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Hypoxia drives plastic divergence in cichlid body shape

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Abstract Organisms experience multiple selective agents that can influence phenotypes through heritable and/or plastic changes, often reflecting complex interactions between phenotype and environment. Environmental factors can directly influence phenotypes, but also indirectly affect phenotypic variation when genetic/plastic change in one trait results in correlated genetic/plastic change in another trait. In fishes, body shape is a trait that might be particularly prone to influence from environmental pressures that act on other morphological features. Variation in dissolved oxygen among aquatic environments has a large impact on the size of the gills and brains of fishes. It is likely that dissolved oxygen interacts with other environmental factors to both directly and indirectly influence patterns of body shape variation. We examined effects of dissolved oxygen on body shape variation among populations of an African cichlid fish (*Pseudocrenilabrus multicolor*) from multiple high- and low-oxygen sites within a single drainage in Uganda. A split-brood laboratory experiment was used to estimate plasticity of gill and brain size, and we used morphometric analyses to identify variation in body shape in F_1 offspring. Several analyses enabled us to identify genetic effects among populations, and effects of oxygen acting either directly on body shape or indirectly through its effects on gill and brain size. A large part of the variation in body shape was due to plastic variation in gill size associated with dissolved oxygen. Fish raised under low oxygen had deeper heads and shorter bodies, and this variation was driven by both direct effects of oxygen and indirect effects of gill size variation. Body shape variation in fishes should reflect interacting effects of multiple environmental factors that act directly or indirectly on morphology. Body shape might be particularly difficult to predict when phenotypes are plastic, because changes among populations would occur rapidly and be unrelated to genetic variation.

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Introduction

Environmental pressures can act in a pleiotropic fashion to produce a phenotype. This can occur when selection acts on variation in multiple traits at the same time, such as when multiple organ systems are functionally integrated. It can also occur when multiple traits undergo plastic responses simultaneously (i.e. plasticity integration; Parsons and Robinson 2006). However, such correlated trait changes are not always adaptive. They instead might be due to developmental constraints imposed when adaptive changes in one trait (i.e. direct effects) cause correlated (i.e. indirect) *maladaptive* (or non-adaptive) changes in another trait. The correlated changes might be genetic, but in the case of a non-adaptive correlated change, it seems intuitive that they might be more likely to be plastic. Some studies of phenotypic variation control for plastic versus genetic effects, but whether these effects are *direct* or *indirect* effects of the environment is not normally assessed.

One trait that is likely to be strongly influenced by correlated morphological changes is body shape. Body shapes vary widely even within taxa, and should reflect adaptations to specific modes of living. For example, water flow might influence whether fish should preferentially use sustained or burst swimming, which in turn influences body and fin shape (Langerhans 2008). Feeding modes (e.g. Schluter and McPhail 1992; Svanbäck and Eklöv 2003; Andersson et al. 2006; Ruehl and DeWitt 2007) and predation pressure (e.g. Langerhans and DeWitt 2004; Andersson et al. 2006; Langerhans 2009) should also affect body shape. What is less well-known is the degree to which these factors, and others, influence other morphological traits that might in turn influence body shape, perhaps imposing a constraint on adaptive body shape evolution. Predictions for body shape variation under different environmental conditions are therefore not straightforward.

An abiotic factor that could affect body shape is dissolved oxygen. Previous studies in fishes have shown bodies to be deeper, and heads to be longer and wider, under low-oxygen (i.e. hypoxic) conditions (Chapman et al. 2000, 2008). While both genetic and plastic variation in these body shape components has been identified between high- and low-oxygen environments (Chapman et al. 2008), it is not known whether this variation is adaptive. Genetic variation in body shape could reflect adaptive evolution, and plastic variation could reflect an adaptive response to the environment. An alternative is that body shape variation could be a maladaptive or non-adaptive response to divergent oxygen regimes that reflects changes in other morphological traits that respond to dissolved oxygen (see Langerhans et al. 2007).

In hypoxic environments, fish can have a number of adaptations to enhance oxygen uptake. These adaptations can be physiological (e.g. anaerobic respiration, higher haematocrit; Cooper et al. 2002; Martínez et al. 2004, 2009), behavioral (e.g. increased use of aquatic surface respiration; Chapman and Liem 1995; Timmerman and Chapman 2004), or morphological (e.g. increased gill surface area and reduced brain mass; Chapman et al. 2000, 2008; Crispo and Chapman 2010). Morphological adaptations are the most likely to influence body shape directly. An enlargement of the gills increases the surface area available for oxygen uptake, but also directly influences body shape, as documented in a study of nine field populations of the cyprinid *Barbus neumayeri* (Langerhans et al. 2007).

A less obvious adaptation to hypoxia is a reduction in brain mass that has been observed in some fishes. The brain is a metabolically-costly organ in vertebrates, and thus a reduction in brain size might result in increased oxygen availability for other functions (e.g. Mink et al. 1981; Chapman and Hulen 2001; Poulson 2001). It is not known whether brain mass influences body shape in fishes. The braincase is considerably larger than the size of the brain in most fish species (review: Kotrschal et al. 1998), so effects might be minimal, unless the braincase and the brain itself change proportionally at the intraspecific level. Larger gill size (Langerhans et al. 2007), and perhaps brain size, should result in an enlargement of the head.

The goal of this study is to examine correlations between body shape and traits associated with respiration in a species of haplochromine cichlid fish, *Pseudocrenilabrus multicolor victoriae* (Seegers). This species is found in a diverse suite of environments, including both free-flowing, high-oxygen rivers and stagnant, hypoxic swamps in southwestern Uganda (Crispo and Chapman 2008, 2010). Fish from hypoxic waters have larger gills to increase surface area for oxygen uptake, which might result in an enlargement of the head (Langerhans et al. 2007; Chapman et al. 2008). The brain, however, is smaller under hypoxia, presumably to reduce the oxygen requirement (e.g. Chapman and Hulen 2001; Poulson 2001; Crispo and Chapman 2010), and this might influence head shape in the opposite direction. We therefore expect a trade-off between respiration (i.e. oxygen acquisition and utilization) and streamlining to occur within this system, but the relationship would depend on the relative effects of gill size and brain size on body shape. Given that gill and brain size are highly plastic (e.g. Chapman et al. 2000, 2008; Crispo and Chapman 2010), differences in body shape among populations in the wild might be due to *plastic* effects acting through gill and brain size, or plastic effects acting directly on body shape, but could also be due to *genetic* differentiation between environment types.

We here identify, in *P. multicolor*, potential genetic differences in body shape among populations, and plastic effects of dissolved oxygen on body shape. We employ path analyses to examine *direct* effects of dissolved oxygen on body shape and *indirect* effects acting through the influence of oxygen on gill and brain size. We build on the earlier study of *Barbus neumayeri* (Langerhans et al. 2007) by using a split-brood design to distinguish between plastic and genetic variation in body shape variation. We used a laboratory-rearing experiment, in which F_1 offspring from multiple populations from each environment type (river and swamp) were raised under high- and low-oxygen conditions (Crispo and Chapman 2010). Through this experiment, we are able to test for (1) plastic effects of dissolved oxygen (both direct and indirect) and (2) a combination of plastic and genetic effects attributable to the environment (high-oxygen river versus hypoxic swamp).

Methods

Sampling and rearing

P. multicolor specimens were live-sampled from sites in Uganda, and the gills and brains were measured on F_1 offspring from a laboratory-rearing study (Crispo and Chapman 2010). Because the sampling and rearing protocols are described in detail in a previous study (above), we will mention them only briefly here. Fish populations were live-sampled in Uganda from four river sites (Bunoga, Rwebakwata, Kahunge, Kamwenge) and two swamp sites (Kantembwe and Kanyantale) from a single drainage (see Fig. 1 in Crispo and Chapman 2010). The swamp sites are characterized by low flow water that remains hypoxic throughout the year (e.g. average monthly dissolved oxygen concentration of 0.1–0.4 mg/l at Kantembwe; see Fig. 3 in Crispo and Chapman 2008). River sites are characterized by free-flowing water that remains relatively well oxygenated throughout the year (average dissolved oxygen concentrations of ~6–7 mg/l at river sites during our May–July sampling season; see Fig. 2 in Crispo and Chapman 2008). Although seasonal variation in dissolved oxygen occurs, rarely, if ever, do dissolved oxygen levels reach as low in the river as they do in the swamp (e.g. the lowest recorded average dissolved oxygen concentration observed at Kahunge was 1.1 mg/l after 1 year of monthly sampling).

P. multicolor were transported live to McGill University, Canada. We then used a split-brood experiment, in which fish from seven families (i.e. offspring from seven males and seven females) were raised from each of the six populations. An exception to this sample size was the Kanyantale population, from which nine families were used. The experiment was divided between 2 years, with four populations raised in the first year (three river and one swamp population; Bunoga, Rwebakwata, Kahunge, and Kantembwe) and two populations raised in the second year (one river and one swamp population; Kamwenge and Kanyantale). F_1 broods were split between low-oxygen treatment tanks and high-oxygen treatment tanks after 1 week of release from the mothers' mouths (*P. multicolor* is a maternal mouth brooder). Our sample thus included 7–9 families from each of 6 populations, for a total of 44 families, each raised under two treatments (one tank per family per treatment; 88 tanks). Oxygen in the low-oxygen treatment was gradually lowered to approximately 0.7–1.2 mg/l via bubbling of nitrogen gas through the water column (using a commercial controller; Point Four Systems Inc., Coquitlam, British Columbia). Oxygen in the high-oxygen treatment remained at approximately 7.5–8.0 mg/l via bubbling of air through the water column. Although these values were slightly higher than those observed in the field (see above), they were used to match levels used in previous studies in this system (Chapman et al. 2000, 2008) and to reduce the risk of fish kills caused by anoxia if the oxygen controller were to overshoot the target oxygen concentration.

After approximately 1 year, the largest 2 males in each tank were sacrificed for morphological analyses. Exceptions occurred for 6 tanks from which only 1 male was sacrificed: Bunoga low-oxygen, Bunoga high-oxygen (two tanks), Kanwenge high-oxygen, Rwebakwata low-oxygen, Rwebakwata high-oxygen. Only 1–2 fish per tank were used because dominance hierarchies reduced the growth of some fish, making it difficult to perform dissections and measurements on these smaller fish. Only males were used because mouth brooding might have influenced morphological traits in females (see Crispo and Chapman 2010). Fish were euthanized with buffered tricaine methanesulfonate (MS222; pH = 7.0) and were preserved in 4% paraformaldehyde (buffered with phosphate buffered saline; pH = 7.0). Details of the gill and brain dissections and measurements are given in Crispo and Chapman (2010). Briefly, brains were dissected out using standard procedures, weighed five times each, and the average of the five measurements was used in the analysis. Gills (four hemibranchs) were dissected from the right side of the fish, and both sides of each hemibranch were photographed using a Lumenera Scientific Infinity camera attached to a dissecting microscope. Five metrics were measured digitally on each side of each of the four hemibranchs, including total gill filament length (TGFL), average gill filament length (AFL), total number of gill filaments (TNF), total hemibranch area (THA), and total perimeter of the hemibranchs (TP) (Muir and Hughes 1969; Hughes 1984; Langerhans et al. 2007; Chapman et al. 2008; Crispo and Chapman 2010). Measurements were made using Motic Images Plus version 2.0. Gill metrics were standardized to a common body mass using an allometric equation:

$$Y_{std} = Y_{obs}\left(M_{avg}/M_{obs}\right)^{\beta}.$$

In this equation, gill metrics are represented by Y; body mass is represented by M; subscripts std, obs, and avg represent the standardized, observed, and average measures, respectively; and the slope of the relationship between the gill metric and body mass is represented by β (for further details, see Crispo and Chapman 2010). All five gill metrics were highly correlated even after standardization, and we thus used principle components (PC) analysis on the $\log_{10}$-transformed gill metrics to reduce the number of dimensions. One component was extracted with an eigenvalue greater than one. All gill metrics were strongly positively correlated with this component, and thus it reflects overall variation in gill size (Crispo and Chapman 2010). We also standardized brain mass to a common body mass (as for the gill metrics) and used these values ($\log_{10}$-transformed) in the analyses below.

Geometric morphometrics

Fish were photographed on their left sides using a digital camera. Fifteen landmarks were digitized (using tpsDig version 2.10), of which four were semilandmarks (Fig. 1; Zelditch et al. 2004). Semilandmark 2 was created by drawing a line from landmarks 1 and 3 and projecting it 90° (using ImageJ version 1.42q); the semilandmark was placed at the point at

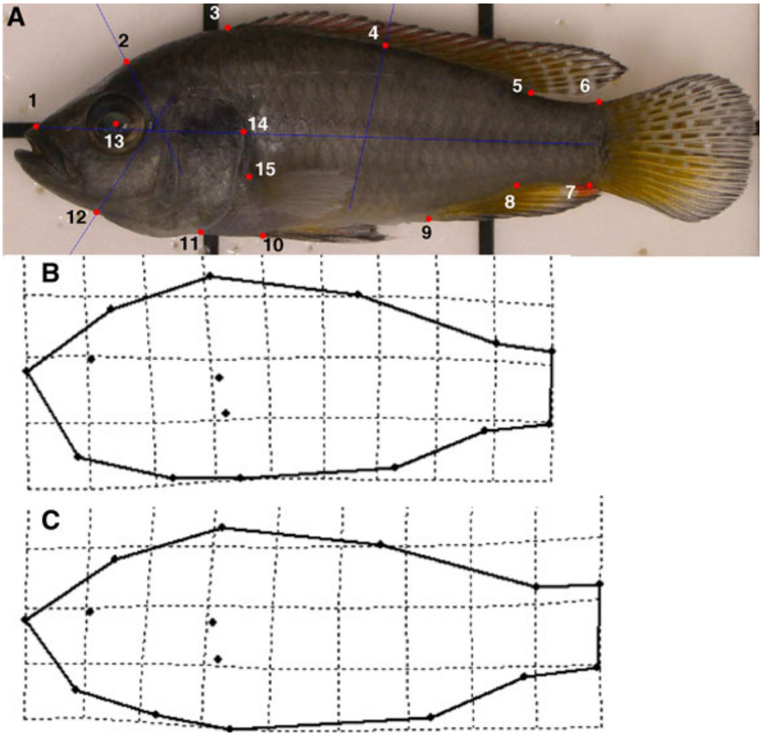

Fig. 1 Positioning of landmarks and semilandmarks for the geometric morphometric analysis (**a**). Semilandmarks include 2, 4, 12, and 14. "Average" fish shape, across all populations, for F_1 offspring raised under low (**b**) or high (**c**) oxygen conditions in a laboratory-rearing experiment

which this line crossed the edge of the body. Semilandmarks 4 and 12 were created in a similar way, but with lines drawn between landmarks 3 and 5, and landmarks 1 and 11, respectively. Semilandmark 15 was created by drawing a line from landmark 1 to the end of the hypural plate, and digitizing the point where this line crossed the operculum edge. Geometric morphometric analysis was performed to obtain partial and relative warp scores and centroid sizes (using tpsRelw version 1.45). Partial warp scores are two-dimensional vectors that represent localized changes in position of the landmarks. Relative warps are linear combinations of partial warps and are independent of one another. We initially analyzed relative warps that explained more than five percent of the variation in the data (p. 168, Zelditch et al. 2004); however, we present results from only those warps that explained more than 15% of the variation in the data.

Statistical analysis

We used JMP (version 8.0) to conduct analyses of covariance (ANCOVAs) to test for population and oxygen treatment effects on each of the relative warps, while controlling for variation among families and variation due to centroid size. Centroid size is a measure of the distance of the landmarks from the center point of the landmark configuration. Family was a random factor nested within population. We included the population-by-treatment interaction in the model; a significant interaction would indicate that oxygen-related plasticity in body shape differs among populations. We also initially included interactions with centroid size, but subsequently removed non-significant interactions (two-way interactions were left in the model if the three-way interaction was significant.) We used the "traditional" method to estimate mean squares. From these analyses, we could determine whether variation in body shape was due to genetic variation among populations (i.e. population effect) or plastic variation related to dissolved oxygen (i.e. treatment effect). We chose to use multiple univariate analyses, in addition to the multivariate analysis below, because we were able to include family-level variation in the univariate models, to control for genetic variation within populations and tank effects within treatments (random nested models cannot be included in the multivariate analysis; Langerhans 2009). Because relative warps are independent of one another, each ANCOVA tested for effects on orthogonal components of body shape.

Additionally, we used JMP to conduct a multivariate analysis of covariance (MANCOVA) to test for effects on overall body shape, to complement the above analyses on specific components of body shape (relative warps). The drawback of using this multivariate test is that random factors cannot be included in the model. Therefore, we did not include the effects of family. We included all partial and uniform warps as response variables. As above, we included population and oxygen treatment as fixed effects and the centroid size as a covariate, and we included the interaction between population and treatment. All interactions with centroid size were non-significant and thus were removed from the model. We extracted the canonical variate (CV) scores for population and treatment, which are similar to discriminant function scores, but reveal the most effective discriminators among populations and between treatments, respectively (i.e. they explain variation among groups instead of among individuals).

To disentangle possible direct and indirect effects of environment and dissolved oxygen on body shape, we used path analyses (using AMOS version 18). We performed two analyses, one on each of the first two relative warps. Variation in these two warps was explained to some extent by oxygen treatment (see "Results"), and both warps explained greater than 15% of the overall variation in body shape. We examined direct effects of gill size (PC1 scores, as above), brain mass (standardized to a common body mass and $\log_{10}$-transformed), and oxygen treatment (high or low) on relative warps for body shape, as well as indirect effects of oxygen through its effects on gill size and brain mass. We did not consider effects of gill size on brain mass, and vice versa, because they were not correlated with each other (results not shown). We ideally would have included population effects, but categorical variables could not be included in the model. We therefore instead included environment type (river versus swamp), which was treated as a binomial variable. As with the oxygen treatment effects, we included both direct effects of environment and indirect effects through the environment's effects on gill size and brain mass. We also included direct effects of centroid size on relative warps. We used maximum likelihood for the estimation of effects, and 10,000 bootstrapped samples to obtain P values for the estimates.

Results

In general, across populations, fish were shorter and had deeper, larger heads when reared under low oxygen conditions, as illustrated in the visualizations of the "average" fish shape in the two rearing environments (Fig. 1). However, univariate analyses on relative warps revealed different effects on different components of body shape. The first relative warp explained 26.06% of the variation in body shape and represented a deepening of the body in the head region, elongation of the head region, and shortening of the body in the posterior region (Fig. 2). Differences in the first relative warp were mainly driven by variation between treatments, by the population-by-treatment interaction, and also by the centroid size-by-population-by-treatment interaction (Table 1; Fig. 2). Relative warp 1 scores were higher under low oxygen, and this treatment effect was more pronounced for some populations than for others (Table 1; Fig. 2). Relative warp 1 scores generally decreased with an increase in centroid size for all populations, with the exception of Kantembwe fish raised under high oxygen and Kamwenge fish raised under low oxygen (results not shown). The second relative warp explained 15.11% of the variation in body shape and represented primarily a decrease in head length, and a decrease in body depth around the mid-section near the insertion of the pelvic fin (i.e. not the depth of the head as was evident in Relative warp 1). Differences in the second relative warp were driven primarily by variation among families and between treatments, but the treatment effect differed among populations (although the population-by-treatment interaction only approached significance; $P = 0.051$; Table 1; Fig. 2). Relative warp 2 scores were typically higher under low oxygen, but the opposite trend was apparent for two populations (Kahunge and Kanyantale; Fig. 2). Relative warp 2 scores typically increased with an increase in centroid size, but again this effect varied among populations. The remaining 24 relative warps each explained less than 15% of the variation in body shape. We also analyzed relative warps 3–6 (each explaining more than 5% of the variation), but the variation was not apparently related to differences between environments or treatments (results not shown).

The multivariate analysis revealed strong effects of population, treatment, and centroid size (Table 2). However, results from this analysis should be interpreted with caution because variation among families was not considered, but could be a source of genetic variation among families and/or tank effects. The most discriminatory differences between fish raised under low versus high oxygen were larger heads and overall deeper bodies in the former, probably due mainly to the increase in gill size under low oxygen (see below;

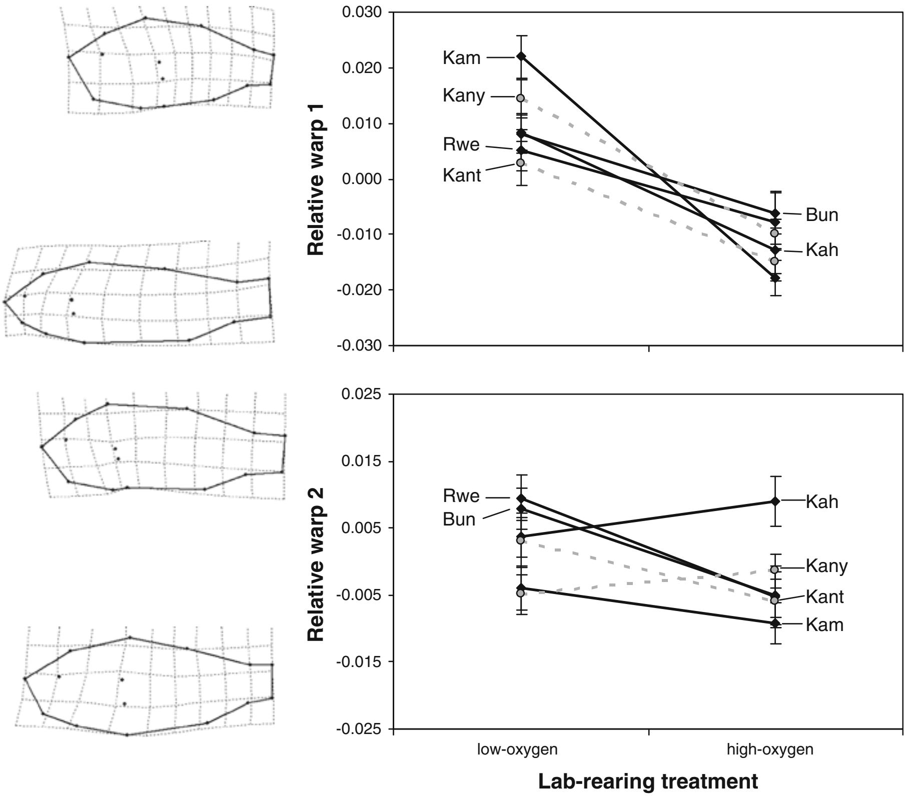

Fig. 2 Adjusted mean (±standard error) relative warp scores for each population, obtained from the analyses of covariance (described in the "Methods"), for F_1 *P. multicolor* raised under high or low oxygen in a laboratory experiment. *Gray points* and *dashed lines* represent swamp populations. *Black points* and *solid lines* represent river populations. The deformation grids represent shape variation between the two most extreme samples in the dataset for each relative warp, magnified by a factor of three. *Bun* Bunoga, *Rwe* Rwebakwata, *Kah* Kahunge, *Kam* Kamwenge, *Kany* Kanyantale, *Kant* Kantembwe

Fig. 3). The most discriminatory differences among populations were a reduction in depth in the posterior region, and an elongation of the head, in two populations (Kamwenge and Kanyantale; Fig. 3).

Path analysis suggested strong negative effects of oxygen on gill size, and positive effects of oxygen on brain mass (Table 3; Fig. 4), as expected based on our previous work (Crispo and Chapman 2010). Gills were larger under low oxygen, and scores for the first relative warp increased with increasing gill size, resulting in deeper and longer heads and shorter bodies (Table 3; Figs. 2, 4). Similarly, oxygen had a direct negative effect on the first relative warp (deeper and longer heads, shorter bodies under low oxygen). Relative warp 1 scores also increased with an increase in brain mass. Both the direct effects of brain mass, and the indirect effects of oxygen through its effects on brain mass, resulted in increased relative warp 1 scores. Relative warp 1 scores also increased with an increase in centroid size. There was no effect of environment type (swamp or river) on gill size, brain mass, or relative warp 1 scores (Table 3; Fig. 4). Gill size and brain mass had no significant effects on relative warp 2 scores (Table 3). The direct effect of oxygen was significant and negative for this warp, meaning that with increased oxygen, bodies became

Table 1 Results from analyses of covariance for F_1 *P. multicolor* from multiple populations raised under high or low oxygen in a laboratory experiment

Effect	*F*	*df*	*P*
WARP 1			
Population	1.242	5,56.3	0.302
Family (population)	1.459	38,105	0.069
Treatment	110.960	1,105	<0.001
Centroid size	3.569	1,105	0.062
Population * treatment	3.695	5,105	0.004
Centroid size * population	0.621	5,105	0.684
Centroid size * treatment	1.270	1,105	0.262
Centroid size * population * treatment	3.382	5,105	0.007
WARP 2			
Population	1.818	5,44.8	0.129
Family (population)	2.332	38,111	<0.001
Treatment	8.915	1,111	0.004
Centroid size	3.125	1,111	0.080
Population * treatment	3.555	5,111	0.051
Centroid size * population	2.813	5,111	0.020

Relative warps were used as the response variables. The *df* are the numerator degrees of freedom, followed by the denominator degrees of freedom

Table 2 Results from the multivariate analysis of covariance for F_1 *P. multicolor* from multiple populations raised under high or low oxygen in a laboratory experiment

Effect	Wilks' λ	*F*	*df*	*P*
Population	0.173	2.106	130,640.6	<0.001
Treatment	2.679	13.293	26,129	<0.001
Centroid size	0.693	3.437	26,129	<0.001
Population * treatment	0.320	1.283	130,640.6	0.028

All partial and uniform warps were used as the response variables. Interactions with centroid size were non-significant and were thus not included in the model. The *df* are the numerator degrees of freedom, followed by the denominator degrees of freedom

deeper near the mid-section (however, this pattern was not apparent for Kahunge or Kanyantale; Fig. 2). Relative warp 2 scores increased with an increase in centroid size, and there were no significant effects of environment type on the second relative warp (Table 3).

Discussion

Plastic effects of dissolved oxygen

Our study revealed plastic effects on body shape. The most striking result was the presence of larger heads under the low oxygen treatment, which was largely, but not completely, due to an increase in gill size under low oxygen. While both gill size and brain mass resulted in

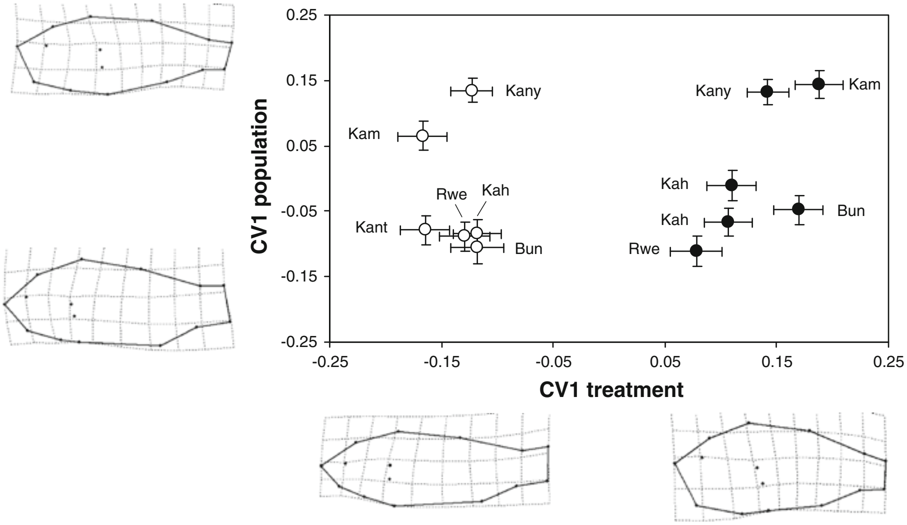

Fig. 3 Canonical variate scores for population (y-axis) and laboratory-rearing treatment (high or low oxygen; x-axis) obtained from the multivariate analysis of covariance (using JMP; described in the "Methods"). Presented are the adjusted mean (±standard error) canonical scores for each F_1 population of *P. multicolor* raised under high or low oxygen in the laboratory experiment. *Solid points* represent fish raised under low oxygen and *open points* represent fish raised under high oxygen. The deformation grids represent shape variation between the two most extreme samples in the dataset for each canonical variate, magnified by a factor of three. *Bun* Bunoga, *Rwe* Rwebakwata, *Kah* Kahunge, *Kam* Kamwenge, *Kany* Kanyantale, *Kant* Kantembwe

an increase in head size, the indirect effect of oxygen was in the opposite direction between these two traits—oxygen was positively correlated with brain mass but negatively correlated with gill size. However, any effects of brain mass were overwhelmed by the effects of gill size on body shape, noted by the increase in head size under low oxygen (despite smaller brains). Although head size increased under low oxygen, body depth near the mid-section *decreased* under the same conditions (relative warp 2; Fig. 2). A possible reason for this plastic effect is that fish raised under high oxygen have increased body condition; however, this response was small in comparison to the effects on head depth, and was population-specific (Table 1; Fig. 2).

A similar study was conducted on an African cyprinid (*Barbus neumayeri*) from a range of water flow and dissolved oxygen regimes (Langerhans et al. 2007). A primary difference between that study and the present one is that the former used wild-caught fish, whereas we used F_1 fish raised under two oxygen treatments, using a split-brood design. We were thus able to partition genetic and plastic effects. While both studies identified strong effects of gill size on body shape, our study revealed that these differences are primarily due to *plastic* changes in *P. multicolor*, as evidenced by the strong treatment effects on both gill size and body shape. This result could have implications for understanding whether dissolved oxygen might influence population structuring and speciation. High levels of plasticity and low levels of genetic differentiation suggest that gene flow might not be restricted between environment types; instead, plasticity might permit high levels of gene flow between environments, or gene flow might favor in the evolution of plasticity over local adaptation (Crispo 2008; Crispo and Chapman 2008, 2010).

Table 3 Standardized regression weights for the direct (DE), indirect (IE), and total (TE) effects from the path analyses

	Effect on gill size			Effect on brain mass			Effect on body shape (RW1)			Effect on body shape (RW2)		
	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE
Oxygen	**−0.899**	–	**−0.899**	**0.482**	–	**0.482**	**−0.406**	−0.248	**−0.654**	**−0.443**	0.223	**−0.220**
Environment	0.023	–	0.023	−0.064	–	−0.064	−0.021	−0.002	−0.023	0.088	<0.001	0.088
Gill size	–	–	–	–	–	–	**0.365**	–	**0.365**	−0.311	–	−0.311
Brain mass	–	–	–	–	–	–	**0.166**	–	**0.166**	−0.117	–	−0.117
Centroid size	–	–	–	–	–	–	**−0.192**	–	**−0.192**	**0.326**	–	**0.326**
r^2			**0.809**			**0.236**			**0.511**			**0.191**

“Oxygen” and “environment” were binomial variables, where a 1 represented high oxygen and high water flow, and a 0 represented low oxygen and low water flow. Gill size was the first principle component obtained from PCA on five gill metrics. The brain mass was $\log_{10}$-transformed. Significant effects ($P < 0.05$) are bold

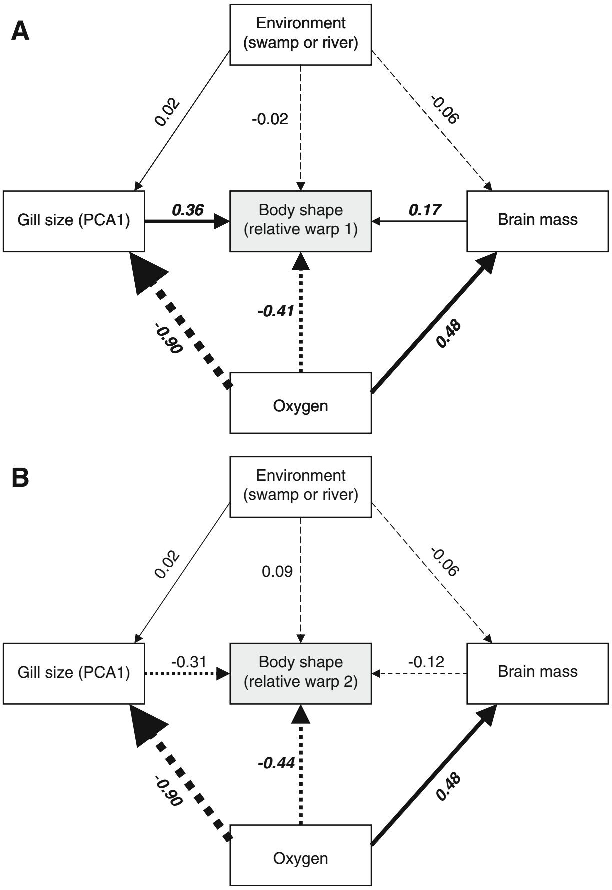

Fig. 4 Path diagrams showing the standardized regression weights for the direct effects on relative warp 1 (**a**) and relative warp 2 (**b**). "Oxygen" and "environment" were binomial variables, where a *1* represented high oxygen and high water flow, and a *0* represented low oxygen and low water flow. Gill size was the first principle component obtained from PCA on five gill metrics (described in the "Methods"). The brain mass was $\log_{10}$-transformed. Significant effects ($P < 0.05$) are *bold*

Genetic variation in body shape

Although we detected evidence for population effects on body shape, the variation was not explained by whether the fish were of swamp or river origin. We expected differences between swamps and rivers due to the potential divergent selection pressures that they should impose. The first selection pressure is dissolved oxygen concentration, which might affect overall activity. Streamlining influences the energetic costs of swimming, and therefore different body shapes might be predicted for environments in which energy expenditure is limited (e.g. Boily and Magnan 2002; Ohlberger et al. 2006). Additionally, we might have expected differences in body shape that evolved through genetic correlations, when selection acts on other structures. However, previous work on this system showed that most of the variation in gill size between environments was due to plasticity (Crispo and Chapman 2010). While genetic differences in brain mass occurred among populations, the brain had a smaller effect on body shape in this study than did gill size.

Any apparent variation among populations was not due to genetic variation related to differences in oxygen acquisition between river and swamp populations. Instead, differences between swamp and river populations probably occur in the wild due to plastic variation in response to dissolved oxygen, and potentially other environmental factors that were not tested in our laboratory experiment.

A second selection pressure that differs between swamps and rivers is water flow, which might affect whether a fish preferentially uses burst or sustained swimming. Theory predicts that fish from fast-flowing waters should use steady swimming, and therefore be more ‘streamlined’, with deep anterior ends tapering towards the caudal end (Langerhans 2008). Fish from slow-moving waters with high structural complexity (e.g. swamps), on the other hand, should use unsteady, burst swimming, and therefore have relatively deeper posteriors (Langerhans 2008). Empirical investigations typically support these predictions (review: Langerhans 2008). While we found no obvious differences between swamp and river fish (for populations combined within habitat), our multivariate analysis did uncover population differences. Specifically, fish from Kanyantale and Kamwenge had slightly more ‘tapered’ bodies than the other four populations (Fig. 3). Kamwenge is a free-flowing river site that remains high in oxygen year-round, so it seems intuitive that fish might be streamlined for contending with continuous water flow. Kanyantale, however, is a stagnant swamp site and we therefore predict that fish should use burst swimming when necessary for evasion or prey capture, but remain relatively inactive during other periods (to conserve oxygen). This result is therefore surprising. A possible reason for the discrepancies in body shape for these two sites is that the parental stock for the rearing experiment were sampled in a different year (May–June 2008) than the other four sites (May–June 2006). However, the experiment and measurements were conducted the same way, by the same experimenter, in both years, so it is not likely that the differences are due to methodological artifacts. Genetic differences in body shape among populations might therefore instead be related to differences in biotic or abiotic environmental features that we have not explored.

Possible implications of body shape variation

As above, we found no evidence for genetic differences in body shape between high-flow and low-flow environments, although we might have predicted differences in streamlining. However, we did not test for plastic effects of water flow in our laboratory experiment, and it remains possible that differences in body shape occur among wild populations, reflecting plastic effects of water flow. The previous study on *B. neumayeri* found direct (deeper body) and indirect (shallower body) effects of water flow that opposed each other, through water flow’s effect on dissolved oxygen (Langerhans et al. 2007). That study, however, did not partition genetic and plastic effects. Laboratory-rearing studies on other species have identified *plastic* effects of water flow on body shape. For example, head depth was lower under high water flow than under low water flow in both Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*), although the effects of water flow on overall body depth differed between the species (Pakkasmaa and Piironen 2001). Previous studies on *P. multicolor* found differences in body depth among wild populations of swamp, lake, and river fish, although it is not clear whether these differences reflect swimming mode (Chapman et al. 2000, 2008). It is thus possible that plastic differences in body shape occur between swamps and rivers in wild populations, potentially influencing swimming abilities.

A recent meta-analysis revealed that body shape predictions for high- versus low-flow environments were met in 42 of 58 cases, or roughly three quarters of the time, at the intraspecific level (Langerhans 2008). Why are the predictions not met in the other quarter of the cases, including this study on *P. multicolor*? One possible reason is that *P. multicolor* does not preferentially use sustained swimming in the high flow environment and burst swimming in the low flow environment, but might instead be a swimming *generalist* (see Videler 1993). For example, they might use low flow areas of high flow environments to "sit-and-wait" until rapid movement is necessary. It is common to capture *P. multicolor* in structured inshore areas of riverine habitats. Another possible reason for body shape predictions not being met is that optimal body shapes cannot be achieved in a particular environment due to multiple interacting factors that influence body shape. Enlarged gills manifested under low oxygen conditions result in deeper bodies and heads. Yet, these interactive environmental effects might not always translate into differences in swimming performance among populations from different environments. Swimming performance tests in *P. multicolor* revealed no difference in critical swimming speed (U_{crit}; the water velocity at which fish can no longer maintain position within a water column) between treatments (high versus low oxygen) or between populations from different environments (river versus swamp) when fish were tested under their home environment. However, hypoxia-reared fish performed better when tested under normoxic conditions than either the same fish measured under low oxygen or high-oxygen reared fish tested under hypoxia (K. Gotanda, E. Reardon, S. Murphy, L. Chapman unpublished data). This better performance of hypoxia-reared fish is likely due to the increased gill surface allowing for increased oxygen uptake capacity to meet the metabolic demands (Pauly 1981; Neill and Bryan 1991; Chapman et al. 2008; Crispo and Chapman 2010). These results suggest that body shape in this species may not be a good predictor of sustained swimming.

The actual effects of body shape on swimming performance vary widely across species and studies, suggesting that general predictions for body shape are context-specific. This variation occurs at both the inter- and intraspecific levels. Interspecific comparisons reveal inconsistencies for the relationship between body depth and swimming speed among species (e.g. Webb and Fairchild 2001; Ohlberger et al. 2006; Leavy and Bonner 2009). Intraspecific studies might be more useful for understanding the effects of body shape differences on swimming performance because other potential effects on swimming (i.e. physiology, behavior, fin size and shape) are more likely to be similar between intraspecific groups. Results from intraspecific studies generally reveal positive associations between body depth and swimming speed, although patterns are sometimes inconsistent. In laboratory-raised brook trout (*Salvelinus fontinalis*), U_{crit} was positively associated with body depth (Rouleau et al. 2010). In Gilthead seabream (*Sparus aurata*), however, fish with *shallower* bodies had higher U_{crit} (Koumoundouros et al. 2009). The latter study compared ontogenetic groups, however, and ontogeny might influence swimming performance independently of body shape. In rainbow fish (*Melanotaenia duboulayi*), lake fish have deeper posterior regions and river fish have deeper anterior regions (McGuigan et al. 2003). Following predictions (Langerhans 2008), river fish had higher sustained swimming abilities (U_{crit}), but this pattern was sex-specific in a congeneric species (*Melanotaenia eachamensis*; McGuigan et al. 2003). In guppies (*Poecilia reticulata*), body depth was positively but weakly associated with U_{crit}, although the direction of the relationship between body depth and burst swimming speed varied (Oufiero and Garland 2009). In the common carp (*Cyprinus carpio*), U_{crit} was positively associated with body depth but negatively associated with head depth (Li et al. 2009), suggesting that predictions for body depth's effects on swimming should be strongly related to the precise positioning of measurements on the fish (difficult to standardize among fish lineages). And finally, in crucian carp (*Carassius carassius*), body depth was positively associated with *both* speed and acceleration (Domenici et al. 2008), contradicting the assertions that functional trade-offs exist between sustained and burst swimming (Blake 1983, 2004; Webb 1984; Videler 1993; Langerhans 2008, 2009). Taken together, these observations suggest that the predictions for body depth on swimming performance are not straightforward, and should depend on the intricacies of individual systems.

Conclusion

Environmental factors can have interactive effects on morphology. Our results clearly show that dissolved oxygen concentration has a large, plastic effect on body shape. The most obvious differences observed in our experiment were larger heads in fish raised under low oxygen, and this effect was largely driven by oxygen's effects on gill size. This finding emphasizes the significance of indirect effects on body shape variation that may not follow predictions for shape variation in response to direct effects of variables such as water flow. While some genetic effects on body shape were observed, the underlying cause of this variation was not clear, and was not related to whether the fish were of swamp or river origin. Future studies should use an integrative approach that examines multiple environmental factors that influence morphology in fish populations.

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