

# **Fish Physiology, Toxicology, and Water Quality**

Proceedings of the Ninth International  
Symposium, Capri, Italy,  
April 24-28, 2006

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# Fish Physiology, Toxicology, and Water Quality

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Capri, Italy, April 24-28, 2006

Edited by

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## **ABSTRACT**

Scientists from Europe, North America and South America convened in Capri, Italy, April 24-28, 2006 for the Ninth International Symposium on Fish Physiology, Toxicology, and Water Quality. The subject of the meeting was “Eutrophication: The toxic effects of ammonia, nitrite and the detrimental effects of hypoxia on fish.” These proceedings include 22 papers presented over a 3-day period and discuss eutrophication, ammonia and nitrite toxicity and the effects of hypoxia on fish with the aim of understanding the effects of eutrophication on fish. The ever increasing human population and the animals raised for human consumption discharge their sewage into rivers and coastal waters worldwide. This is resulting in eutrophication of rivers and coastal waters everywhere. Eutrophication is associated with elevated ammonia and nitrite levels, both of which are toxic, and the water often becomes hypoxic. Aquatic hypoxia has been shown to reduce species diversity and reduce total biomass.

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## **FOREWORD**

### **Symposia on Fish Physiology, Toxicology, and Water Quality; a Brief History**

Vance Thurston and Rosemarie Russo were good friends long before I knew them, and I have known Vance since the 1970's. We would go to Russia together and we had a number of joint research projects. In the early 1980's Rose and I were driving from Athens to Atlanta when she told me she had been asked to organize a science exchange program with several research institutes in China. She asked if I knew anyone in China because she knew I had been a guest of the central government in China and had worked at Zhongshan University in Guangzhou, PRC for several months. The end result was that UBC held the cooperative agreement to encourage research collaboration between environmental scientists from the Peoples' Republic of China and scientists from North America and Europe, under the USA-PRC environmental protection agreement.

The First Symposium was held at Zhongshan University, Guangzhou, PRC, in September 1988, with the help of Professor Lin Hao-ran of Zhongshan University, and attracted scientists from Europe, Canada, and the U.S., as well as many scientists from the PRC and Hong Kong. This was the beginning of the series of international symposia organized by Vance, Rose, and myself, sponsored by the US Environmental Protection Agency through the Athens Laboratory. The Guangzhou Symposium was memorable for its audio equipment: the sound was such that the lectures could be heard by people on boats passing down the Pearl River. The Second Symposium was held two years later in September 1990 in Sacramento, California, with the help of Professor Joe Cech. We had an excellent dinner in the Train Museum; the positive response from all participants illustrated the world wide acceptance of Californian cuisine. The location for the Third Symposium was Nanjing University in Nanjing, PRC, in November 1992 and this time we had the skilled help of Professor Jin Hong-jun. Vance, the great entertainer, sang songs during dinner and as usual brought us together as a group. Vance made a special effort for the Fourth Symposium, held in Bozeman, Montana, in September 1995, to encourage participation of scientists from Europe, as well as from the PRC, North America, and Mexico. Vance drove a van from Bozeman to San Francisco with Chinese delegates on board to show them various sites of ecological interest in North America. We returned to China for the Fifth Symposium, which was held at the City University of Hong Kong, in November 1998 with the able help of Professor Rudolf Wu. The Sixth Symposium was held in La Paz, Mexico, in January 2001. In addition to attracting a large audience from the Mexican scientific community, the 30 papers accepted for presentation represented 15 countries, more than any previous Symposium. The Seventh Symposium, in Tallinn, Estonia, was affectionately dedicated to the memory of Robert Vance Thurston, who died unexpectedly on February 16th, 2002, at the age of 75. Dr. Arvo Tuvikene was a great help in putting that Symposium together, along with Gretchen Rupp from Montana State University. Vance was very active in international environmental research projects in the Baltic republics, the former Soviet Union, and Mexico. He was practically an honorary citizen of Lithuania for the many projects he had there and the computers and other equipment he provided to their scientists. Rose and I organized the Eighth Symposium in Chongqing, China in October 2004 in association with Dr. George

Bailey of Athens EPA and the able help of Professor Gao Yuqi of the 3<sup>rd</sup> Military Medical University in Chongqing.

The functions of these Symposia are twofold, the first to exchange scientific information and the second to remove political barriers between scientists from different countries and promote collaboration. This has been achieved. Now we come to the Ninth Symposium in this long-standing series, to be held in Capri, Italy with the able assistance of Professor Bruno Tota and Laurajean Carbonaro.

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# Interdemic variation in gill morphology of a eurytopic African cichlid

by

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## Introduction

The physical environment has a major influence on the ecology of organisms. For fishes, the availability of dissolved oxygen (DO) is one abiotic factor that can exert a strong selective force by affecting habitat quality, growth, survival, and reproduction. Oxygen scarcity (hypoxia) occurs naturally in systems characterized by low levels of ambient light and mixing. For example, heavily vegetated swamps, flooded forests, and the hypolimnion of deep lakes are habitats particularly prone to oxygen limitation. Low DO can be acute in (but not limited to) tropical waters where high temperatures elevate rates of organic decomposition and reduce oxygen tensions in the water. Unfortunately, environmental degradation is increasing the occurrence of hypoxia as the influx of municipal wastes and fertilizer runoff accelerates eutrophication and pollution of water bodies (Prepas and Charette, 2003). Increasing hypoxia is a threat to fresh waters and coastal waters worldwide; and oxygen depletion in deeper waters, one side effect of this process, can lead to fish kills and a massive reshaping of fish communities (Prepas and Charette, 2003). It has therefore become increasingly important to understand the effects of hypoxia on aquatic organisms.

Despite much interest in the physiological and biochemical adaptations of fishes to hypoxic stress, the significance of dissolved oxygen in driving divergence among populations remains largely unexplored. Strong selection for hypoxia tolerance may lead to variation among populations that experience divergent aquatic oxygen environments. This may lead to further diversification if the benefits accrued by higher respiratory performance in hypoxic habitats lead to sub-optimal performance in normoxic waters. Our studies of East African fishes have demonstrated that alternative dissolved oxygen (DO) environments provide a strong predictor of interpopulational (interdemic) variation, particularly with respect to respiratory traits (e.g., gill size) and associated characters. In a series of studies comparing populations from low- and high-oxygen environments, we found total gill size (surface area and/or total gill filament length) to be larger in swamp-dwelling populations of the cyprinid *Barbus neumayeri* (Chapman *et al.*, 1999; Schaack and Chapman, 2003), the mormyrids *Gnathonemus victoriae* and *Petrocephalus catostoma* (Chapman and Hulen, 2001), the cichlid

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*Pseudocrenilabrus multicolor victoriae* (Chapman *et al.*, 2000; Chapman *et al.*, 2002), and the air-breathing African catfish *Clarias liocephalus* (McCue, 2001) relative to open-water populations. This interdemic variation in gill traits could be purely genetically based or due to environmental influences on gene expression (i.e. phenotypic plasticity; DeWitt and Scheiner, 2004). If there is phenotypic plasticity, it may be determined either at a critical period of ontogeny, or be a phenotypic response that remains labile throughout an individual's lifetime.

Recent studies support an element of environmentally-induced plasticity in gill morphology in response to DO regime. In a study of the African cichlid *P. multicolor*, we compared gill size of a population from a stable hypoxic habitat with one of a stable well-oxygenated habitat (Chapman *et al.*, 2000). In addition, we compared siblings (split-brood) raised under hypoxic or well-oxygenated conditions. The response to hypoxia was an increase in gill area, both in the field (29%) and in the plasticity experiment (18%). The difference in the magnitude of the response between field and experimental fishes may reflect differences in selection pressures between populations, and/or a combination of inherited changes and plasticity (Chapman *et al.*, 2000). For the sea bass (*Dicentrarchus labrax*) Saroglia *et al.* (2002) reported higher gill surface area associated with lower oxygen partial pressure of the water in which the bass were reared for 3 months, providing further evidence of phenotypic plasticity in fish gills in response to DO availability. The maintenance of plasticity in gill morphology of divergent populations may preserve the possibility for future evolutionary responses. This could be particularly relevant in aquatic systems of the Lake Victoria basin of East Africa that is characterized by a volatile history, peppered by volcanic explosions and changing lake levels (Beadle, 1981; Stager *et al.*, 1986; Johnson *et al.*, 1996).

The species flock of endemic haplochromine cichlids in Lake Victoria represents one of the most rapid, extensive, and recent radiations of vertebrates known (Kaufman 1992; Kaufman *et al.*, 1997; Seehausen *et al.*, 2002). By contrast, a small number of eurytopic cichlid species inhabit a broad range of habitats (rivers, streams, lakes, and swamps) throughout the Lake Victoria watershed and adjacent areas. Mechanisms facilitating the eurytopic distribution of these species remain largely unknown; however, strong patterns of morphological variation across populations suggest locally adapted phenotypes (Smits *et al.*, 1996; Chapman *et al.*, 2000). In this study, we quantified interdemic variation in the gill morphology of the widespread African cichlid *Astatoreochromis alluaudi*. We compared gill metrics of a population from a hypoxic lake habitat with one of a well-oxygenated hyper-eutrophic lake (Chapman *et al.*, 2000). In addition, we compared siblings (split-brood) reared under hypoxic or well-oxygenated conditions for one population.

### *Study Site and Species*

*Astatoreochromis alluaudi* is a widespread haplochromine cichlid that can be found in a range of habitats in the Lake Victoria basin of East Africa including fast flowing rivers, lakes, wetlands, and streams (Greenwood, 1959; Chapman *et al.*, 1996a,b). It is a species well known for a high degree of plasticity in the pharyngeal jaw apparatus and associated muscles in response to the nature of its prey base. When introduced to a hard prey diet (e.g., mollusks) *A. alluaudi* develops a large pharyngeal mill with hypertrophied muscles; while a softer diet is associated with a smaller jaw and trophic muscles (Greenwood, 1965a; Huysseune *et al.*, 1994). Smits *et al.* (1996) reported that *A. alluaudi* feeding on snails showed a 31% increase in

head volume compared to fish that fed on insects. Given the widespread distribution of this species and the extraordinary plasticity of its pharyngeal jaw apparatus, we anticipated a similarly high level of variation in its gill apparatus in response to variation in aquatic oxygen. We selected two field populations of *A. alluaudi* from Uganda. Both are lake populations but one (Lake Nabugabo) experiences very low oxygen conditions in dense wetland bays and the other (Lake Saka) is found in areas of high oxygen.

Lake Nabugabo, a satellite of Lake Victoria ( $24 \text{ km}^2$ , approximately  $0^\circ 45' \text{ S}$  and  $31^\circ 45' \text{ E}$ ), is characterized by an extensive stretch of shoreline macrophytes (mainly *Misanthidium violaceum* and *Vossia cuspidata*), interrupted by stretches of forests (dominated by *Ficus* spp.) and sand beaches. Small bays surround the east side of the lake and provide an ideal environment for the development of the bladder-wort *Utricularia*. The lake lies within an extensive wetland that was formerly a bay on the western shore of Lake Victoria (Fig. 1, Greenwood, 1965b). Long shore bars that isolate Lake Nabugabo from Lake Victoria were created during water-level fluctuations about 4,000 years ago (Greenwood, 1965b). Today, water from Lake Nabugabo drains southeastward via the Lwamunda swamp before it seeps through the sand bar into Lake Victoria. *Astatoreochromis alluaudi* was first reported from Lake Nabugabo by Greenwood (1965b) based on a survey conducted by a Cambridge expedition. However, only one specimen was collected at that time with no records of habitat use. In recent studies, *A. alluaudi* has been reported in the wetland ecotones of the lake and the papyrus-choked Juma River, the main tributary to the lake (Chapman *et al.*, 1996a,b; Rosenberger and Chapman, 1999; Schofield and Chapman, 1999). In a quantitative survey of habitat use of fishes in Lake Nabugabo conducted in 2000 (see Chapman *et al.*, 2003 for details), we found that *A. alluaudi* were restricted in their distribution to wetland ecotones, primarily in small bays characterized by dense growth of *Ceratophyllum* and bordered by emergent wetland grasses. In the 2000 survey, the DO level where *A. alluaudi* were captured averaged  $3.5 \text{ mg l}^{-1}$  (upper 50 cm of the water column) with an average water temperature of  $24.4^\circ \text{ C}$ . This current distribution pattern of this species may reflect the introduction of the predatory Nile perch in the early 1960's. Many haplochromine cichlids were largely restricted to wetland ecotones, which serve as refuges from Nile perch predation (Chapman *et al.*, 1996a,b, 2002, 2003).

Lake Saka is found at the northernmost extreme of the crater lakes in western Uganda ( $0^\circ 40' \text{ N}$  and  $30^\circ 15' \text{ E}$ , elevation of 1,520 m) and has a maximum length of 1.4 km and maximum width of 1.0 km. A small crater forms an embayment at the southeast corner of the lake (Melack, 1978). Several wetlands drain into Lake Saka, and the drainage basin has been almost totally cleared for agricultural production by subsistence farmers and a large Ugandan government prison farm (Crisman *et al.*, 2001). Unlike Lake Nabugabo, this is a highly eutrophic lake characterized by supersaturated DO conditions during the peak light of the day. Crisman *et al.* (2001) reported DO in surface waters often exceeding  $15 \text{ mg l}^{-1}$  (180% saturation) during 1995-1998. In a quantitative survey of the fish fauna of Lake Saka conducted in 2000, we found that *A. alluaudi* was most abundant in wetland ecotones with average surface DO concentration of  $10.4 \text{ mg l}^{-1}$ . Nile perch were also stocked into Lake Saka in the early 1970s, but currently persist at low numbers.

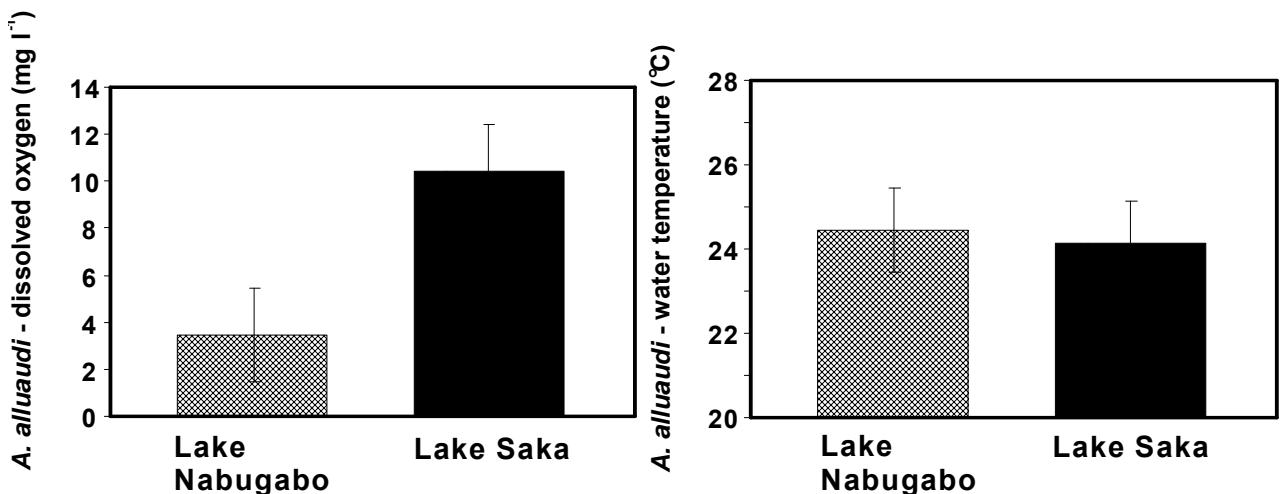


Figure 1. Mean levels of dissolved oxygen concentration ( $\text{mg l}^{-1}$ ) and water temperature ( $^{\circ}\text{C}$ ) measured in the upper 50 cm of the water column at sites where *Astatoreochromis alluaudi* were captured in a survey (2000) of Lake Nabugabo and Lake Saka, Uganda. (Unpublished data from Chapman and colleagues.)

## Methods

### *Field Collections*

In both lakes (Nabugabo and Saka), *A. alluaudi* were captured using metal minnow traps. Fish were euthanized with an overdose of MS222 in the field and preserved in buffered paraformaldehyde ( $40 \text{ g l}^{-1}$ ).

### *Rearing Experiment*

As part of a larger lab-rearing experiment to explore direct and indirect tradeoffs between trophic structures and the respiratory apparatus of *A. alluaudi*, F1 offspring from Lake Nabugabo were raised under low ( $\approx 1.3 \text{ mg l}^{-1}$ ) and high ( $\approx 7.4 \text{ mg l}^{-1}$ ) DO. Water temperature averaged  $24.5^{\circ}\text{C}$ . We used a split brood design with the F1 offspring of three sets of parents to provide family level replication; but the number of families was limited due to complexity of key target traits (gill surface area). Brooding pairs were held in separate, normoxic aquaria until young were released from the female's mouth. When a brood was released from a female, each brood was divided into two groups of 10 individuals and groups randomly allocated to one of 6 aquaria (20-l) between two treatments. After 2 months, each tank was cropped to 6 individuals, by randomly removing and euthanizing offspring. *Astatoreochromis alluaudi* in Lake Nabugabo are not molluscivorous, as snails are extremely rare in this system (Beadle, 1981; Efitre *et al.*, 2001). In the rearing experiment reported in this paper, fish from Lake Nabugabo were fed tetramin food flakes once per day. Fish were raised for approximately 1 year and then euthanized (MS222) and preserved in buffered paraformaldehyde.

### *Gill morphometry*

Gill metrics were estimated for 10 specimens from each field population. We measured the following gill characters: total gill filament length (TGFL), average lamellar density (ALD), average lamellar area (ALA), total hemibranch area (THA), and total gill surface area (TGSA). For the rearing experiment, we measured three specimens from each family, and we included only measures for TGFL and THA. TGSA and lamellar characters on lab-reared fish will be the focus of future studies. Total gill filament length was measured using standard methods modified after Muir and Hughes (1969) and Hughes (1984a). For each fish, the branchial basket was removed, and the four gill arches from the left side of the basket were separated. For each hemibranch of the gill arches, the length of every 5<sup>th</sup> gill filament was measured (Fig. 2a). Two successive measurements along a hemibranch were averaged and multiplied by the number of filaments in the section between the two filaments. Filament lengths were summed for the four hemibranchs and multiplied by 2 to produce an estimate of total gill filament length (TGFL). Lamellar density was measured in the dorsal, middle, and ventral parts of every 10<sup>th</sup> filament of the second gill arch on the left side (Fig. 2b). The total number of lamellae (on one side of the filament) and average lamellar density (ALD) were estimated using a weighted mean method that takes into account the difference in length of different filaments (Muir and Hughes, 1969; Hughes and Morgan, 1973). For every 10<sup>th</sup> filament starting at filament 5, the length and height of 5 secondary lamellae was measured at the top, middle, and bottom sections of the filament (Fig. 2b). Average values of these characters for each filament were converted to estimates of lamellar area using a regression determined through the dissection of several lamellae from various sections of the second gill arch from two or more specimens from each population. The sum of the total lamellar area for all sections of the second arch was divided by the total number of lamellae and multiplied by 2 to produce a weighted average bilateral surface area on one side of the filament (ALA). Total gill surface area (TGSA) was determined using the formula:  $TGSA = TGFL \times 2 \times ALD \times ALA$ . To estimate hemibranch area, we digitized the area of the gill filaments on the 8 hemibranchs on 1 side of the fish. This was multiplied by 2 to produce an estimate of the total hemibranch area (THA, not including the bony arches, Fig. 2a). For all characters, images were captured with a Leica stereoscope and Infinity I camera, and linear and areal dimensions measured with Motic Images software version 2.0.

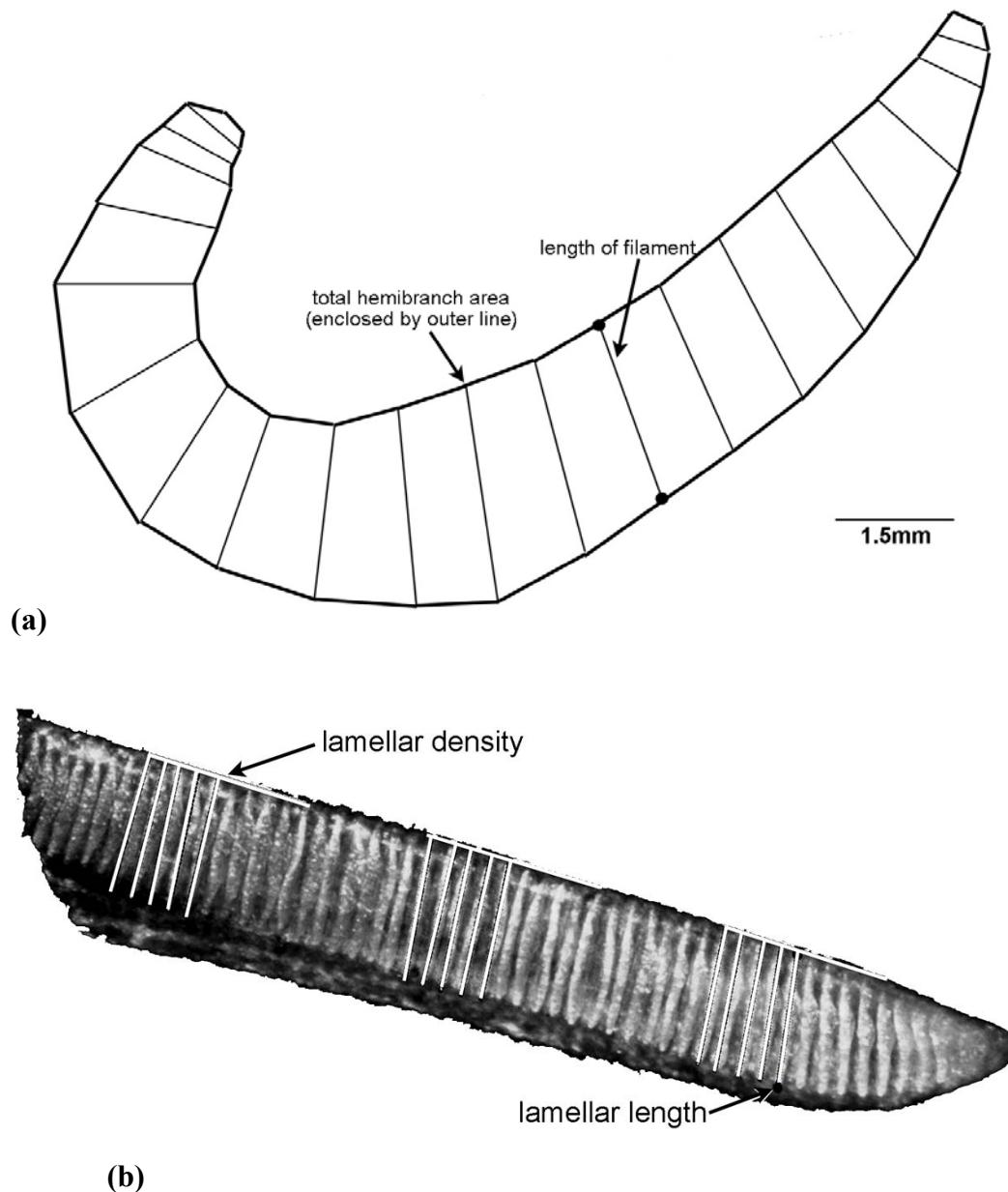


Figure 2. Illustration of measurements used for analyses of gill size and shape of *Astatoreochromis alluaudi*. (a) The length of filaments was used to estimate total gill filament length (TGFL); the total hemibranch area (THA) was estimated from the outer line. (b) Average lamellar density (ALD) was estimated by measuring the length encompassed by 10 filaments at the base, middle, and upper part of selected filaments. Lamellar length (shown here) and height (not shown here) were measured for 5 lamellae at the base, middle, and upper part of selected filaments and used in the calculation of average lamellar area (ALA).

Palzenberger and Pohla (1992) reviewed the literature on gill morphometry of fishes. From their data set for 28 non-air-breathing freshwater species (with multiple populations for eight species), they extracted the mean slope of significant regressions for gill morphometric parameters and body weight. They set the lowest and highest mean values within each parameter range to 0% and 100% respectively to create a range of values for each gill character. This permitted them to express the values of a species as a percentage within the range of values for freshwater fishes. We used their parameter estimates for total gill surface area and total gill filament length to estimate these characters for each field population expressed as a percentage of freshwater fishes.

## Results

### *Field populations*

Gill characters were measured on 10 fish from each field population selected to maximize range in body mass (Lake Nabugabo: mean body mass = 5.20 g, range = 1.427 g to 11.277 g; Lake Saka: mean = 10.14 g, range = 2.486 to 15.06 g). For the two populations, total gill filament length, average lamellar area, hemibranch area, and total gill surface area were positively correlated with body size (Table I). Average lamellar density was negatively related to body size in both groups (Table I). ANCOVA indicated no difference in the slopes of the bilogarithmic relationships between *A. alluaudi* of the Lake Nabugabo and Lake Saka populations for total gill filament length, total hemibranch area, lamellar density, or lamellar area, though there was trend toward heterogeneity in slopes for the latter (Table I). Intercepts differed for all four of these characters between field populations (Table I). When adjusted for body mass total gill filament length, hemibranch area, and lamellar area were greater in fish from the hypoxic waters of the Lake Nabugabo wetland, than in fish from the well-oxygenated waters of Lake Saka (Table I, Fig. 3a,b). Interestingly, gill lamellar density was lower in *A. alluaudi* from Lake Nabugabo (Table I, Fig. 3c). The slopes of the bilogarithmic relationship between total gill surface area and body mass differed between the two populations, with gill surface area increasing more slowly with body size in the Lake Saka population. Given heterogeneity in the slopes, we did not test for a difference in the intercepts; however, for a fish of the average size of the two populations, and using the independent regressions for each population (5.8 g), total gill surface area was estimated at  $35.87 \text{ cm}^2$  for *A. alluaudi* from Lake Nabugabo and  $15.65 \text{ cm}^2$  for *A. alluaudi* from Lake Saka (Fig. 3d).

Data on *A. alluaudi* were converted into a percentage of the estimated range of all species following Palzenberger and Pohla (1992) for total gill filament length and total gill surface area. Total gill filament length, expressed as a percentage of freshwater fishes averaged 67% for Lake Nabugabo, and 56% for Lake Saka. For total gill surface area, *A. alluaudi* from Lake Saka averaged only 20% of the range for freshwater fishes, while those from Nabugabo averaged 54% of the range.

Table I. Summary of linear regression analyses and analyses of covariance (ANCOVA of relationships between gill morphometric characters and body mass (g) for *Astaoreochromis alluaudi* from Lake Nabugabo (low-oxygen site) and Lake Saka (high-oxygen site). Both gill characters and body mass were  $\log_{10}$  transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean body mass of 6 g and a common regression line). If slopes were heterogeneous, then we did not test for a difference in intercepts (Int.). \*For total gill surface area, the means represented the predicted value from the population-specific regression lines, since slopes were heterogeneous.

Character	Site	n	Slope	Int.	r	p	ANCOVA		Slope p	ANCOVA F	Int. p	Adj. means
							F	p				
<b>Total gill filament length (mm)</b>	Nabugabo	10	0.641	2.012	0.978	<0.001	2.008	0.176	146.665	<0.001	2552.70	
	Saka	10	0.471	2.891	0.979	<0.001					1782.38	
<b>Hemibranch Area (mm<sup>2</sup>)</b>	Nabugabo	10	0.671	2.184	0.995	<0.001	0.342	0.567	150.676	<0.001	504.66	
	Saka	10	0.526	3.002	0.994	<0.001					321.36	
<b>Lamellar Density (no. per mm)</b>	Nabugabo	10	-0.17	1.589	0.807	0.008	0.195	0.665	29.269	<0.001	28.71	
	Saka	9	-0.14	1.677	0.786	0.007					36.90	
<b>Lamellar area (mm<sup>2</sup>)</b>	Nabugabo	10	0.641	-2.467	0.893	0.001	4.429	0.053	23.83	<0.001	0.0099	
	Saka	9	0.339	-2.488	0.869	0.001					0.0055	
<b>Total gill Surface area (cm<sup>2</sup>)</b>	Nabugabo	10	0.723	1.003	0.97	<0.001	8.669	0.01	*****	*****	30.76*	
	Saka	9	0.529	0.791	0.971	<0.001					15.49*	

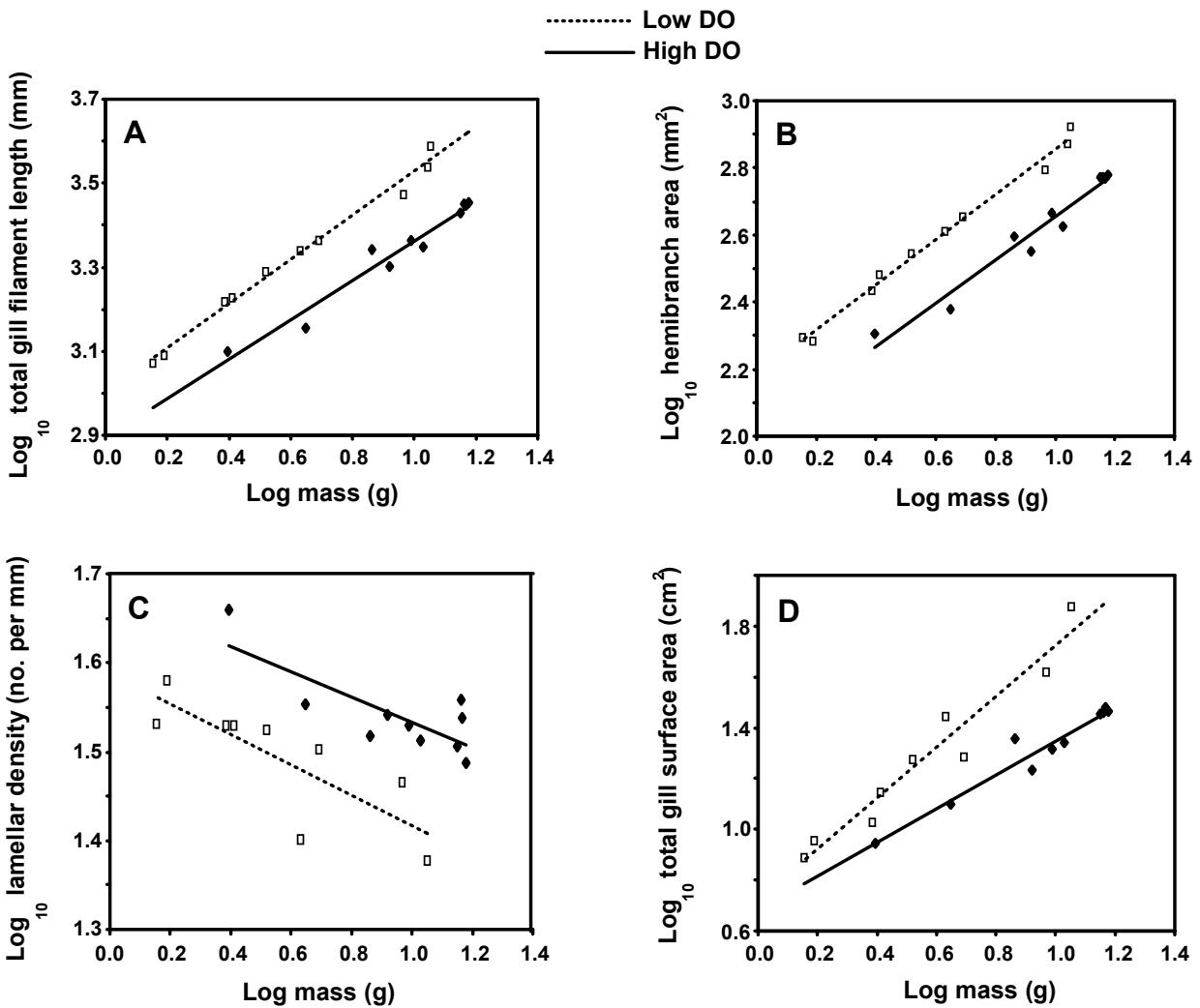


Figure 3. Bilogarithmic plots of gill metrics and body mass for *Astatoreochromis alluaudi* from two field populations: Lake Nabugabo (low oxygen), Lake Saka (high oxygen). (a) total gill filament length (mm), (b) total hemibranch area ( $\text{mm}^2$ ), (c) average lamellar density (number of lamellae per mm), and (d) total gill surface area ( $\text{cm}^2$ ).

#### Lab-rearing study

For our lab-rearing study, full siblings whose parents originated from Lake Nabugabo were raised under low- and high-DO. Total gill filament length and total hemibranch area were measured for 3 fish per family per treatment selected to maximize range in body size (normoxia: mean body weight=5.4 g, range=1.7 to 10.4 g; hypoxia: mean=4.8 g, range=3.4 to 9.2 g). For both the normoxia- and hypoxia-raised fish, total gill filament number and hemibranch area were positively related to body mass (Table II). Analyses of covariance indicated no difference in the slopes of the bilogarithmic relationships between the hypoxia and normoxia groups for total gill filament length and hemibranch area (Table II); however, the intercepts differed between groups. For a fish of a given body mass, total gill filament length

and hemibranch area were greater in fish grown under extreme hypoxia than in fish grown under normoxia (Table II, Fig. 4a,b).

Table II. Summary of linear regression analyses and analyses of covariance (ANCOVA) of relationships between gill morphometric characters and body mass (g) for F1 offspring of *Astatooreochromis alluaudi* from Lake Nabugabo. Both gill characters and body mass were  $\log_{10}$  transformed. The mean values represent antilogged adjusted means calculated from the ANCOVA analyses (sample means adjusted for a common mean body mass of 6 g and a common regression line).

Character	DO	n	Slope	Intercept	r	p	ANCOVA		Slope	ANCOVA		Intercept	Adj.
							F	p	p	F	p	means	
<b>Total gill</b>	Hypoxia	9	0.422	3.094	0.905	<0.001	0.537		0.476	122.776	<0.001	2371.4	
<b>Filament</b>	Normoxia	9	0.48	2.92	0.985	<0.001						1733.8	
<b>length (mm)</b>													
<b>Hemibranch</b>	Hypoxia	9	0.528	2.302	0.902	<0.001	2.904		0.11	156.36	<0.001	449.78	
<b>area (<math>\text{mm}^2</math>)</b>	Normoxia	9	0.677	2.021	0.994	<0.001						295.80	

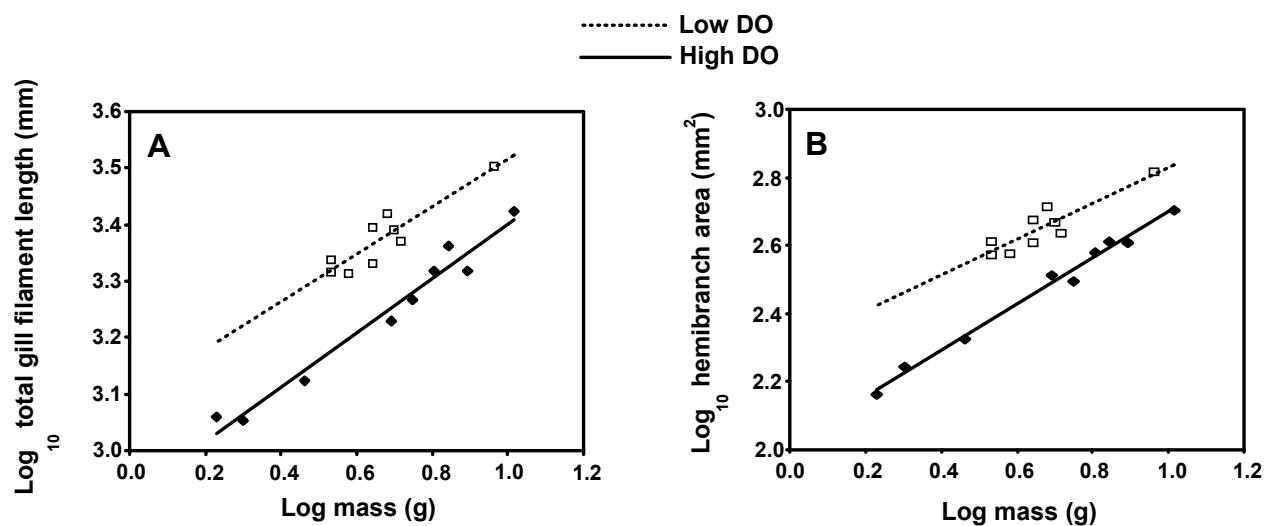


Figure 4. Bilogarithmic plots of gill metrics and body mass for F1 offspring *Astatooreochromis alluaudi* from Lake Nabugabo raised under low- and high-oxygen conditions. (a) total gill filament length (mm) and (b) total hemibranch area ( $\text{mm}^2$ ).

## Discussion

### *Aquatic Oxygen as a Predictor of Morphological Divergence*

Several studies based on interspecific comparisons of non-air-breathing fishes have suggested that large gill respiratory surface may reflect hypoxic conditions in their environment (Gibbs and Hurwitz, 1967; Galis and Barel, 1980; Fernandes *et al.*, 1994; Mazon *et al.*, 1998). There is now a growing body of evidence to support similar patterns of variation within species. Significant variation in total gill surface area and/or other metrics of gill size has been reported among populations of widespread species that inhabit alternative oxygen environments, including representatives of several families: Cichlidae, Cyprinidae, Mormyridae, and Poeciliidae (Chapman *et al.*, 1999, 2000, 2002; Chapman and Hulen, 2001; Schaack and Chapman, 2003; Timmerman and Chapman, 2004). In *A. alluaudi*, patterns of variation in gill metrics between populations from alternative aquatic oxygen environments support this trend. *Astatoreochromis alluaudi* is one of the few extremely widespread haplochromine cichlids in East Africa, in stark contrast to the enormous number of stenotypic endemic cichlids in great lakes of the region. The remarkable morphological variation in its trophic and respiratory characters may have facilitated its eurytopic distribution. The haplochromine cichlid *Pseudocrenilabrus multicolor* inhabits a similarly broad range of habitats (rivers, streams, lakes, and swamps), and is also characterized by strong interdemographic variation in gill morphology, trophic morphology, and other morphological traits (Chapman *et al.*, 2000, 2002).

The difference in total gill filament length between *A. alluaudi* from lakes Nabugabo and Saka (30%) was much lower than the difference in total gill surface area (50%), and notably the total gill surface area of fish from Lake Saka fell within the lower range (20<sup>th</sup> percentile) of freshwater fishes (derived from equation in Palzenberger and Pohla, 1992). Lakes Nabugabo and Saka differ not only in dissolved oxygen availability but also in other site characters that may contribute to differences in gill morphology between lakes. For example, Lake Saka is subject to very high concentrations of a number of potentially toxic blue-green algae (aka cyanobacteria), including *Microcystis aeruginosa*, *Oscillatoria* sp. and *Cylindrospermopsis* sp., with *M. aeruginosa* the dominant species by biomass (E. Phlips and L. Chapman unpubl. data). Since the gill comprises over half the body surface area of a fish and is characterized by a thin barrier between the blood and the water, most chemical transfer between the fish and the aquatic environment occurs across the gills (Hughes, 1984b; Wood and Soivio, 1991; Randall and Brauner, 1993). Thus, fishes living in waters with high levels of algal toxins may be selected for decreased gill surface area. To provide additional support for the hypothesis of oxygen caused population differentiation, we performed the rearing experiment to directly test for oxygen effects on gill morphology while holding other environmental parameters constant.

### *Developmental Plasticity in Fish Gills*

Phenotypic plasticity often evolves because it allows organisms to mitigate environmental variation (DeWitt and Scheiner, 2004). We found a strong element of developmental plasticity in total gill filament length and total hemibranch area in *A. alluaudi* in response to the dissolved oxygen environment in which it was raised. *Astatoreochromis*

*alluaudi* grown under normoxia exhibited a total gill filament length and a hemibranch area smaller (27% and 34%, respectively) than fish raised under hypoxic conditions. Two lines of evidence to suggest this plasticity in gill morphology is adaptive. First the induced morphological responses are in the direction one would predict to increase oxygen uptake capacity. Second, the response was similar in the lab-reared fish to that observed between the two field populations from alternative oxygen environments. The next steps in this work will focus on quantification of gill surface area for this rearing experiment and the interaction of environmentally-induced variation and population effects by comparing fish from both lakes Nabugabo and Saka reared under low- and high-oxygen conditions.

The maintenance of plasticity in these divergent populations may preserve the possibility for future evolutionary responses and foster population colonization and persistence in novel environments (Schlichting and Pigliucci, 1998; Yeh and Price, 2004). Swamps grade into lakes and rivers in the Lake Victoria basin, and small changes in water levels can produce large changes in available habitat and in connectivity. Thus, fish lineages may experience alternative oxygen environments either within or among generations. We found high levels of developmental plasticity in the gill morphology of two other species of East African fishes that persist in variable DO environments. These other species include the cichlid *P. multicolor* (Chapman *et al.*, 2000) and the cyprinid *Barbus neumayeri* (L. Chapman unpubl. data). And, other studies have demonstrated high levels of plasticity in gill traits in fishes (Schwartz, 1995; Sargolia *et al.*, 2002) and larval salamanders (Bond, 1960; Burggren and Mwalukoma, 1983). Thus, environmentally-induced gill proliferation may be a widespread response to sub-lethal hypoxic stress.

The degree of developmental plasticity in response to alternative DO environments in *A. alluaudi* may differ depending on other features of the environment or the natal history of the population. When fed on hard prey (e.g., molluscs) *A. alluaudi* will develop a massive pharyngeal mill with hypertrophied muscles, whereas a softer diet leads to reduction in pharyngeal jaw size and associated musculature (Greenwood, 1965a; Huysseune *et al.*, 1994). Smits *et al.* (1996) found that the total head volume in snail-eating *A. alluaudi* was 31% larger than in fish from an insect-eating population, and they reported internal reallocations of the respiratory apparatus (change in the shape of the gills). Thus, gill proliferation may be compromised to some degree when *A. alluaudi* is faced with dual challenge of hypoxia and a mollusk-dominated diet. We are currently exploring this interaction in *A. alluaudi* (Chapman, Galis, and DeWitt, unpubl. data).

Despite an apparent advantage to gill proliferation in response to hypoxic stress, an important issue is understanding what maintains these divergent respiratory phenotypes in the field. Why not have large gills in all environments? Fitness trade-offs, whereby the phenotype with the highest performance in one habitat performs sub-optimally in the alternative environment, may contribute to the maintenance of variation among field populations (Van Buskirk *et al.*, 1997; DeWitt and Scheiner, 2004). Trade-offs between feeding and respiratory structures seem very likely in fishes because of their generally compact, laterally compressed head morphology. Our studies on two East African fishes, the cichlid *P. multicolor* and the cyprinid *B. neumayeri*, suggest potential trade-offs between respiratory phenotypes. For example, we demonstrated that adaptive change in gill size (large gills) in fish from hypoxic

waters correlates with reduced size of key trophic muscles and feeding performance relative to small-gilled conspecifics (Chapman *et al.*, 2000; Schaack and Chapman, 2003). These trade-offs may lead to fitness costs in the field that impose habitat-specific selection pressures on dispersers.

Recent models of the role of phenotypic plasticity in driving genetic evolution argue that moderate levels of adaptive plasticity are optimal for evolution in novel environments by enhancing population persistence and placing populations under directional selection leading to potentially higher adaptive peaks (Price *et al.*, 2003). The widespread distribution of some East Africa cichlids such as *A. alluaudi* and *P. multicolor* may reflect broad environmental tolerances due to their phenotypically plastic responses to environmental variation. However, these plastic responses may be assimilated genetically on the long term if populations are under directional selection towards new adaptive peaks (Price *et al.*, 2003; West-Eberhard, 2005).

## Summary

The significance of variation in dissolved oxygen in driving phenotypic divergence is a largely unexplored aspect of aquatic biodiversity. However, there is now strong evidence that alternative oxygen environments are a strong predictor of intraspecific variation in fishes, particularly in respiratory traits and associated characters. Developmental plasticity seems to play a large role in explaining variation in gill morphology among populations, and may be an important mechanism contributing to the widespread distribution of species that cross strong dissolved oxygen gradients. Future studies on widespread African cichlids that explore the interaction of genetic and environmentally-induced morphological variation in multiple populations should elucidate the potential that an initially plastic response to a novel oxygen environment may be followed by genetic changes in the same direction.

One of the many challenges facing freshwater fishes is the increasing occurrence of hypoxia, which has lead to fish kills, changes in fish distribution, and a massive reshaping of some fish communities. Thus, it has become increasingly important to understand the consequences of low-oxygen stress on fish populations. Interpopulational variation and phenotypic plasticity in respiratory traits may contribute to species persistence in the face of environmental change.

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