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LETTER

Phenotypic plasticity and the possible role of genetic assimilation: Hypoxia-induced trade-offs in the morphological traits of an African cichlid

Lauren J. Chapman,[1,3] Frietson Galis[2] and Jason Shinn[1]
[1]Department of Zoology, University of Florida, Gainesville, Florida 32611, U.S.A.
E-mail: ljchapman@zoo.ufl.edu
[2]Institute of Evolutionary and Ecological Sciences, Leiden University, PO Box 9516, 2300 RA, Leiden, The Netherlands.
[3]Wildlife Conservation Society, 185th Street and Southern Boulevard, Bronx, New York 10460, U.S.A.

Abstract

In this study we investigate the possible role of phenotypic plasticity and genetic assimilation in the process of adaptation and evolutionary change in the cichlid *Pseudocrenilabrus multicolor victoriae*. In the field we compared a population of a stable hypoxic habitat with one of a stable well-oxygenated habitat. In the laboratory, we compared individuals from the same mother raised under hypoxic or well-oxygenated conditions to examine phenotypic plasticity. Morphological parameters of three categories were measured: (a) the gill apparatus, (b) the surrounding structural elements, and (c) the outer shape of the fish. Swamp-dwelling fish had a 29% greater total gill surface area than fish from the well-oxygenated habitat due to their larger gill filament length and greater lamellar area. In the plasticity experiment, total gill surface area was 18% greater in the hypoxia group due to a larger number of longer filaments. Surrounding elements and outer shape also differed between the field populations and between fish grown under hypoxic and well-oxygenated conditions, but there was disparity between the field results and the plasticity experiment. The disparity between field and experimental fish may be due to: (a) differences in selection pressures between populations, (b) different constraints for genetic and plasticity changes, or (c) selection against plastic responses to hypoxia. Our results suggest that both (a) and (c) are involved.

Keywords

Africa, Cichlidae, genetic assimilation, gill morphology, hypoxia, phenotypic plasticity, trophic morphology.

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INTRODUCTION

An initial phenotypically plastic response to a novel environmental condition may be followed by genetic changes in the same direction, a process referred to as genetic assimilation. However, the importance of the role of genetic assimilation in the evolution of organisms is debatable (e.g. Waddington 1953, 1956; Williams 1966; Gerard *et al.* 1993; Eshel & Matessi 1998; Pál 1998; Schlichting & Pigliucci 1998). Early papers were concerned with plastic responses to severe perturbations producing large phenotypic changes with a macro-evolutionary importance. Waddington (1953, 1956) experimentally showed how perturbations such as a heat shock and exposure to ether vapour induced hidden variation in *Drosophila melanogaster*, variation that then became inherited after artificial selection. Recent research on heat-shock proteins highlights the potential evolutionary importance of this type of genetic assimilation (Rutherford & Lindquist 1998; Wagner *et al.* 1999). Arguably, an even more important evolutionary phenomenon may be the assimilation of changes of traits in response to common environmental fluctuations (Eshel & Matessi 1998). Williams (1966) has argued that such assimilation leads to canalization and, thus, to an unfavourable loss of flexible response. However, genetic assimilation can also lead to a shift of the reaction norm that is not necessarily less plastic (Rollo 1994; Eshel & Matessi 1998; Pal 1998; Schlichting & Pigliucci 1998). When conditions in the new environment fluctuate sufficiently, selection will favour genetic systems that preserve phenotypic plasticity (Levins 1965; Slatkin & Lande 1976). Genetic assimilation that preserves plasticity will be favourable in the long term; first, because it

preadapts populations to possible sudden, permanent changes of the environment that resemble some rare environmental condition in the old situation, and second, because it preserves the possibility for future evolutionary responses (Matsuda 1982; Hall 1992; Rollo 1994; Schlichting & Pigliucci 1998; see also Stearns 1983; Roth 1992). There is clearly a need for experimental data to test how common it is that evolution proceeds by genetic assimilation.

The present study on *Pseudeucrenilabrus multicolor victoriae* Seegers is the first in a series of studies where we try to test some of the above theoretical predictions. *Pseudocrenilabrus multicolor* is a small mouth-brooding haplochromine cichlid fish found in the Nile river system and Lake Victoria basin. It occupies a wide range of habitats including fast-flowing rivers, intermittent streams, lakes, and dense swamps. The habitats differ widely in stability and dissolved oxygen (DO) availability and provide an excellent system to test the importance of plasticity and genetic assimilation.

In this first study we compare a population from a stable hypoxic habitat with one from a stable well-oxygenated habitat. In addition we compare individuals from a habitat with a fluctuating oxygen level raised under hypoxia or normoxia (a split-brood experiment). We measure morphological parameters of three different categories: (a) the gill apparatus, (b) the surrounding structural elements, and (c) the outer shape of the fish. The rationale for these three categories is that an increase in gill size under hypoxia should either increase body size and/or decrease the space taken up by surrounding elements.

We also investigate whether differences in the responses induced in the plasticity experiment are in the same traits and in the same direction as differences between the field populations (as is expected when genetic assimilation has taken place). Where we find differences between field and experimental fishes we try to answer whether they are due to (a) differences in selection pressures between populations other than hypoxia, (b) different constraints for genetic and plasticity changes, and/or (c) selection against plastic responses to hypoxia.

MATERIALS AND METHODS

To examine natural variation in the gill morphology of *P. multicolor*, fish were collected from Lake Kayanja, Uganda (high oxygen site) and the dense interior of the wetland surrounding Lake Manywa, Uganda (low oxygen site). We had two wetland sites in Lake Manywa. The first site was 50–100 m from swamp-water ecotone where DO levels were low (2.5–3.8 mg L^{-1}, referred to as Manywa 100), but much higher than the second collection site at 500–550 m, where DO levels were extremely low (0.4 mg L^{-1}, referred to as Manywa 550). *Pseudocrenilabrus multicolor* were collected from the marginal areas of Lake Kayanja where DO averaged 6.1 mg L^{-1}.

For the growth experiment we used fish from a stock group originally collected from the Mpanga River, Uganda. Fish were collected from a section of open river characterized by strong seasonal changes in DO concentration. The brood of a single female was divided between a control tank held at normoxia (< 7.5 mg L^{-1}) and an experimental tank held at 1.0 mg L^{-1} for 5 months.

Gill morphological measurements were made using standard methods modified after Muir & Hughes (1969) and Hughes (1984); and described in Chapman & Chapman (1998). Gill characters did not differ between the two Manywa groups (Manywa 100 and Manywa 550). We therefore combined the Manywa sites for all gill comparisons with the high oxygen site. For the surrounding structural elements, we used only Manywa 100 specimens for comparison with Lake Kayanja and selected a series of characters that were most likely to be affected by change in gill size.

RESULTS

Size of the gill surface area

Fish from hypoxic conditions showed a greater total gill surface area than fish from well-oxygenated waters, both in the field and in the plasticity experiment. Total gill surface area was 29% larger in the swamp-dwelling fish from Lake Manywa than in the Kayanja population, and 18% larger in fish grown under hypoxia than normoxia (Fig. 1A, B, Table 1).

Parameters constituting the total gill surface area

The larger total gill surface area in swamp-dwelling fish was a result of an increase in the length of the gill filaments and in the size of the secondary lamellae, which were 26% larger than in the Kayanja population (Fig. 1C, Table 1). In the plasticity experiments the increase in the total gill surface area was due to an increase in both the length and the number of filaments (Fig. 1D, Table 1). However, in contrast to the field comparison, the size and density of the secondary lamellae were unchanged (Table 1).

Morphological parameters of surrounding structures

In the plasticity experiment both the m. retractor dorsalis and m. levator externus 4 exhibited a reduced cross-sectional area (reduced width and/or depth, Fig. 2B, Table 2). This will probably affect the maximum possible pharyngeal biting force that can be exerted by these muscles. The m. sternohyoideus exhibited a reduced depth

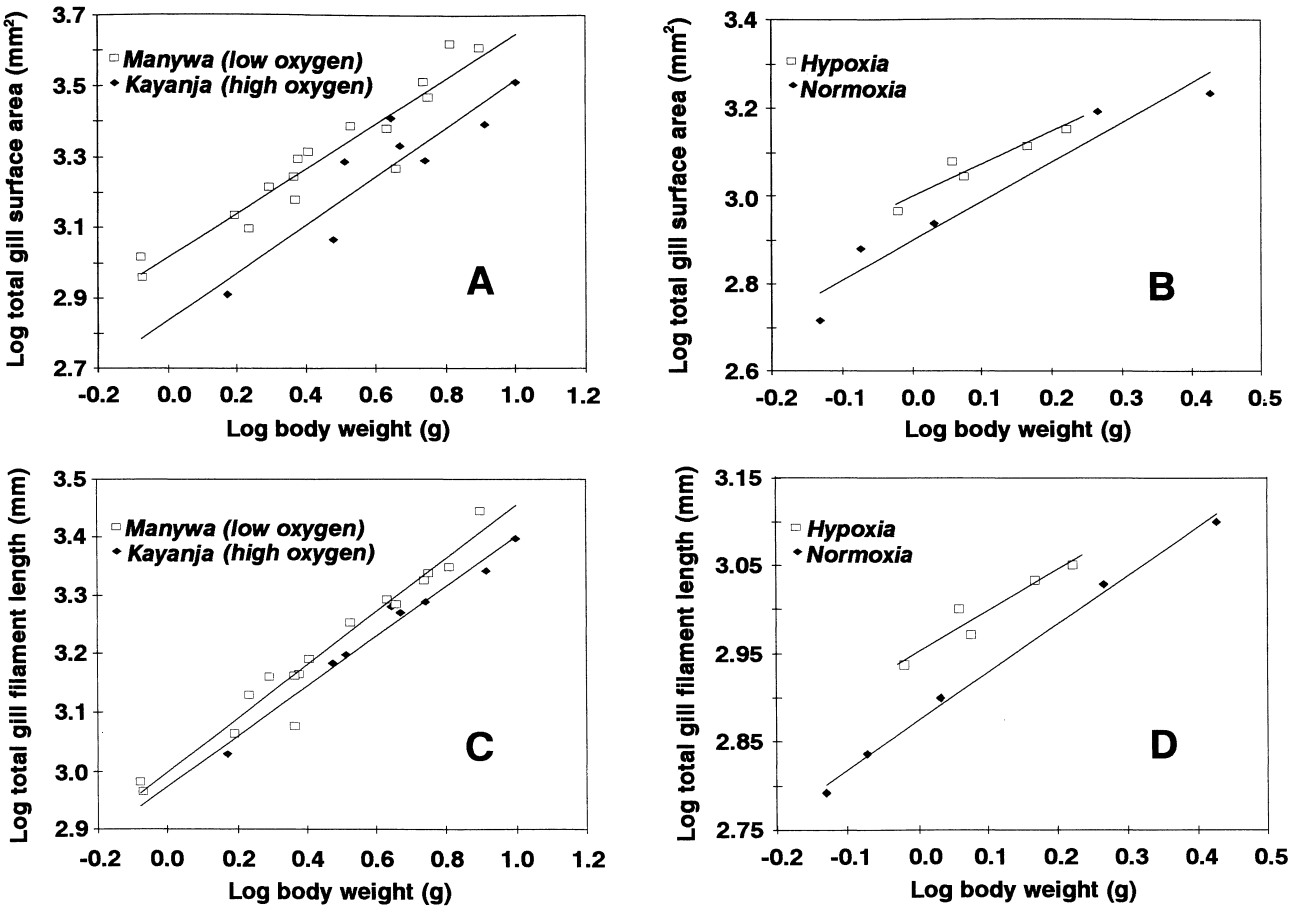

Figure 1 Bilogarithmic plots of total gill surface area (mm^2) and body weight (g) for *Pseudocrenilabrus multicolor* from (A) the hypoxic waters of the Lake Manywa swamp and the well-oxygenated ecotone of Lake Kayanja and (B) a lab-rearing experiment where fish from a split brood were grown under hypoxia (1 mg L^{-1}) and normoxia. Parts (C) and (D) represent the bilogarithmic relationship between total gill filament length (mm) and body weight (g) for the same groups of fish.

and length under hypoxia but a greater width. The length of the m. geniohyoideus muscle was greater in fish raised under hypoxia than those raised under normoxia, which may relate to the larger head of these fishes (see below). The parameters of the upper pharyngeal jaw were unchanged, but the lower pharyngeal jaw showed a reduced thickness (Fig. 2D, Table 2).

In the field population from the hypoxic site only one muscle was reduced in cross-sectional area relative to fish from the well-oxygenated site, the m. levator externus 4 (Fig. 2A, Table 2). As in the plasticity experiment, the length of the m. geniohyoideus muscle was greater in the swamp-dwelling fish. The upper pharyngeal jaws exhibited a decrease in the width, length, and depth in the swamp-dwelling fish (Table 2), which is presumably correlated to a decrease in the maximum force that the jaw can exert.

Outer shape

In the field, body depth was greater in the fish from the low-oxygen site (Table 2). In contrast, in the plasticity experiments, the width and the length of the head were greater in hypoxia-raised fish, while body depth was unaffected (Table 2).

DISCUSSION

Increase in gill surface area in response to hypoxia

An increase in gill surface area is a common evolutionary response of fishes to hypoxia (Galis & Barel 1980). The 29% larger gill surface area of *P. multicolor* from the Manywa swamp when compared with fish from the well-oxygenated site thus conforms to this expectation. The

Table 1 Summary of analyses of covariance of relationships between gill characters and body weight for groups of *Pseudocrenilabrus multicolor*

	Field			Laboratory		
Character	Population	Mean size	*P* value	Group	Mean size	*P* value
Total gill filament number	High DO	572.80	0.057	Normoxia	467.74	0.006
	Low DO	554.63		Hypoxia	486.41	
Mean gill filament length (mm)	High DO	2.69	<0.001	Normoxia	1.82	<0.001
	Low DO	3.06		Hypoxia	2.06	
Total gill filament length (mm)	High DO	1545.25	0.003	Normoxia	853.10	<0.001
	Low DO	1698.24		Hypoxia	1000.00	
Average lamellar density (per mm)	High DO	30.83	0.002	Normoxia	35.16	0.878
	Low DO	29.11		Hypoxia	35.24	
Total number of lamellae	High DO	49090.79	0.329	Normoxia	27989.81	<0.001
	Low DO	50933.09		Hypoxia	35237.09	
Average lamellar area (mm^2, 1 side only)	High DO	0.0081	<0.001	Normoxia	0.0082	0.575
	Low DO	0.011		Hypoxia	0.0085	
Total gill surface area (mm^2)	High DO	1548.82	<0.001	Normoxia	972.47	0.022
	Low DO	2187.76		Hypoxia	1191.24	
Gill filament density (per mm)	High DO	5.65	0.150	Normoxia	7.82	0.052
	Low DO	5.90		Hypoxia	7.31	
Gill filament area (Arch I) (mm^2)	High DO	18.92	0.015	Normoxia	7.96	<0.001
	Low DO	20.84		Hypoxia	10.94	

The field comparison represents populations from a low oxygen (Lake Manywa swamp) and a high oxygen (Lake Kayanja ecotone) site. The laboratory comparison represents groups reared under normoxia and extreme hypoxia (1 mg L^{-1}). Both gill characters and body weight were $\log_{10}$ transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean body weight and a common regression line for the two groups). In all cases, the slopes were homogeneous. Lake Kayanja, $n = 8$; Lake Manywa $n = 16$; normoxia treatment, $n = 5$; hypoxia treatment, $n = 5$.

developmental response to hypoxia in our plasticity experiment was not as large (18%) compared with that found between the field populations.

Disparity between the developmental and evolutionary response to hypoxia

In the field, the larger gill surface area in the low-oxygen site was due to longer filaments and larger secondary lamellae. In the experiment, the increase was due to more and longer filaments. The greater lamellar size allows the Manywa swamp fishes to have a larger gill area without as great an increase in the space necessary for the gill apparatus as was observed in the plasticity experiment. This is because the larger lamellar area did not result in an increased distance between adjacent filaments. The same phenomenon of disparity between the field comparison and the plasticity experiment was found for structures surrounding the gills and outer shape. In the field, size of the surrounding structures was less affected than in the experimental fish; hence, in the field, feeding performance is likely to be less compromised.

Factors explaining the disparity between the developmental and evolutionary response to hypoxia

Different selection pressures

The simplest explanation for the observed disparities would be that selection factors other than dissolved oxygen availability are different between Lake Kayanja and the Manywa swamp. This could lead to interdemic variation that has a genetic and/or an environmental basis. Such differences in selection factors are likely to be found in characters such as the turbidity and ionic strength of the water, diet, and predator pressure.

Different constraints

Constraints on plastic changes may differ from those on hereditary changes. On one hand, it can be that there is no genetic variation for traits in which there is plasticity. This is extremely unlikely as it is hard to imagine how selection could maintain plasticity and lead to the absence of genetic variation at the same time. Selection experiments can provide a definite answer. On the other hand, there may be no plasticity for certain traits for which there is genetic

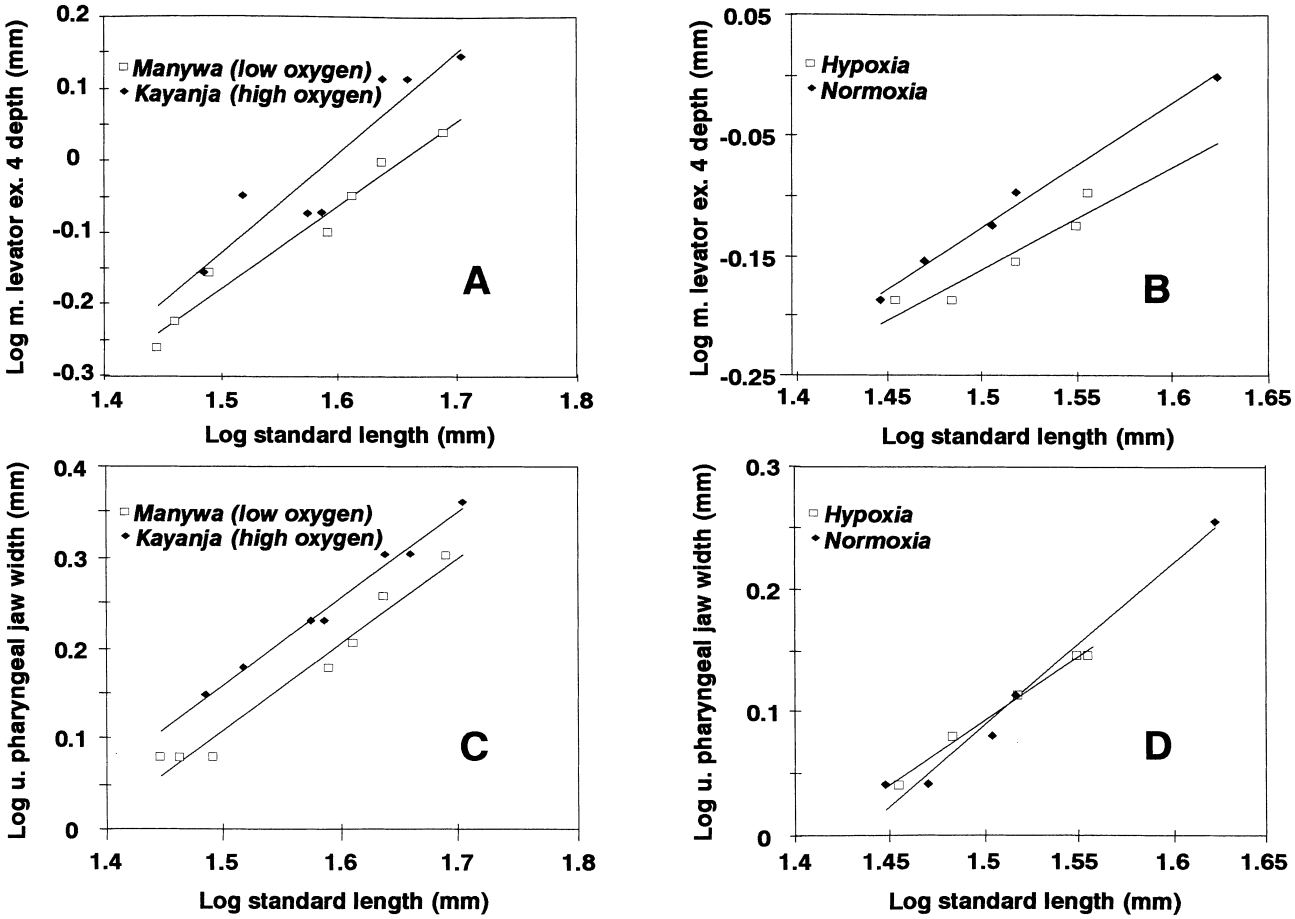

Figure 2 Bilogarithmic plots of the depth of the m. levator ex. 4 muscle (mm) and standard length (mm) for *Pseudocrenilabrus multicolor* from (A) the hypoxic waters of the Lake Manywa swamp and the well-oxygenated ecotone of Lake Kayanja and (B) a lab-rearing experiment where fish from a split brood were grown under hypoxia (1 mg L^{-1}) and normoxia. Parts (C) and (D) represent the bilogarithmic relationship between the width of the upper pharyngeal jaw (mm) and standard length (mm) for the same groups of fish.

variation. Although this is an interesting option, this is unlikely to explain the differences that we found. For example, the large variation in lamellar area in individuals of the split-brood hypoxic treatment suggests that there is no lack of plasticity in this character. However, further plasticity experiments that are currently under way on fishes from a stable hypoxic site and a stable normoxic site will provide more clarity in this respect.

Combined response of plastic and inherited changes

A combination of inherited changes and plasticity changes may allow for a finer-tuned response to severe hypoxic conditions in the Manywa swamp population. If we compare differences between the Manywa fishes and the fishes of the hypoxic treatment, it seems that in the Manywa fish, a larger increase in gill area has been realized with a less detrimental effect on the functioning of the fish. Not all plastic changes may be equally adaptive. The above-mentioned functional morphological considerations suggest a suboptimal response against hypoxia in our plasticity experiments. Selection against the plastic response of one of the characters would change the internal conditions in the fish and, presumably, the conditions for the plastic responses of other characters. A combined response of plasticity and genetic traits may allow a fine-tuning exceeding that for plastic responses alone, resulting in an improved overall adaptiveness.

CONCLUSIONS

This study provides evidence for phenotypic plasticity in response to severe hypoxia in *P. multicolor*. Gill morphology as well as structures surrounding the gills and outer shape differed between the field populations and between fish grown under hypoxia and normoxia, but there was disparity between the field results and the plasticity experiment.

Table 2 Summary of analyses of covariance of relationships between nonrespiratory characters and standard length for groups of *Pseudocrenilabrus multicolor*

	Field			Laboratory		
Character	Population	Mean size	*P* value	Group	Mean size	*P* value
Body depth (mm)	High DO	12.56	<0.001	Normoxia	10.57	0.267
	Low DO	13.30		Hypoxia	10.74	
Head width (mm)	High DO	7.52	0.802	Normoxia	5.48	0.030
	Low DO	7.57		Hypoxia	5.74	
Head length (mm)	High DO	******	******	Normoxia	11.48	0.030
	Low DO	******		Hypoxia	11.97	
Eye diameter (mm)	High DO	3.55	0.008	Normoxia	3.12	0.222
	Low DO	3.42		Hypoxia	3.14	
M. sternohyoideus – length (mm)	High DO	5.53	0.348	Normoxia	4.99	0.019
	Low DO	5.47		Hypoxia	4.79	
M. sternohyoideus – width (mm)	High DO	3.55	0.254	Normoxia	3.52	<0.001
	Low DO	3.60		Hypoxia	3.78	
M. sternohyoideus – depth (mm)	High DO	3.01	0.030	Normoxia	2.90	0.006
	Low DO	3.13		Hypoxia	2.69	
M. geniohyoideus – length (mm)	High DO	5.45	0.005	Normoxia	4.75	0.031
	Low DO	5.81		Hypoxia	5.00	
M. geniohyoideus – width (mm)	High DO	0.89	0.079	Normoxia	0.50	0.917
	Low DO	0.84		Hypoxia	0.50	
M. geniohyoideus – depth (mm)	High DO	0.54	0.535	Normoxia	0.84	0.733
	Low DO	0.52		Hypoxia	0.83	
M. levator ex. – length (mm)	High DO	2.84	0.914	Normoxia	2.39	0.001
	Low DO	2.83		Hypoxia	2.25	
M. levator ex. – width (mm)	High DO	0.64	0.082	Normoxia	0.46	0.394
	Low DO	0.59		Hypoxia	0.45	
M. levator ex. – depth (mm)	High DO	0.96	0.007	Normoxia	0.77	0.002
	Low DO	0.82		Hypoxia	0.71	
M. retractor dorsalis – length (mm)	High DO	******	******	Normoxia	1.62	0.923
	Low DO	******		Hypoxia	1.63	
M. retractor dorsalis – width (mm)	High DO	1.61	0.077	Normoxia	1.28	0.944
	Low DO	1.67		Hypoxia	1.27	
M. retractor dorsalis – depth (mm)	High DO	0.72	0.724	Normoxia	1.38	0.001
	Low DO	0.73		Hypoxia	1.26	
Lower pharyngeal jaw – length (mm)	High DO	2.72	0.215	Normoxia	2.49	0.228
	Low DO	2.80		Hypoxia	2.42	
Lower pharyngeal jaw – width (mm)	High DO	3.94	0.403	Normoxia	3.30	0.998
	Low DO	3.98		Hypoxia	3.30	
Lower pharyngeal jaw – depth (mm)	High DO	0.97	0.671	Normoxia	0.56	0.016
	Low DO	0.96		Hypoxia	0.50	
Upper pharyngeal jaw – length (mm)	High DO	2.20	0.002	Normoxia	1.67	0.675
	Low DO	2.04		Hypoxia	1.66	
Upper pharyngeal jaw – width (mm)	High DO	1.71	<0.001	Normoxia	1.28	0.912
	Low DO	1.52		Hypoxia	1.27	
Upper pharyngeal jaw – depth (mm)	High DO	1.18	0.009	Normoxia	0.53	0.891
	Low DO	1.08		Hypoxia	0.53	

The field comparison represents populations from a low oxygen (Lake Manywa swamp) and a high oxygen (Lake Kayanja ecotone) site. The laboratory comparison represents groups reared under normoxia and extreme hypoxia (1 mg L^{-1}). Both gill characters and standard length were $\log_{10}$ transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean body weight and a common regression line for the two groups). Adjusted means are not presented when the slopes of the relationships differed. Lake Kayanja, $n = 7$; Lake Manywa, $n = 7$; normoxia treatment, $n = 5$; hypxoia treatment, $n = 5$.

A much-discussed hypothesis assumes that evolutionary change will often proceed as an initial phenotypically plastic response to a novel environmental condition followed by genetic changes in the same direction (genetic assimilation). Our study reveals that assimilation is likely to have happened for some parameters (e.g. longer gill filaments), but more experiments are necessary to confirm this. We do present field evidence to suggest that assimilation of plastic traits does not always take place after long-term selection and that long-term selection in combination with phenotypic plasticity may provide a better adapted response to changed environmental conditions than plasticity alone.

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BIOSKETCH

Lauren J. Chapman has research interests in the fields of aquatic ecology, aquatic conservation, and physiological ecology, with particular emphasis on the respiratory ecology of fish, dispersal, phenotypic plasticity, and the role of wetlands in the maintenance of fish faunal structure and diversity.

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