness and uncovering evidence for convergent evolution of organismal function would further ascertain the adaptive significance of performance metrics documented here.

4.2 | Oxygen acquisition in sulphidic and nonsulphidic habitats

Habitats with high levels of H_2S are typically characterized by hypoxia (Bagarinao, 1992), which exacerbates the toxic effects of H_2S itself (Bagarinao & Lantin-Olaguer, 1998). Fish in sulphidic habitats should therefore be selected for efficient oxygen acquisition, and we predicted that they would exhibit higher maximum gill ventilation rates and higher buccal volumes, ultimately maximizing the volume of water that can be pumped over the gas-exchange surfaces. Although we did find that fish from sulphidic habitats exhibited higher rates of water being pumped per unit time, this was driven by differences in buccal volumes between populations. In fact, we documented a trade-off between ventilation frequency and buccal volume, where larger buccal volumes were associated with lower ventilation frequencies. This finding parallels a similar trade-off between the ability to either generate high fluid speeds or high volumetric flow rates during suction feeding in centrarchid fishes (Higham, Day, & Wainwright, 2006).

The higher capacity for ventilation in fish from sulphidic habitats adds further evidence for the importance of trait modifications associated with oxygen acquisition, distribution and use during adaptation to sulphide spring environments. On a molecular level, *P. mexicana* populations in sulphidic habitats exhibit differential expression of and positive selection on oxygen transport genes (Barts et al., 2018) and upregulation of genes associated with anaerobic ATP production (Kelley et al., 2016). On a behavioural level, fish from sulphidic habitats heavily engage in aquatic surface respiration (Plath et al., 2007), which is a common response in hypoxia-tolerant fish species (Kramer & McClure, 1982; Lewis, 1970; Timmerman & Chapman, 2004). And on a morphological level, increased head size is correlated not only with ventilation efficiency (documented here), but also with significant increases in gill surface areas (Tobler et al., 2011). Interestingly, evolutionary increases in head size and concomitant changes in gill surface area are common in other lineages of sulphide spring poeciliids (Tobler & Hastings, 2011) and other fishes exposed to chronic hypoxia (Bouton, Visser, & Barel, 2002; Chapman, Galis, & Shinn, 2000; Chapman & Liem, 1995). Whether variation in head size (and presumably correlated differences in buccal volumes) also impacts ventilation frequency (negatively) and the volume of water pumped through time (positively) in other fishes remains to be investigated. In essence, our study adds additional evidence for how increases in head size might be adaptive in the context of hypoxia (increased ventilation capacity), but we still fail to understand the putative costs associated with these traits in normoxic environments.

4.3 | Functional trade-offs and trait–performance correlations

Our experiments have revealed clear trade-offs between different modes of swimming (as previous studies), but no evidence for trade-offs between swimming and ventilation. In fact, there was positive association between critical swimming speed and gill ventilation frequency. This is not surprising because an increased capacity to acquire oxygen should directly impact aerobic performance (Kiceniuk & Jones, 1977). Selection on ventilation efficiency consequently may not be expected to negatively impact fish locomotion, or vice versa. Instead, there may be functional facilitation between ventilation and steady swimming performance (Ghalambor et al., 2003).

The final intriguing observation from our study is the significant correlation between phenotypic traits (body shape) and organismal performance. The major axis of covariation between body shape and performance particularly related to variation in head size and caudal peduncle proportion resemble body shape differences between populations (see Figure S1). This suggests that morphological differences between populations are directly related to performance variation documented here. However, it is important to note that physiological traits correlated with morphology may also be important. Previous studies documented significant physiological differences between fish from sulphidic and nonsulphidic populations (Passow, Arias-Rodriguez, et al., 2017a; Passow, Henpita, et al., 2017b), which may impact locomotion and ventilation. The fact that body shape cannot be the sole predictor of performance variation in our study is evidenced by the fact that we could not detect any differences in locomotion and ventilation between males and females, even though *P. mexicana*—like all other poeciliids (Culumber & Tobler, 2017)—exhibit pronounced sexual dimorphism. Although the lack of sex differences in our study may be a consequence of limited statistical power, other studies have suggested that compensatory mechanisms mitigate costs associated with sexual dimorphism and equalize performance between the sexes (Husak & Swallow, 2011; Oufiero, Jugo, & Garland, 2014).

5 | CONCLUSIONS

Our study revealed that previously documented phenotypic differences between *P. mexicana* from sulphidic and nonsulphidic

habitats are linked to differences in organismal function in the context of locomotion and ventilation. Although documented trade-offs between steady and burst swimming performance largely match the findings of previous studies, we also documented how variation in head size impacts ventilation in fish. Hypoxia-induced morphological variation is common in fishes, but its functional significance for ventilation remains largely unexplored. Future studies should also focus on characterizing trade-offs and facilitation among different aspects of organismal function (*e.g.* locomotion, ventilation, foraging and reproduction) (Ghalambor et al., 2003). A better understanding of interdependencies between different aspects of organismal performance will improve our understanding of phenotypic evolution, just like the recognition of pleiotropic effects has in the past (Yamamoto, Byerly, Jackman, & Jeffery, 2009).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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