

RESEARCH ARTICLE

Context-dependent relationships between swimming, terrestrial jumping and body composition in the amphibious fish *Kryptolebias marmoratus*

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ABSTRACT

Understanding the mechanisms that create phenotypic variation within and among populations is a major goal of physiological ecology. Variation may be a consequence of functional trade-offs (i.e. improvement in one trait comes at the expense of another trait) or alternatively may reflect the intrinsic quality of an organism (i.e. some individuals are simply better overall performers than others). There is evidence for both ideas in the literature, suggesting that environmental context may mediate whether variation results from trade-offs or differences in individual quality. We tested this overarching ‘context dependence’ hypothesis by comparing the aquatic and terrestrial athletic performance of the amphibious fish *Kryptolebias marmoratus* captured from two contrasting habitats, a large pond and small burrows. Overall, pond fish were superior terrestrial athletes but burrow fish were better burst swimmers, suggestive of a performance trade-off at the population level. Within each population, however, there was no evidence of a performance trade-off. In burrow fish, athletic performance was positively correlated with muscle content and body condition, consistent with the individual quality hypothesis. In pond fish, there was only a relationship between glycolytic white muscle and aquatic burst performance. Notably, pond fish were in better body condition, which may mask relationships between condition and athletic performance. Overall, our data highlight that population-level trends are insufficient evidence for the existence of phenotypic trade-offs in the absence of similar within-population patterns. Furthermore, we only found evidence for the individual quality hypothesis in one population, suggesting that patterns of phenotypic covariance are context dependent.

KEY WORDS: Trade-offs, Biomechanics, Individual variation, Muscle, Jumping, Mangrove rivulus

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INTRODUCTION

Individuals within a population can differ considerably in numerous aspects of their phenotype (e.g. morphology, behaviour), and understanding the proximate causes and ultimate consequences of individual variation has emerged as a major goal for biologists (Bennett, 1987; Bolnick et al., 2003; Dingemanse and Doctermann, 2013; Metcalfe et al., 2016). Individual variation has long been recognized as a key component of natural selection and adaptation (Darwin, 1859), and more recent studies have also demonstrated that phenotypic differences between individuals of the same population can influence population dynamics (Bjørnstad and Hansen, 1994; Allgeier et al., 2020) and ecosystem function (Hart et al., 2016; Raffard et al., 2017; Allgeier et al., 2020). However, the proximate mechanisms underpinning patterns of individual variation within populations are often unclear. Why do the trait values of some individuals fall above the population mean while those of other individuals fall below it?

Several hypotheses have been proposed to explain the existence of diverse phenotypes within populations. The ‘quality hypothesis’ posits that individual variation reflects individual quality, and that high- and low-quality individuals exist within populations. High-quality status is conferred when intrinsic differences between individuals (e.g. genetics, environmental experiences) shape the phenotypic traits that determine individual fitness (Wilson and Nussey, 2010). High-quality individuals should therefore fall above the population mean for many or all fitness-linked performance traits. For example, male plainfin midshipman (*Porichthys notatus*) that exhibited high reproductive success (i.e. large brood size) also had a large body size, built large nests and were highly successful at attracting females (Bose et al., 2018).

In contrast to the quality hypothesis, the ‘trade-off hypothesis’ states that individuals within a population use alternative adaptive solutions to maximize their performance and fitness, resulting in divergent phenotypes. Multiple adaptive peaks can occur as a result of trade-offs caused by morphological and/or physiological specialization, whereby the specialization of traits to improve one performance axis is mechanically linked to decreased performance in another (Agrawal, 2019; Cohen et al., 2020). For example, bluegill sunfish (*Lepomis macrochirus*) in littoral habitats exhibited greater manoeuvrability and poorer steady-state swimming performance compared with those in pelagic habitats because the morphological features (e.g. body shape) that maximized manoeuvrability were incompatible with steady-state swimming (Ellerby and Gerry, 2011; Gerry et al., 2011). Trade-offs can similarly result from the differential allocation of resources to various life processes. For instance, allocating energy to maximize reproduction can limit the energy available for somatic growth or activity (Koch and Wieser, 1983; Hayward and Gillooly, 2011),

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and allocating energy to the viscera may also impair survival if this increases predation risk or impedes locomotion (Witter and Cuthill, 1993; Plaut, 2002). Although trade-offs are considered one of the most critical factors in shaping the organismal phenotype, empirical studies often find positive or non-significant relationships between traits, rather than negative trade-offs as expected (Bono et al., 2017; Agrawal, 2019; Cohen et al., 2020). Consequently, a major goal of physiological ecology is understanding the conditions under which trade-offs are expressed.

The environment may mediate the expression of trade-offs and/or the variation in individual quality (West-Eberhard, 2003). In variable environments, for example, trade-offs may be minimal as these environments are often inhabited by generalist species that perform well under a wide range of conditions (Gilchrist, 1995; Kitahara et al., 2000; Kassen, 2002; Devictor et al., 2008). Environmental food availability can also mask or reveal trade-offs, particularly among life history traits. Allocating energy to one life history process (e.g. reproduction) often occurs at the detriment of another (e.g. growth) in energy-limited environments, whereas benign or energy-rich environments can mask such energetic trade-offs (Zera and Harshman, 2001). Overall, however, the context dependence of both the quality hypothesis and the trade-off hypothesis is poorly understood, especially in wild populations.

Amphibious fishes are powerful models to study potential performance trade-offs because they regularly transition between two habitats that differ dramatically in physical properties: water and land (Gordon et al., 1969; Graham, 1997; Wright and Turko, 2016). Moving between buoyant aquatic environments and gravity-dominated terrestrial environments poses biomechanical challenges for the musculoskeletal system of fishes (Martinez, 1996; Gillis and Blob, 2001; Turko et al., 2017). Indeed, many studies have demonstrated that divergent morphological and/or physiological features are often required to enhance locomotion in each medium, such as modified appendages (Harris, 1960; Standen et al., 2014) or differences in muscle activity patterns (Perlman and Ashley-Ross, 2016; Foster et al., 2018) and movement kinematics (Gillis, 1998; Hsieh, 2010). Nevertheless, very few studies have explicitly explored trade-offs between aquatic and terrestrial modes of locomotion in fishes, despite strong suspicions that such trade-offs exist (Shine and Shetty, 2001; Gibb et al., 2013; Styga et al., 2017). For example, terrestrial acclimation resulted in oxidative (red) muscle hypertrophy in the amphibious mangrove rivulus (*Kryptolebias marmoratus*), which improved terrestrial locomotor performance. This effect was reversed when fish returned to water, suggesting that the morphological features that enhance terrestrial locomotion may be detrimental for locomotor movement in aquatic environments (Brunt et al., 2016; McFarlane et al., 2019). In the present study, we used *K. marmoratus* to determine whether the trade-off hypothesis or the quality hypothesis would explain variation in athletic ability within wild populations, and whether the prevailing hypothesis is context dependent and thus changes across dramatically different environments.

Kryptolebias marmoratus is a euryhaline amphibious killifish that inhabits small pools (e.g. ponds or crab burrows) throughout mangrove swamps of the tropical western Atlantic (Taylor, 2012; Tatarenkov et al., 2017). These fish frequently leave water (emerge) to escape unfavourable aquatic conditions (e.g. hypoxia, hydrogen sulphide; Regan et al., 2011; Rossi et al., 2019a), disperse or forage (Taylor, 1990; 2012), and can survive out of water for several weeks (Taylor, 1990; Turko et al., 2019). Consequently, effective locomotion in both aquatic and terrestrial settings is critical. We studied wild *K. marmoratus* from two genetically

distinct populations that live in dramatically different habitats (Turko et al., 2018). The first habitat consisted of brackish water-filled crab burrows that are often extremely hypoxic and rich in toxic hydrogen sulphide (Turko et al., 2018; Rossi et al., 2019a). Fish from these burrow habitats are known to spend up to 90% of their time on land (Turko et al., 2018). The second habitat was a freshwater pond, which is often more benign in terms of abiotic conditions (Turko et al., 2018; Rossi et al., 2019a). Fish from the freshwater pond spend only one-third as much time on land as those in crab burrows (Turko et al., 2018). We assessed terrestrial and aquatic locomotor performance in fish from both populations. In either population, the trade-off hypothesis predicts a negative correlation between terrestrial and aquatic locomotor performance, while the quality hypothesis predicts a positive slope. To understand whether muscle phenotype underlies performance variation in water and on land, we also measured the size and number of aerobic (red) and anaerobic (white) muscle fibres. The trade-off hypothesis predicts a negative correlation between the amount of red versus white muscle. Finally, we measured body condition of each individual. The quality hypothesis predicts a positive correlation between body condition and locomotor performance, such that individuals in good condition are also good athletes.

MATERIALS AND METHODS

Collection information

In December 2016, we collected *Kryptolebias marmoratus* (Poey 1880) ($N=41$, 27.5 ± 0.7 mm standard length, 0.33 ± 0.02 g) from two contrasting habitats (pond and crab burrows) on Long Caye, Lighthouse Reef, Belize, that we have studied previously (Turko et al., 2018; Rossi et al., 2019a; Fig. S1). The pond site ($17^{\circ}13.24'N$, $87^{\circ}35.53'W$) is a shallow open freshwater pool filled with algae and woody debris. The crab burrow site ($17^{\circ}13.08'N$, $87^{\circ}35.65'W$) is a densely vegetated forest dominated by red mangroves, *Rhizophora mangle*, and containing abundant brackish water-filled burrows excavated by land crabs *Cardisoma guanhumi* (Sutton et al., 2018).

Water temperature, salinity and dissolved oxygen at each site were measured (YSI Multiparameter Meter, Hanna Instruments, Woonsocket, RI, USA) 1–2 times per day over 7 days. At the pond versus burrow sites, water temperature was 27.0 ± 1.2 versus $29.4\pm 1.8^{\circ}C$, salinity was 0.9 ± 0.2 versus $22.8\pm 5.2\text{‰}$, and dissolved oxygen was 46.7 ± 49.0 versus $11.0\pm 5.7\%$ air saturation (means \pm s.d.). Fish were captured using a combination of minnow and Taylor traps and then held in 120 ml plastic containers for 24–48 h prior to experimentation. Fish captured from the pond habitat were held in water collected directly from the pond, while fish collected from crab burrows were held in seawater that was diluted with rainwater to approximate natural conditions (18–20%). Fish were held under a natural photoperiod (approximately 12 h:12 h light:dark) at ambient temperature ($27\text{--}29^{\circ}C$); all experiments were similarly conducted at ambient temperature. All experiments were approved by the Belize Fisheries Department and the University of Guelph animal care committee.

Aquatic performance

Aquatic burst performance was measured by stimulating fish to emerge using aquatic hypercapnia (Robertson et al., 2015) and measuring the magnitude of the jump. Individual fish were placed in a 10 cm \times 10 cm \times 22 cm tank filled to 6 cm depth with water. Salinity of the water was adjusted to match that of each collection location (i.e. 1‰ for pond fish, 20‰ for burrow fish). Fish were filmed at 500 frames s $^{-1}$ using two high-speed cameras positioned at a 90 deg angle to one another (Fastec IL5SM8, Fastec Imaging

Table 1. Aquatic athletic performance variables used in the principal component analysis

	Jump height (mm)	Total jump distance (mm)	Nose velocity (mm ms ⁻¹)	Nose acceleration (mm ms ⁻²)
Pond	38.8±3.2	39.9±3.6	1.61±0.14	0.42±0.05
Burrows	55.1±2.5	60.0±2.7	2.54±0.10	0.67±0.04
<i>P</i> -value	0.0003	<0.0001	<0.0001	0.0003
PC1 contribution (%)	26.7	27.7	27.7	18.0
PC1 loading strength	0.91	0.93	0.93	0.75
PC1 quality of representation	0.83	0.86	0.86	0.56

All values have been adjusted to a 27.5 mm long fish (the average size of fish used in this study) and are presented as means±s.e.m. *P*-values are the results of Student's *t*-tests, and principal component values show the loading of each variable on the first principal component (PC1) used for subsequent analyses. Bold indicates significant differences between populations.

Corporation, San Diego, CA, USA). To consistently elicit a robust and rapid emersion response, ~15 g of ENO (sodium carbonate, sodium bicarbonate, citric acid; GlaxoSmithKline) was dissolved in 750 ml of water to saturate it with CO₂ prior to introducing the fish. Water was changed between each trial to ensure consistent jump conditions across all fish. To allow for 3D reconstruction of video data, cameras were calibrated via direct linear transformation using custom Matlab code (DLTcal.m; Hedrick, 2008). Seven points along the midline of each fish were digitized including the nose and tail (DLTdv.m; Hedrick, 2008). Vertical jump height, total jump distance, velocity and acceleration were calculated for each jump. The jump was chosen as the first emersion attempt that brought the fish's full head clear of the water surface (Regan et al., 2011). Most fish entirely left the water within a few seconds of being placed in the filming arena, and all trials were complete within 5 min. After each trial, fish were returned to their individual holding containers to recover overnight before terrestrial athletic performance was measured.

Terrestrial performance

The day after aquatic performance was measured (~14–18 h later), terrestrial athletic performance was assessed by chasing fish to exhaustion in a terrestrial arena as described previously (Brunt et al., 2016; McFarlane et al., 2019; Rossi and Wright, 2020). Briefly, individual fish were quickly transferred from their holding containers to a plastic arena (30×60 cm) lined with damp filter paper. The salinity of the water used to dampen the paper was adjusted to match that of each collection location. Fish were encouraged to jump with gentle prods to the caudal peduncle until exhaustion (no response to 5 sequential prods ~1 s apart). Great care was taken to ensure that these prods did not interfere with the jumping motion. All trials were video recorded using a tripod-mounted video camera (GoPro Hero3, 60 frames s⁻¹) mounted directly above the experimental arena. Video files were subsequently imported into ImageJ software (Schneider et al., 2012) and the coordinates of the tip of the snout were measured before and after each jump to calculate displacement. Only tail-flip

jumps were included in our analysis. Rare instances of other repositioning behaviours (e.g. 'squiggles'; Pronko et al., 2013) were excluded as these resulted in minimal displacement (less than ~1 body length). From each trial, we calculated the distance of the longest jump, the mean of the longest 5 jumps, the mean jump distance, the median jump distance and the total distance travelled. After each trial, fish were euthanized using tricaine methanesulphonate, weighed, and photographed for subsequent measurement of body length. The body cavity was then opened via a ventral incision and samples were fixed in 10% neutral buffered formalin for 24 h, then transferred to 70% ethanol for long-term storage.

Organ investment and muscle histology

To assess investment in energetically expensive tissues, we carefully dissected the liver and gonads from each fish, quickly blotted away excess ethanol, and weighed each to 0.001 g. As a result of fixation and ethanol storage, our measurements underestimate the mass of each organ in living fish, but careful dissection and accurate weighing of fresh tissues was not logistically possible in the field. At the whole-body level, fixed tissue weighed 7.8±0.8% less than fresh tissue (mean±s.e.m.) and these values were highly correlated (*r*=0.992). After dissection, a transverse steak of the axial muscle was taken from directly behind the dorsal fin and routinely embedded in paraffin wax for histology (Turko et al., 2018). Sections (5 µm) were cut on a rotary microtome and stained with haematoxylin and eosin. The size and number of red and white muscle fibres were quantified as previously described (Rossi et al., 2018; Rossi et al., 2019b). Red muscle fibres were easily distinguished from white muscle fibres on haematoxylin- and eosin-stained sections owing to their small size and triangular orientation at the lateral line (Fig. S2).

Statistical analysis

To account for both differences in body size among individuals and differences in how each trait scales with body size (Fig. S3), we first used standard length to calculate residual values for each trait. Mean

Table 2. Terrestrial athletic performance variables used in the principal component analysis

	Longest jump (mm)	Top 5 jumps (mm)	Mean jump (mm)	Median jump (mm)	Total distance (cm)	Number of jumps	Endurance (s)
Pond	29.0±0.7	26.2±0.5	8.5±0.2	6.2±0.2	1580±44	187±4	390±19
Burrows	24.7±0.6	21.7±0.5	7.1±0.2	5.6±0.3	1077±45	151±4	260±10
<i>P</i> -value	<0.0001	<0.0001	0.0001	0.057	<0.0001	<0.0001	<0.0001
PC1 contribution (%)	13.0	16.2	16.7	10.1	21.0	11.7	11.3
PC1 loading strength	0.77	0.86	0.88	0.68	0.98	0.73	0.72
PC1 quality of representation	0.60	0.74	0.77	0.46	0.96	0.53	0.52

All values have been adjusted to a 27.5 mm long fish (the average size of fish used in this study) and are presented as means±s.e.m. *P*-values are the results of Student's *t*-tests, and principal component values show the loading of each variable on the first principal component (PC1) used for subsequent analyses. Bold indicates significant differences between populations.

Table 3. Muscle and body condition performance variables used in the principal component analysis

	White muscle				Red muscle				Body condition		
	Feret diameter (μm)	Fibre area (μm^2)	Number of fibres	Total area (mm^2)	Feret diameter (μm)	Fibre area (μm^2)	Number of fibres	Total area (μm^2)	Body mass (mg)	Gonad mass (mg)	Liver mass (mg)
Pond	35.2 \pm 1.4	1641.8 \pm 46.0	1567 \pm 44	2.26 \pm 0.22	3.8 \pm 0.1	15.0 \pm 0.5	134 \pm 5	2003 \pm 83	343 \pm 6	11.9 \pm 1.0	15.5 \pm 1.8
Burrows	37.6 \pm 1.7	1470.7 \pm 59.6	1689 \pm 28	3.14 \pm 0.23	3.8 \pm 0.1	16.0 \pm 0.5	105 \pm 4	1731 \pm 104	317 \pm 11	4.4 \pm 0.5	4.8 \pm 0.3
<i>P</i> -value	0.29	0.029	0.025	0.0089	0.68	0.16	<0.0001	0.048	0.042	<0.0001	<0.0001
PC1 contribution (%)	8.9	10.4	35.4	45.3	32.1	28.3	7.6	32.0	24.2	35.3	40.5
PC1 loading strength	0.38	-0.41	0.76	0.86	0.91	0.86	0.45	0.91	0.70	0.84	0.90
PC1 quality of representation	0.14	0.17	0.58	0.74	0.83	0.74	0.20	0.83	0.48	0.71	0.81

All values have been adjusted to a 27.5 mm long fish (the average size of fish used in this study) and are presented as means \pm s.e.m. *P*-values are the results of Student's *t*-tests, and principal component values show the loading of each variable on the first principal component (PC1) used for subsequent analyses. Bold indicates significant differences between populations.

standard length was nearly identical between populations (burrow 27.5 \pm 1.1 mm, pond 27.4 \pm 0.6 mm, *t*-test *P*=0.92). This is a common statistical approach to correct for variation in body size that performs well when variation in body size is small and similar across groups, criteria met by our dataset (Reist, 1985; McCoy et al., 2006; Revell, 2009; Berner, 2011; Nakagawa et al., 2017). We took a conservative approach and assumed a common size–trait slope between populations when performing size adjustment. To confirm that our results were robust to this method of body size adjustment, we repeated our analyses allowing the slope to vary between populations; this second approach produced identical qualitative results (data not shown). Next, we used 5 separate principal component analyses (PCAs) to summarize the 22 size-adjusted athletic performance and body composition variables using the *prcomp* command in R statistical software (version 4.0.2; <http://www.R-project.org/>). In each case, the first principal component (PC1) explained much of the phenotypic variation we measured (aquatic performance 77.6%, terrestrial performance 65.3%, red muscle 65.0%, white muscle 40.7%, condition 66.6%) Furthermore, all traits loaded on PC1 strongly and in the same

direction (Tables 1–3). We therefore used PC1 of each aspect of athletic performance and body composition in subsequent analyses. To compare phenotypes between burrow and pond populations and to test for relationships among phenotypic traits, we used simple linear models that included the traits of interest and their interaction with habitat. When the phenotype by habitat interaction term was non-significant (*P*>0.05), we re-ran the model without the interaction to increase our power to detect main effects. Effect sizes of the relationships we evaluated among phenotypic traits cannot be simply expressed as an *R*² value because we included the habitat term in all models. Instead, we calculated the relative importance of each predictor variable (i.e. the portioned *R*²) using the method *lmg* in the package *relaimpo* (Grömping, 2006).

Within-population trait variation can sometimes be masked by principal component analyses when among-population differences are large relative to within-population variation (Du, 2019). Given that a main goal of our study was detecting potential within-population trade-offs, we repeated all analyses using separate PCAs for each population to test the robustness of our results. This second approach did not qualitatively affect our results (Fig. S4).

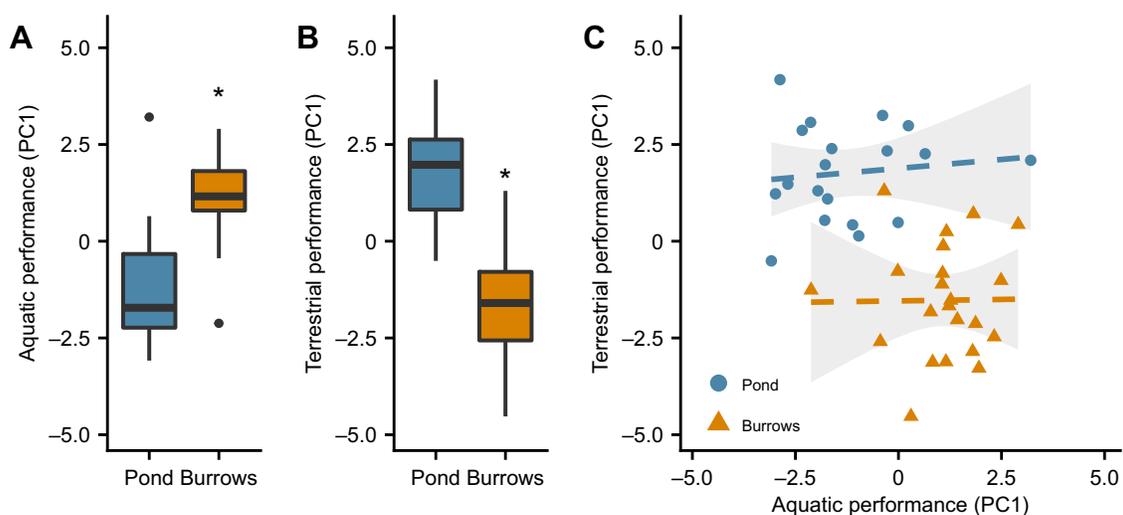


Fig. 1. Athletic performance of two wild populations of *Kryptolebias marmoratus*. Aquatic (A) and terrestrial (B) performance are shown as principal components (PCs) that assimilate several performance variables. For each boxplot, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the quartiles (i.e. 25th and 75th percentiles), whiskers show the highest and lowest values within 1.5 \times the interquartile range, and points show outliers. Asterisks represent significant differences among populations (*P*<0.05). (C) There was no significant relationship between aquatic and terrestrial performance after accounting for differences among habitats (*P*>0.05). Dashed lines show the best linear fit within each population; shaded grey regions are 95% confidence intervals.

RESULTS

At the population level, aquatic burst performance of *K. marmoratus* was better in fish captured from crab burrows than in those from the large pond ($F_{1,39}=30.65$, $P<0.00001$; Fig. 1A; Fig. S3A–D). In contrast, terrestrial athletic performance was higher in pond fish ($F_{1,39}=59.47$, $P<0.00001$; Fig. 1B; Fig. S3E–K). At the individual level, however, there was no relationship between aquatic and terrestrial performance after accounting for differences between habitats ($F_{1,38}=0.15$, $P=0.70$, lmg-partitioned $R^2=0.12$; Fig. 1C).

Crab burrow fish had more white glycolytic muscle than pond fish ($F_{1,39}=13.18$, $P=0.0008$; Fig. 2A; Fig. S3L–O), but there was no difference in red aerobic muscle between populations ($F_{1,39}=0.50$, $P=0.48$; Fig. 2B; Fig. S3P–S). At the individual level, the amount of white muscle was a significant predictor of aquatic burst performance ($F_{1,38}=6.97$, $P=0.012$, lmg-partitioned $R^2=0.22$; Fig. 2C) but red muscle was not ($F_{1,38}=0.39$, $P=0.54$, lmg-partitioned $R^2=0.003$; Fig. 2D), after accounting for differences

between habitats. Terrestrial performance was not related to the amount of white muscle ($F_{1,38}=0.59$, $P=0.45$, lmg-partitioned $R^2=0.11$; Fig. 2E). The relationship between red muscle and terrestrial performance depended on population (interaction $F_{1,37}=6.58$, $P=0.015$; Fig. 2F). In fish from crab burrows, there was a significant positive relationship between the amount of red muscle and terrestrial performance (*post hoc* $t=2.46$, $P=0.019$), but there was no significant relationship in pond fish ($t=-1.44$, $P=0.16$).

Pond fish were in significantly better body condition than crab burrow fish ($F_{1,39}=43.06$, $P<0.0001$; Fig. 3A). At the individual level, there was no relationship between body condition and aquatic burst performance after accounting for differences between habitats ($F_{1,38}=1.25$, $P=0.27$, lmg-partitioned $R^2=0.17$; Fig. 3B). Body condition was positively correlated with terrestrial performance in crab burrow fish (interaction $F_{1,37}=6.69$, $P=0.014$; *post hoc* $t=2.54$, $P=0.015$; Fig. 3C) but not pond fish ($t=-0.94$, $P=0.35$; Fig. 3C). Body condition was not significantly related to the amount of white

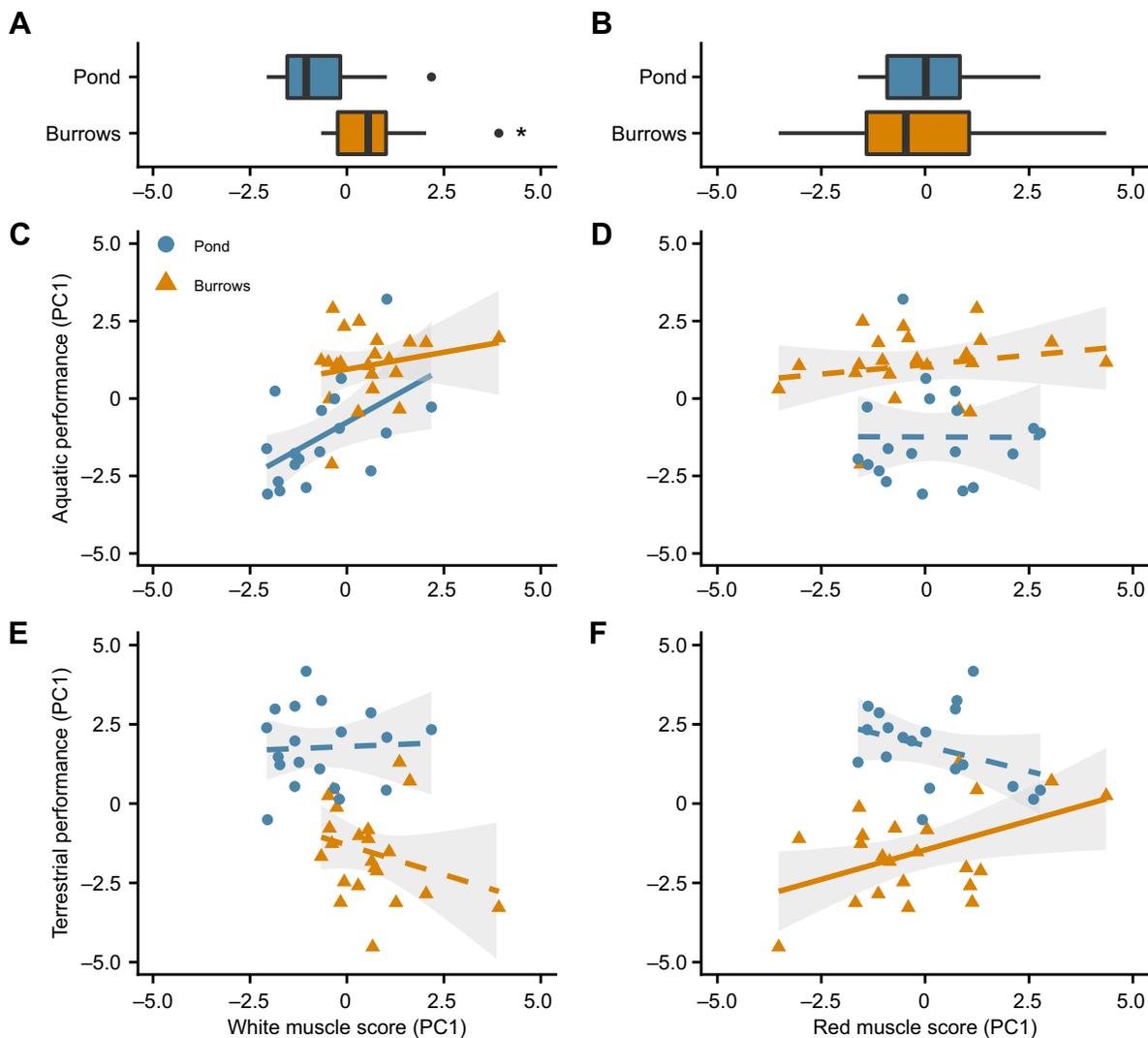


Fig. 2. Muscle phenotypes and athletic performance of two wild populations of *K. marmoratus*. White glycolytic (A) and red oxidative (B) muscle phenotypes are shown as principal component scores that assimilate several variables describing the size and number of muscle fibres. For each boxplot, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the quartiles (i.e. 25th and 75th percentiles), whiskers show the highest and lowest values within 1.5× the interquartile range, and points show outliers. Asterisks represent significant differences between populations ($P<0.05$). (C–F) Relationships between muscle phenotype (white: C,E; red: D,F) and athletic performance (aquatic: C,D; terrestrial: E,F). Significant linear relationships ($P<0.05$) are shown with solid lines; non-significant ($P>0.05$) relationships are shown with dashed lines. Shaded grey regions are 95% confidence intervals.

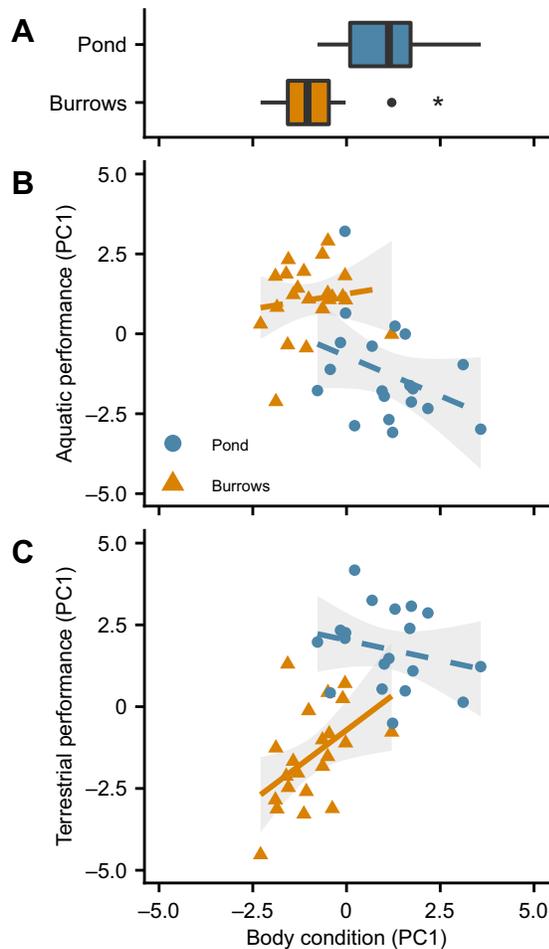


Fig. 3. Body condition and athletic performance of two wild populations of *K. marmoratus*. Body condition (A) is shown as principal component scores that assimilate variables describing body size, mass and organ investment. For each boxplot, the bold horizontal line in the middle of the box represents the median, the top and bottom of the box represent the quartiles (i.e. 25th and 75th percentiles), whiskers show the highest and lowest values within 1.5 \times the interquartile range, and points show outliers. Asterisks represent significant differences between populations ($P < 0.05$). (B,C) Relationships between body condition and athletic performance (aquatic, B; terrestrial, C). Significant linear relationships ($P < 0.05$) are shown with solid lines; non-significant ($P > 0.05$) relationships are shown with dashed lines. Shaded grey regions are 95% confidence intervals.

($F_{1,38}=3.91$, $P=0.055$, lmg-partitioned $R^2=0.18$; Fig. 4A) or red muscle in either population ($F_{1,38}=1.78$, $P=0.19$, lmg-partitioned $R^2=0.05$; Fig. 4B). There was no relationship between the amount of red and white muscle ($F_{1,38}=0.01$, $P=0.92$, lmg-partitioned $R^2=0.003$; Fig. 4B).

DISCUSSION

Our goal was to test whether variation in athletic ability among and within wild populations of *K. marmoratus* could be explained by the quality hypothesis or the trade-off hypothesis and whether these hypotheses depend on environmental conditions. At the population level, pond fish were better terrestrial athletes than crab burrow fish, whereas crab burrow fish had better burst locomotor abilities in water. These population-level results suggest a functional trade-off between terrestrial and aquatic locomotor performance, but we found no evidence for this trade-off among individuals within either population. Given this lack of individual-level relationships, we

suggest that there is no causative relationship between these aspects of locomotor performance. Rather, we found that both between- and within-population differences in aquatic burst performance could be explained by the amount of anaerobic white muscle. Crab burrow fish tended to have more white muscle than pond fish, and in both populations, individuals with more white muscle also tended to be better athletes in water. There was a positive relationship between the amount of aerobic red muscle and terrestrial performance in crab burrow fish, but between-population differences in terrestrial performance could not be explained by differences in muscle. There was also no relationship between red and white muscle phenotypes within or between populations. Our results suggest that aquatic and terrestrial performance are functionally independent in *K. marmoratus*, perhaps because they are largely powered by different subsets of muscle. Finally, we found that pond fish were in significantly better body condition than crab burrow fish. Body condition was positively correlated with terrestrial performance in crab burrow fish but not in pond fish, possibly because pond fish were in very high body condition overall. Taken together, our findings suggest that the quality hypothesis best explains variation in athletic ability in fish from the crab burrow habitat, as those in better body condition were better terrestrial athletes. In contrast, we found no evidence for the quality hypothesis in pond fish, and no evidence for the trade-off hypothesis in either population. We conclude that environmental context appears to mediate the relationship between body condition and athletic performance in this species.

Aquatic versus terrestrial performance trade-offs

At the population level, we found strong correlational evidence for a trade-off between aquatic and terrestrial performance. Crab burrow fish exhibited better burst locomotor abilities in water, while both burst and endurance terrestrial locomotor performance were higher in pond fish. Numerous studies have demonstrated that population-level swimming performance of fishes can diverge in habitats with different abiotic and biotic conditions (Langerhans, 2009; Ellerby and Gerry, 2011; Oufiero et al., 2011; Ingley et al., 2016). However, at the level of individual fish within each population, we found no statistical relationships between aquatic and terrestrial performance, which strongly suggests that traits that improved aquatic performance (e.g. amount of white muscle) were not strongly linked to terrestrial performance, and vice versa. Our measurement of aquatic performance was limited to burst swimming, but on land we measured both burst and endurance performance. Both types of terrestrial performance were positively correlated (i.e. both loaded in the same direction in our PCA), indicating that our data provide no evidence for burst–burst or burst–endurance trade-offs. There are many reasons why trait relationships that emerge across populations may not be reflected at the level of individuals. For instance, when environmental conditions differ between populations, phenotypic plasticity and/or local adaptation may cause traits to diverge regardless of whether there are functional links among them (Savolainen et al., 2013; Peiman and Robinson, 2017; Agrawal, 2019). The presence of trade-offs may even constrain phenotypic divergence if both traits contribute to fitness. Overall, our findings highlight that a negative correlation between populations is insufficient evidence to conclude that a trade-off is present. To be confident about causation, the same negative correlation should also be evident at the individual level and there should be a mechanistic explanation for why the trade-off exists.

The skeletal musculature of *K. marmoratus* was a relatively good predictor of locomotor performance. Fish with more white muscle had better burst swimming abilities in water at both the population

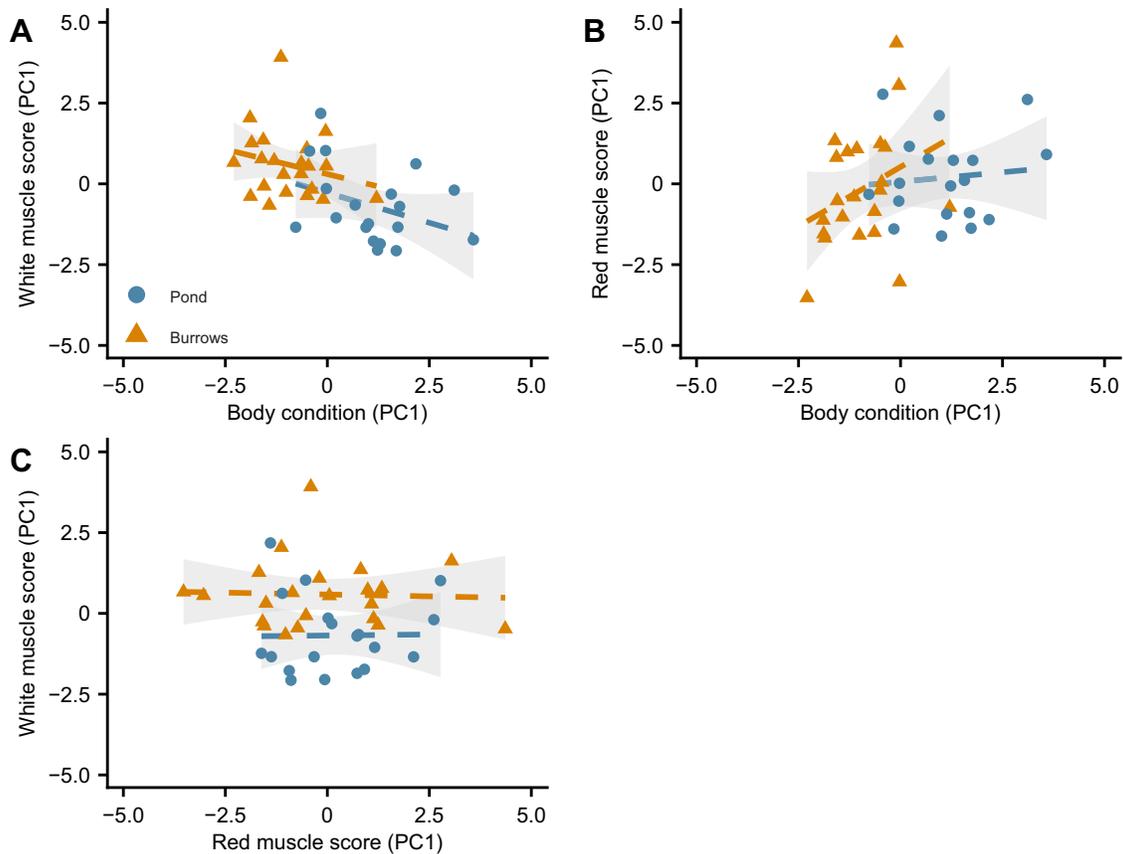


Fig. 4. Body condition and muscle phenotype of two wild populations of *K. marmoratus*. There was no significant relationship between body condition and white (A) or red (B) muscle score. (C) There was also no relationship between red and white muscle score. Dashed lines show the best linear fit within each population (all $P > 0.05$). Shaded grey regions are 95% confidence intervals.

and individual level. White muscle is the primary muscle type recruited in rapid escape responses in fishes (Domenici and Blake, 1997). We also found that the amount of red muscle was positively related to terrestrial performance in the crab burrow population, consistent with several laboratory studies that have demonstrated that *K. marmoratus* with larger red muscle fibres tend to have better jumping abilities on land (Brunt et al., 2016; McFarlane et al., 2019; Rossi et al., 2019b, 2020). At the level of individual fish, there was no relationship between red and white muscle phenotype within either population, which suggests that these muscle types can respond independently to natural selection and/or environmental context (i.e. phenotypic plasticity). Functional independence of these muscle types may also partly explain why we found no relationship between aquatic and terrestrial athletic performance. Interestingly, fish from the pond habitat were better terrestrial athletes than crab burrow fish despite having the same amount of red muscle. One possibility is that terrestrial performance diverged between populations owing to differences in the red muscle that were not evident from our histological analysis. Abiotic conditions (e.g. dissolved oxygen, temperature) can dramatically alter many physiological factors that determine red muscle performance, including capillarity (Johnston and Bernard, 1984), mitochondrial density (Johnston and Bernard, 1982; Egginton and Sidell, 1989; Sanger et al., 1990), myofibrillar volume (Johnston and Maitland, 1980) and activity of important metabolic enzymes (Johnston and Bernard, 1982). Another possibility is that fish differed in age among populations, as age has previously been shown to influence

jumping ability (Styga et al., 2017; Rossi et al., 2019b), although we have no reason to suspect this is the case. Assessment of these other performance traits was beyond the scope of our current study but would be an interesting avenue for future work.

Population-level divergence in aquatic and terrestrial locomotion may reflect behavioural differences among fish from each habitat. We previously found that fish captured from crab burrows spent almost 90% of their time out of water, triple the amount of time spent by fish from the pond habitat (Turko et al., 2018). However, video footage captured from within a crab burrow habitat revealed that although fish leave water frequently, they may remain relatively inactive on land (see movie 1 in Turko and Wright, 2015). Spending time out of water may therefore serve as a refuge from severe aquatic conditions for crab burrow fish, rather than reflect an active terrestrial lifestyle. Consistent with this interpretation, in this study we found that crab burrow fish were much better at exiting water than pond fish. Pond fish, in contrast, do not leave the water as often (Turko et al., 2018), but are more likely than burrow fish to experience weeks to months of drought during the dry season (A.J.T., T.A.B., S.C., D.S.T., P.A.W. and E.M.S., personal observation; Fig. S1). During this time without water, effective movement overland may be critical for seeking moist refuges or new aquatic environments, and we hypothesize that this explains why pond fish were better terrestrial athletes overall. Thus, we suggest that both the local environment and the intrinsic emersion behaviour of *K. marmoratus* may be important determinants of locomotor ability.

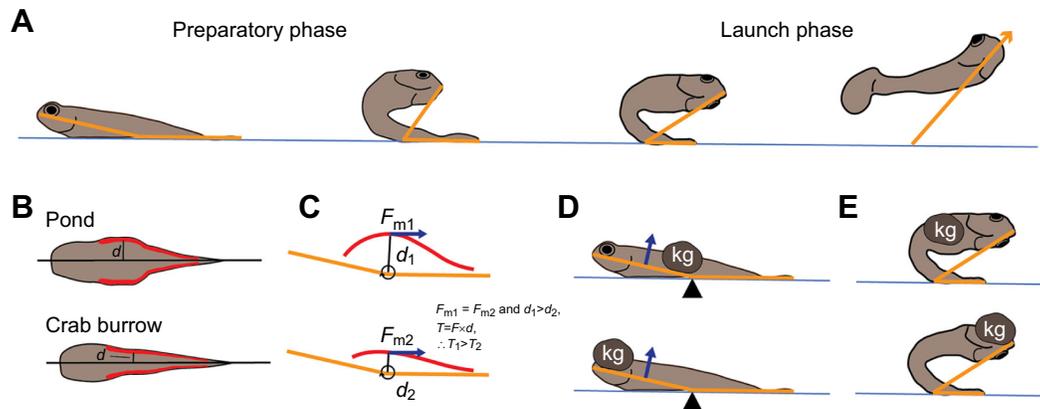


Fig. 5. Terrestrial jumping mechanics in *K. marmoratus*. (A) Body shape change throughout a jump. Mechanical advantage acts on a terrestrially jumping fish as a factor of the location of the muscle relative to the bending axis of the fish (B,C) and as a factor of the distribution of the fish's mass (D,E). In the absence of differences in red muscle mass, mechanical advantage may explain differences in performance between pond and burrow fish. To initiate a terrestrial jump, fish lift their heads off the substrate (A, preparatory phase). To lift its head, the fish must produce torque (T), which is the product of muscle force (F) multiplied by the distance (d) the force is applied from the axis of rotation (denoted by the circular arrow in C). Fish with wider girth will have red muscle positioned farther from the spine and therefore have a greater mechanical advantage (B,C). This means that in the initial part of the jump (A, preparatory phase), muscles of wider fish need to produce less force to lift the head off the ground. In addition, the fish's mass distribution impacts the ratio of in- and out-lever arms used in jumping. Mass that is located close to the axis of rotation (symbolized by 'kg' added mass D and E) has a small out-lever, increasing the mechanical advantage of the muscle and allowing it to decrease its effort to lift a given load. In addition, the mass of the animal distributed closer to the tail helps to keep the tail loaded throughout the preparatory phase of the jump, possibly increasing the efficiency of translating muscle force into ground reaction forces, resulting in an increase in jump length.

Body condition and athletic performance

Pond fish were in better overall body condition than crab burrow fish, possibly because they spend more time in water and may therefore have had increased feeding opportunities (Turko et al., 2018). However, the pond habitat is also ephemeral (Fig. S1). Temporary habitats often favour individuals that develop rapidly and reproduce before the conditions deteriorate (Errea and Danulat, 2001; Blažek et al., 2013; Vrtilek et al., 2019), which may explain why we found greater gonadal investment in the pond fish. Similarly, increased energy stores in the form of larger livers in pond fish may extend the survival time of *K. marmoratus* on land during prolonged periods of drought (Turko et al., 2019; Rossi and Wright, 2020). In contrast, the relatively harsh abiotic aquatic conditions in the crab burrow habitat may have contributed to the poorer body condition in this population. Exposure to aquatic hypoxia, for example, has been shown to impair reproduction and reduce liver mass in numerous fishes, including other killifishes (e.g. Cheek et al., 2009; Borowiec et al., 2015). Additionally, crab burrow fish were previously found to have a ~30% higher routine metabolic rate than pond fish, and this increased energetic demand may result in less energy that can be allocated to the gonad and liver (Turko et al., 2018).

One of our most striking observations was that, despite similar amounts of red muscle between populations, pond fish could jump almost 50% further on land than crab burrow fish. These population differences are difficult to explain based entirely on physiological effects, and we speculate that biomechanical differences between populations may be involved. Three mechanical factors contribute to terrestrial jump performance: muscle position, body mass distribution and reduction of slip (Fig. 5). In pond fish with wider bodies, red muscle is located further from the rotational axis, providing a greater mechanical advantage and thereby producing more jump torque with equivalent muscle force production (Fig. 5B,C). Body mass distribution in pond fish is also located closer to the axis of rotation, while in crab burrow fish, body mass is distributed closer to the head. Mass that is located close to the axis of rotation has a small out-lever compared with mass located towards

the head, again increasing the mechanical advantage of the muscle and minimizing its effort to lift a given load (Fig. 5D). The distribution of mass in pond fish may also provide the benefit of reducing slip. Keeping mass on the ground for longer during the launch phase of the tail flip jump more efficiently transfers muscle force to ground reaction force at the tail (Fig. 5E). Overall, the addition of an optimally located large visceral mass may provide enough mechanical and frictional advantage to outweigh its added mass cost, helping to mechanically explain why higher condition pond fish perform much better than crab burrow fish despite no difference in their red muscle content. Of course, there may be a limit to the mechanical advantage benefit, as at some point the cost of added mass will outweigh the mechanical advantage it provides. This may explain why we found no positive relationship between body condition and terrestrial performance in pond fish.

Conclusions

We tested whether the quality hypothesis or the trade-off hypothesis could explain variation in the athletic performance of *K. marmoratus* from two wild populations. We found a negative population-level association between aquatic and terrestrial modes of locomotion, superficially consistent with the trade-off hypothesis. Trade-offs are not expressed at the level of a population, however, but should rather be thought of as traits with consequences for individuals within those groups (Agrawal, 2019; Cohen et al., 2020). We found no relationship between aquatic and terrestrial performance at the individual level, and therefore conclude that trade-offs do not determine locomotor variation in these populations. Rather, we found some evidence for the quality hypothesis, but only in one population. Overall, our results highlight that patterns of phenotypic covariance can be highly environmentally dependent.

Finally, our results suggest functional independence between aquatic and terrestrial modes of locomotion, possibly because they are powered by different subsets of muscle (i.e. low phenotypic integration). Natural selection has been hypothesized to favour low levels of phenotypic integration in heterogeneous environments (Schlichting, 1989; Earley et al., 2012). Our data are consistent with

this idea, and the capacity of *K. marmoratus* to adjust (via plasticity or selection) aquatic and/or terrestrial performance without negative consequences for performance in the alternative environment is likely to be advantageous for life in highly variable mangrove forests.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.J.T., G.S.R., T.A.B., S.C., D.S.T., P.A.W., E.M.S.; Methodology: A.J.T., G.S.R., T.A.B., E.M.S.; Formal analysis: A.J.T., G.S.R., E.M.S.; Investigation: A.J.T., G.S.R., E.M.S.; Resources: A.J.T., P.A.W., E.M.S.; Data curation: A.J.T., G.S.R., E.M.S.; Writing - original draft: A.J.T., G.S.R.; Writing - review & editing: T.A.B., S.C., D.S.T., P.A.W., E.M.S.; Visualization: A.J.T., G.S.R., E.M.S.; Supervision: P.A.W., E.M.S.; Funding acquisition: P.A.W., E.M.S.

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Data availability

Data are available from the University of Guelph Research Data Repository: <https://doi.org/10.5683/SP3/UM2MQL>.

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