Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance

Sarah Schaack and Lauren J. Chapman

Abstract: Adaptive change in one character may affect associated, but functionally unrelated, characters in adaptive or nonadaptive ways. For example, adaptive change in gill size (i.e., larger gills in hypoxic habitats) may alter adjacent feeding structures and therefore have indirect effects on feeding behavior. This study examines whether differences in total gill filament length among populations of the African cyprinid *Barbus neumayeri* correlate with variation in trophic structures and feeding performance. Morphological measurements revealed larger total gill filament length in populations from low-oxygen habitats. Differences in external and internal morphology of the trophic apparatus correlated with those found in gills and suggest a reduction in food capture and processing ability in fish with larger respiratory apparatus. In paired feeding trials on a novel prey type, large-gilled fish spent more time feeding than small-gilled fish of the same body size without ingesting more food. This suggests less efficient food uptake in large-gilled fish from hypoxic habitats. We suggest that variation in gill size facilitates the broad habitat distribution of *B. neumayeri* but may limit the success of phenotypes crossing physicochemical gradients.

Résumé : Le changement évolutif d’un caractère peut affecter, de façon adaptative ou non, d’autres caractères associés, mais de fonctionnement indépendant. Par exemple, un changement adaptatif de la taille des branches (i.e. plus grandes dans les habitats hypoxiques) peut entrainer des modifications des structures adjacentes associées à l’alimentation et ainsi affecter indirectement le comportement alimentaire. Nous examinons ici si les différences dans la longueur totale des filaments branchiaux chez les différentes populations est-africaines du cyprin *Barbus neumayeri* sont reliées aux variations des structures trophiques et de la performance alimentaire. Des mesures morphologiques ont révélé que la longueur totale des filaments branchiaux est supérieure chez les populations des habitats pauvres en oxygène. Les différences morphologiques internes et externes de l’appareil trophique sont en corrélation avec les différences de taille des branches, ce qui laisse supposer que les poissons à appareil respiratoire de plus grande taille sont moins efficaces pour capturer et manipuler leurs aliments. Au cours d’expériences alimentaires appariées où des poissons ont été exposés à un nouveau type de proie, les poissons à branches de grande taille ont mis plus de temps à se nourrir que les poissons à corps de même taille, mais à petites branches, sans pour autant consommer plus de nourriture. Cela semble indiquer que la consommation de nourriture est moins efficace chez les poissons à grandes branches des milieux hypoxiques. Nous croyons que la variation de la taille des branches favorise la répartition étendue de *B. neumayeri*, mais qu’elle peut entraver le succès des phénomètes qui traversent des gradients physico-chimiques.

[Traduit par la Rédaction]

Introduction

Direct relationships have been detected between environmental and morphological variation in many taxa (e.g., Laurent and Perry 1991; Schlosser et al. 1998; Chapman et al. 1999). Direct relationships, however, may be compromised when (i) a given structure is used for more than one function and is subject to multiple selection pressures (e.g., Lowell 1987) or (ii) adjacent structures perform different functions so that change in one structure influences a second character (e.g., Mazon et al. 1998). Such functional morphological trade-offs, although identified in the literature as a rich area for ecomorphological research (West-Eberhard 1989; Smits et al. 1996; Foster et al. 1998), have rarely been investigated beyond morphological comparisons (Galis and Barel 1980; Liem 1980; Barel et al. 1989; Witte et al. 1990; exceptions, Bouton et al. 1998, 1999).

In previous studies, functional morphological trade-offs between species have been used (retrospectively) to suggest mechanisms of divergence in many groups of fishes (e.g., Wainwright 1996; Huckins 1997; Grubich 2000). However, empirical data illustrating the role of trade-offs in generating or maintaining variation at the intraspecific level exists only for a few species (e.g., *Lepomis gibbosus* (Mittelbach et al. 1999; Robinson et al. 1996); *Salvelinus alpinus* (Adams 1998); *Gasterosteus* spp. (Schlueter 1993, 1995; Day and McPhail 1996); *Cichlasoma citrinellum* (Meyer 1989)). These studies have focused primarily on trade-offs associated with trophic specialization, interspecific competition, and benthic vs. limnetic habitat use. Trade-offs between

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feeding and respiratory structures may also be very important in fishes because of their generally compact, laterally compressed head morphology (Barel 1983; Cech and Massingill 1995).

In this study, we compared total gill filament length (TGFL), trophic morphology, and feeding performance between populations of the African cyprinid, Barbus neumayeri, from high- and low-oxygen sites. Barbus neumayeri is widely distributed in the mosaic of swamps, streams, and rivers in East and Central Africa (Greenwood 1962, 1966; Olowo and Chapman 1996). These heterogeneous environments provide a network of adjacent habitats with varying levels of dissolved oxygen (DO) that correlate with geographic variation in TGFL among populations of B. neumayeri (Chapman et al. 1999); Barbus neumayeri from sites with lower DO were characterized by larger TGFL. Chapman et al. (1999) also found evidence for low dispersal rates among small- and large-gilled populations using mark and recapture. Although movement rates were theoretically high enough to homogenize gene frequencies among populations, genetic analyses using randomly amplified polymorphic DNA (RAPDs) markers indicated significant genetic differences among sites (Chapman et al. 1999). The authors suggested that the observed genetic differences among populations may not reflect insufficient dispersal, but habitat-specific selection pressures on dispersers.

Differentiation among populations may be maintained or increased if locally adapted populations are reproductively isolated from one another. Reproductive isolation among populations would result if large- (low-oxygen) and small-gilled (high-oxygen) individuals cannot successfully disperse between environments. Dispersal of small-gilled fish into swamp habitats may be limited by hypoxia. Large-gilled fish, however, are not likely to be limited by oxygen levels encountered in stream habitats. However, gene flow from swamps to stream populations may be limited by functional morphological trade-offs between large gills and trophic structures. If such a trade-off exists, large-gilled fish may face a competitive disadvantage when foraging alongside small-gilled conspecifics. Such a disadvantage may reduce the success of large-gilled fish moving into higher oxygen habitats and thereby provide a second, different barrier to gene flow among populations.

In this study, we expand on earlier work on B. neumayeri (Chapman et al. 1999; Chapman and Liem 1995; Olowo and Chapman 1996) by examining the relationships among dissolved oxygen, gill morphology, and feeding morphology and performance. A priori predictions were that larger gills of swamp-dwelling fish would correlate with larger external features (e.g., head width, head length, and body depth), reduced internal musculature of the trophic apparatus, and reduced feeding efficiency. Identifying correlations between respiratory and trophic morphology, as well as understanding the link between this structural variation and performance, is essential to revealing patterns of local adaptation and distribution in this species and will help to illuminate the role of heterogeneous environments in the process of diversification.

**Materials and methods**

**Study system**

This study was conducted in Kibale National Park, western Uganda (0°41′N and 30°19′–30°20′E; Fig. 1). The park is characterized by equatorial moist forest but also includes a mosaic of wetland, grassland, pine plantation, thicket, and colonizing forest (Butynski 1990). The park is drained by two major everflowing rivers (Dura and Mpanga) that are tributaries of Lake George. Distinct wet and dry seasons are distributed bimodally each year (May–August and December–February) are drier than other months.

The study site was the Rwembaita Swamp system, one of the larger papyrus swamps in the park (approximately 6.5 km in length). This swamp drains into the Njuguta River, a tributary of the larger Mpanga River (Fig. 1). Terminal brush umbels of papyrus (Cyperus papyrus) reach up to 5 m in height, forming a closed canopy. Minimal exposure to incident light, low levels of mixing, and high rates of organic decomposition produce extremely hypoxic conditions in swamp water (Carter 1955; Thompson 1976; Beadle 1981; Chapman and Liem 1995; Chapman et al. 1998).

The ecolonal gradient between the Rwembaita Swamp and two connected streams (inlet Stream West and Njuguta River) provides an opportunity to examine interdecim variation over a small spatial scale. Four sites were sampled for this study: one high- and one low-oxygen site in both the eastern and western regions of the swamp (Figs. 1A and 1B). In the west, sites were located on either side of the sharp transition between the well-oxygenated waters of the stream and the hypoxic waters of the swamp (high–west and low–west, Fig. 1A). In the east, water leaving the swamp flows through an open-water channel (low–east), picking up oxygen as it flows through the forest, until it meets the well-oxygenated water of the Njuguta River (high–east, Fig. 1B). The “meeting of the waters” between each pair of high- and low-oxygen sites is well defined by a change in water clarity and DO content.

Barbus neumayeri (Cyprinidae) is one of the many members of the genus Barbus widely distributed in swamps, streams, rivers, and lakes of East Africa (Greenwood 1962, 1966; Balirwa 1984; Olowo and Chapman 1996). It is a small, opportunistic feeder (maximum length 12.5 cm) with an omnivorous diet including plant matter, detritus, flowers, insects, and other invertebrates (M. Cech and R. A. Massingill). This species is known from the laboratory using water samples collected on the same day. Flow was estimated visually based on a 0–3 scale (0 = no current, 1 = low flow, 2 = fast flow, and 3 = very fast flow).

**Environmental characteristics of swamp and stream habitats**

Characteristics of all sites were measured and recorded bi-weekly from May to October 1998 and 1999. Dissolved oxygen and water temperature were measured approximately 10 cm below the surface at several locations between 1000 and 1400 using a YSI Model 95 meter. Conductivity (YSI Model 30; YSI Inc., Yellow Springs, Ohio), pH (Chek-mit™), and transparency (modified Secchi disk) were measured in the laboratory using water samples collected on the same day. Flow was estimated visually based on a 0–3 scale (0 = no current, 1 = low flow, 2 = fast flow, and 3 = very fast flow).

**Functional morphology**

Specimens for were collected from all sites over a 1-week
Fig. 1. Four collection sites near Makerere University Biological Field Station in Kibale National Park, Uganda: (A) high–west and low–west, and (B) high–east and low–east.

period (May 1999), preserved in paraformaldehyde (35 g L⁻¹), and transported to the University of Florida.

**Gill morphometrics**

To evaluate if gill size correlates with differences in trophic morphology and feeding performance, we measured TGFL. This metric reflects the number and (or) length of the gill filaments but, unlike gill surface area, does not account for variation in lamellar density or area. We selected TGFL, however, because it is more likely to reflect spatial constraints of large gills on neighboring structures and because it has been well documented that *B. neumayeri* from hypoxic waters have a larger TGFL than fish from well-oxygenated sites (Chapman and Liem 1995; Chapman et al. 1999). In addition, other studies demonstrating patterns of interdecim variation in gill morphometrics have found that larger TGFL correlates with larger gill surface area (*Poecilia latipinna*, Timmerman 2001; *Pseudocrenilabrus multicolor victoriae*, Chapman et al. 2000).

To quantify TGFL, the entire gill was extracted from the left side of each fish, and each of the four gill arches was separated and laid flat on a microscope slide to be measured. For each hemibranch, the length of every 5th gill filament was measured. Two successive measurements were averaged to estimate the length of intermediate filaments. Filament lengths were summed for the eight hemibranchs and multiplied by 2 to produce an estimate of TGFL. Linear regression was used to detect relationships between TGFL and body mass on log₁₀-transformed data for each population of *B. neumayeri*. Analysis of covariance (ANCOVA) was used to compare log₁₀ TGFL between populations with log₁₀ body mass as the covariate. Adjusted means (sample means
Table 1. Descriptions of measurements and functional significance of morphological features measured for populations of *Barbus neumayeri* from high- and low-oxygen sites in Kibale National Park, Uganda.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description of measurement</th>
<th>Functional significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (depth)</td>
<td>Deepest point of body</td>
<td>Streamline morphology (locomotion)</td>
</tr>
<tr>
<td>Eye (width)</td>
<td>Anterior to posterior</td>
<td>Vision (resolving power, sensitivity)</td>
</tr>
<tr>
<td>Head (length)</td>
<td>Snout to posterior edge of operculum</td>
<td>Streamline morphology (locomotion); opercula used for pumping, jaw opening, and Gill protection</td>
</tr>
<tr>
<td>Head (width)</td>
<td>Widest point over opercula</td>
<td>Feeding (suction, grinding); lifts skull, transmits force to pharyngeal teeth during food intake and processing</td>
</tr>
<tr>
<td>Epaxial (depth)</td>
<td>Horizontal septum to the dorsal surface from posterior edge of the operculum</td>
<td>Feeding (suction, grinding); lowers mandible compressing oral–buccal cavity during food intake and processing</td>
</tr>
<tr>
<td>Geniohyoideus (length)</td>
<td>Dentary to the ceratohyal; measured along the muscle tissue</td>
<td>Feeding (crushing, grinding); lowers mandible, moves suspensoria compressing buccal–pharyngeal cavity during mastication</td>
</tr>
<tr>
<td>Geniohyoideus (width)</td>
<td>Midway, along frontal plane</td>
<td></td>
</tr>
<tr>
<td>Sternohyoideus (length)</td>
<td>Hyoid to shoulder girdle; measured to the pharyngo-plethralis externus</td>
<td></td>
</tr>
<tr>
<td>Sternohyoideus (width)</td>
<td>Midway, along frontal plane</td>
<td></td>
</tr>
<tr>
<td>Sternohyoideus (depth)</td>
<td>Midway, along transverse plane</td>
<td></td>
</tr>
<tr>
<td>Pharyngeal jaw (length)</td>
<td>Ceratobranchial of the fifth branchial arch (measured along the cord of the arc)</td>
<td>Feeding (shearing and crushing); food processing in pharyngeal cavity</td>
</tr>
</tbody>
</table>

*From van der Meer and Anker (1984).*
*From Barel (1993).*
*From Sibbing (1969).*
*From Osse (1969).*
*From Barel (1983).*

adjusted for a common mean body mass and a common regression line were calculated and specific pairwise comparisons between sites were made post hoc using least-squared differences (LSD).

**Body shape and trophic morphology**

Eight external features and internal morphological parameters associated with food capture, manipulation, and processing were selected for the morphological comparison (Table 1). Three muscles related to food processing were measured: epaxial, geniohyoideus, and sternohyoideus (for detailed descriptions of muscle complexes and functional significance in other fish, see Osse (1969), Anker (1978), and Sibbing (1982, 1988)). These muscles are associated with various aspects of food intake, control of hyoid movement, and intraoral processing including suction, grinding, and crushing (Sibbing 1982, 1988), and differences in size may reflect differences in force, velocity, or amplitude of muscle action for fish performing these movements while capturing and processing prey. All muscles were measured on the right side of the fish. Elongate muscles (geniohyoideus and sternohyoideus) were measured in multiple dimensions (length, width, and height) when possible without distortion. Width of elongate muscles was measured along the frontal plane of the body, whereas depth was measured along the transverse plane.

Measurements were log₁₀-transformed before analysis. Linear regressions were used to assess the relationship between each feature and body size (with SL (standard length) as the dependent variable). Multiple analysis of covariance (MANCOVA) was used (using SL as a covariate) to look for overall effect of site, DO level, and geographical location, as well as intraregional, two-way comparisons of high- and low-oxygen sites.

**Feeding performance**

Fish were collected from high- and low-oxygen sites in the western region of Rwembaite Swamp (high-west and low-west) and transported to the laboratory at the Makerere University Biological Field Station. Fish were paired by size (within 2 mm) and marked individually with tattoo ink (Chapman and Liem 1995).

Behavioral trials (*n* = 29) were conducted in two aquaria, each of which was divided into a holding compartment and a trial compartment (with a partition that allowed for water exchange). Aquaria were covered to minimize visual stimuli and incident light, and the fourth side of the aquaria was fitted with an observational blind. Spatial grids on the aquaria were used to determine location during the trial.

Fish were acclimated for a minimum of 2 weeks in the holding compartment (mean DO, 5.0 ± 0.2 mg·L⁻¹; temperature, 19.3 ± 0.2°C). In natural habitats, *B. neumayeri* forage by swimming along the bottom substrate and periodically diving into the benthos and taking in a mouthful of detritus that may or may not contain benthic invertebrates (larvae and adults; Schaack 2002). To be able to quantify food

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² Pharyngeal jaw height was excluded from multivariate tests due to unequal sample size.
intake, a food item with invariant nutritional value similar to benthic detritus was used for the behavioral trials. Fish were fed Tetramin Tropical Flake Food™ (Tetra, Blacksburg, Va.) that had been mixed with water, causing it to sink to the bottom of the tank and appearing much like the layer of detritus above which

\[ B. \text{ neumayeri} \]

ormally forage. This food item was therefore novel (i.e., neither population had previous experience with flake food) but similar to the substrate in which both populations forage in their natural habitat. Food was introduced (0.5 g) daily at 1700. Pairs of individually marked fish (one from each site) were transferred to a plastic mesh chamber and deprived of food for ~48 h before each trial to ensure that all individuals tested were in a postabsorptive state and gut contents recovered after the trial would accurately reflect food intake during the trial.

Fish were allowed to acclimate to the trial chamber for a minimum of 45 min. Trials were randomized between tanks and for time of day. The trial was initiated by the introduction of moistened flake food to the bottom of the trial compartment. All behaviors and locations were recorded every 20 s for each fish (alternating every 10 s). Each trial resulted in 180 sets of instantaneous observations of behaviors exhibited by each fish over time.

Some behaviors were binned into categories relevant to foraging (feeding, looking, nonforaging) and activity level (moving, nonmoving), and others were compared individually (e.g., chewing). Behaviors, activity level (moving, nonmoving), and spatial use (number of grid square shifts per fish per trial) were compared using a paired Student's t test. Repeated measures ANOVA (using Helmert contrasts, P < 0.05) was used to test for differences in mean feeding frequency over the course of the trial. Fish were anesthetized after the trial and preserved immediately. Gut contents were later recovered, dried, and weighed to measure total food intake. A paired Student's t test was used to compare total food intake (dry mass, g) to assess efficiency between fish from high- and low-oxygen sites based on the feeding behavior observed.

**Results**

**Environmental characteristics of swamp and stream habitats**

The environmental characteristics for each site are presented in Table 2 (data based on measurements taken between May and October 1998 and 1999). DO, pH, conductivity, water temperature, and water transparency were lower at the swamp sites than in the nearby well-oxygenated sites. For most characteristics, the magnitude of difference was similar between sites in the eastern and western regions. It should be noted, however, that the difference in DO level between high- and low-oxygen sites in the west was more extreme than between those in the eastern portion of the system (paired Student’s t test of differences from May to Oct of 1998 and 1999 for DO levels recorded within 2 days of each other; difference between high–east/low–east = 4.1 ± 0.5 mg L⁻¹, between high–west/low–west = 5.6 ± 0.4 mg L⁻¹; t = 1.77, P = 0.002, n = 28). This difference was mainly due to the much lower DO concentration (0.8 mg L⁻¹) in the western region of the swamp than in the eastern region (2.7 mg L⁻¹). Water flow was higher in both the swamp and stream site of the western pair relative to the eastern sites.

**Functional morphology**

**Gill morphometrics**

At all sites, TGFL of \( B. \text{ neumayeri} \) increased with body mass (Fig. 2). For fish of a given body mass, mean TGFL was greater in fish from the hypoxic habitats than in fish from well-oxygenated waters based on paired comparisons in western (12% difference, \( P = 0.024 \)) and eastern (9% difference, \( P = 0.038 \)) regions. There was no difference in mean TGFL between the two stream sites (\( P = 0.182 \)); however, gills of fish from low–west were marginally larger than those of fish from low–east (\( P = 0.073 \)), which may reflect lower mean DO levels in low–west.

**Body shape and trophic morphology**

Linear regressions performed on measurements (data not shown) indicated strong, positive correlations between morphological features and body size. Multivariate analysis of covariance (MANCOVA) was performed on 12 characters (mass, total length, body depth, eye width, head length and width, geniohyoides length and width, sternohyoideus length, width, and height, and epaxial height) using SL as a covariate (see Table 3 for adjusted means). The MANCOVA revealed a significant overall oxygen effect (slopes equal, Wilks' \( \lambda = 0.623, F = 1.362, P = 0.243, n = 44; \) intercepts different, Wilks' \( \lambda = 0.196, F = 9.543, P < 0.001 \)). There was also an effect of geographical location (for slopes, Wilks' \( \lambda = 0.591, F = 1.558, P = 0.164; \) for intercepts, Wilks' \( \lambda = 0.194, F = 9.671, P < 0.001 \)). Table 3 includes individual character differences associated with these main effects. The interaction between oxygen and geographical location was also significant (intercepts, Wilks' \( \lambda = 0.305, F = 5.327, P < 0.001 \)), indicating that differences between high- and low-oxygen sites were not always consistent between eastern and western regions.
Table 3. Summary of adjusted means (± SE), main and interactive effects based on MANCOVA of morphological characters of *B. neumayeri* (*n* = 44; size range 36.9–81.3 mm) collected from four sites (high-west, low-west, high-east, and low-east) in the Rwembaita Swamp system of Kibale National Park, Uganda.

<table>
<thead>
<tr>
<th>Character</th>
<th>Oxygen</th>
<th>Geographical location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>0.580</td>
<td>0.546</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>1.843</td>
<td>1.844</td>
</tr>
<tr>
<td>Body depth (mm)</td>
<td>1.163</td>
<td>1.145</td>
</tr>
<tr>
<td>Eye width (mm)</td>
<td>0.614</td>
<td>0.607</td>
</tr>
<tr>
<td>Head</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>1.194</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.948</td>
</tr>
<tr>
<td></td>
<td>Epaxial height</td>
<td>0.572</td>
</tr>
<tr>
<td>Geniohyoides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>0.793</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.136</td>
</tr>
<tr>
<td>Sternohyoides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.310</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td>0.306</td>
</tr>
</tbody>
</table>

Note: All measurements were log_{10}-transformed before analysis. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

Fig. 2. Log_{10} total gill filament length (TGFL, mm) versus log_{10} body mass (g) for *Barbus neumayeri* from (a) western (high-versus low-oxygen, *n* = 19), and (b) eastern (high- versus low-oxygen, *n* = 24) sites in the Rwembaita Swamp system of Kibale National Park, Uganda. Significant difference among groups is indicated by ANCOVA (slope, *F* = 0.63, *P* = 0.604; intercept, *F* = 5.27, *P* = 0.004). Estimated marginal means (± SE) for each site are presented in boxes.

**Intraregional comparisons**

Although an effect of DO was identified in the MANCOVA, the strong geographical effect (and oxygen × geographic location interaction) indicated that differences among high- and low-oxygen sites may differ between the eastern and western regions of Rwembaita Swamp. To detect differences between high- and low-oxygen sites within each geographical location separately, we performed intraregional MANCOVA comparisons for each character.

In the comparison of high-west and low-west (slopes, Wilks’ *λ* = 0.433, *F* = 0.764, *P* = 0.675, *n* = 22; intercepts, Wilks’ *λ* = 0.130, *F* = 4.457, *P* = 0.021), there was an oxygen effect overall and for several individual characters (mass, high > low, *F* = 41.078, *P* < 0.001; body depth, high > low, *F* = 20.396, *P* < 0.001; head length, high < low, *F* = 8.123, *P* = 0.01; geniohyoid width, high > low, *F* = 6.115, *P* = 0.023; sternohyoid width, high > low, *F* = 14.304, *P* = 0.001; sternohyoid height, high > low, *F* = 5.759, *P* = 0.027). Stream-dwelling fish from high-west tended to have a larger mass, deeper bodies, shorter heads, wider geniohyoides, and larger sternohyoides muscles in two dimensions (width and height). Most of these features also differed between fish from high- and low-oxygen sites in the combined analysis.

In a comparison of high-east to low-east, there was a significant interaction between oxygen and SL, preventing further testing of differences between intercepts (Wilks’ *λ* = 0.105, *F* = 4.972, *P* = 0.021, *n* = 22) but indicating that there may be important allometric differences between fish from these two sites. Univariate comparisons indicated that the overall slope difference may reflect allometric differences in head length. Head length increases more quickly as a function of SL in the swamp relative to the stream site. For other characters, univariate analyses showed that slopes were similar between sites and intercepts only differed for geniohyoides length and sternohyoides height and width (*F* = 6.494, *P* = 0.020; *F* = 53.664, *P* < 0.001; *F* = 7.255, *P* = 0.014, respectively). Of these three characters, only the
Fig. 3. Mean (± SE) proportion of observations of feeding from paired trials for *B. neutamari* collected from high-west (open bars) and low-west (solid bars) in the Rwembaita Swamp system of Kibale National Park, Uganda (n = 29 paired trials, 180 observations per fish per trial). Asterisks indicate significant differences (P < 0.05) based on paired Student’s t test.

![Graph showing feeding proportion](image)

Fig. 4. Results from feeding performance trials (n = 29) of *B. neutamari* from high-west and low-west regions of the Rwembaita Swamp system in Kibale National Park, Uganda. (a) Mean proportion of behaviors recorded as “feeding” during trials (n = 29). Significant differences are indicated by asterisks between groups and by letters (a, b for high-west; a’, b’ for low-west) between time intervals within groups (P < 0.05). (b) Comparison of mean total food intake (mg, dry mass, n = 28); error bars represent ±SE.

![Graph showing feeding performance](image)

difference in one feature (geniohyoides length) was similar to results of the combined analysis. Additionally, the difference in sternohyoideus width in the east (swamp > stream) was inconsistent with the difference reported for the intraregional comparison in the west.

These results suggest that differences between fish from high- and low-oxygen sites in the west were stronger and may have driven the overall oxygen effect reported in the combined analysis. It should be noted that the difference in TGFL was also greater in the pairwise comparison of high- and low-oxygen sites in the west, and this may reflect the lower DO in low-west versus low-east.

**Feeding performance**

Overall, fish originating from the swamp (low-west) were observed feeding on and chewing food more frequently than fish collected from the stream habitat (high-west) during the feeding trials (Fig. 3). Behaviors unrelated to feeding did not differ between groups (data not shown). Although not more active, low-west fish changed position within the trial chamber more frequently than high-west ("spatial movement", t = 2.059, P = 0.049), although this result was not highly significant. For statistical analyses over the course of the 1-h trial, trials were divided into five 12-min intervals (Fig. 4a). Fish from the low-oxygen site (larger gills) fed more during the first two intervals (interval 1, P = 0.023; interval 2, P = 0.038; Fig. 4a). Helmert contrasts revealed that within each group, mean proportion of feeding behaviors differed only between the first and second intervals (repeated measures ANOVA, P < 0.001 for swamp fish, P = 0.022 for stream fish), after which feeding dropped off and remained lower for the rest of the trial (Fig. 4a).

The mean amount of food ingested by stream and swamp fish during the trials did not differ (paired Student’s t = 2.055, P = 0.225, n = 28 pairs; Fig. 4b). Thus, although swamp fish spent more time feeding and processing food, they did not consume more food during the course of these trials, suggesting a lower feeding efficiency.

**Discussion**

Studies of functional–morphological trade-offs can demonstrate how adaptive change in one character may impact a second, functionally unrelated character in an adaptive or nonadaptive way. Our results demonstrate that variation in TGFL of *B. neutamari* correlated with levels of dissolved oxygen are also correlated with differences in associated trophic structures (Figs. 5a and 5b). The smaller muscles observed in swamp (large-gilled) fish from low-west may indicate a reduction in suction, crushing, and grinding ability. This is supported by feeding trials demonstrating that
swamp fish from low-west fed more frequently than stream fish (from high-west) but did not ingest more food. Although variation in gill size may facilitate the broad use of various habitats by B. neuromyeri, it is possible that reduced foraging efficiency may limit the success of specific phenotypes crossing physicochemical gradients between habitats.

**Dissolved oxygen and gill size**

Fish collected from well-oxygenated stream habitats had larger gills (TGFL) than fish of the same size collected from nearby hypoxic swamp habitats (see Fig. 5a). The difference between populations in the western comparison was larger (12%) than the difference between populations in the east (9%). These differences may reflect the differences in DO, which were more pronounced in the western region, or simply the lower DO level in low-west versus low-east. Flow regimes also differed between the two regions; current was higher in both the high- and low-oxygen sites in the east compared with the west, where current was negligible. It is also possible that environmental characters other than those measured here may contribute to the patterns we observed in morphological traits between high- and low-oxygen sites and between geographical locations.

The patterns reported here support previous correlations between TGFL and oxygen availability in B. neuromyeri (Chapman and Liem 1995; Chapman et al. 1999) and other species (Gnatholomus victoriae, Chapman and Hulen 2001; Pseudocrenilabrus multiclor victoriae, Chapman et al. 2000). Chapman and Liem (1995), however, reported a larger difference in TGFL for B. neuromyeri from high- and low-oxygen sites in the Rwembata Swamp system. This may reflect differences in the developmental history and selection environment experienced by fish during the years previous to each study (e.g., higher levels of rainfall and DO during the 1990s; L.J. Chapman, unpublished data). If gill size does impact trophic structure, earlier data reporting more extreme differences in TGFL suggest that there is potential for even greater interdemic differences in trophic morphology than we observed in our comparisons.

**Body shape and trophic morphology**

Multiple analysis of covariance revealed differences in morphology between high- and low-oxygen sites and between the eastern and western geographical regions. The two differences that emerged between high- and low-oxygen sites were, both in the head region (swamp fish had longer heads, shorter geniohyoideus, and less depth of the sternohyoideus muscle), suggesting a potential association with increased gill size and low oxygen specifically. Differences in muscle size between populations in any dimension may have functional significance in terms of performance, assuming no compensatory change in other dimensions. Geographical differences (in the two features, epaxial and geniohyoideus) may reflect genetic distance between fish from eastern and western areas of the system, or other geographically distinct environmental features.

The directionality of differences between stream and swamp fish supported our initial predictions based on the potential spatial constraints of large gills (i.e., swamp fish had longer heads and smaller internal trophic musculature) in most cases. The difference in cross section (width and height) of the sternohyoideus muscle is important because the strength of a muscle is largely determined by its cross-sectional area, as opposed to its length (Galis et al. 1994). It is possible that larger gills may be volumetrically displacing muscles in the compact head region or that large gills may impede mandibular movement, reducing biting capacity and resulting in smaller muscle mass. Alternatively, the morphological differences between high- and low-oxygen sites may reflect other sources of variation among sites such as prey availability and diet, current flow, visibility, or depth.

For some traits, there was an interaction between DO level and geographical location. These differences in morphology may reflect the difference in magnitude of the oxygen contrast in high-west/low-west (larger) versus high-east/low-east (smaller) if gills do impact trophic morphology. The intra-regional comparison reiterates a clear effect of oxygen in the western comparison but an interaction between oxygen and SL in the east, suggesting differences are not consistent across size classes in the eastern region. Alternatively, morphological change correlated with DO could be influenced by environmental factors other than oxygen, and therefore patterns may be inconsistent between geographic regions. Although there is no evidence in the literature suggesting pH or conductivity would influence gross morphology of gills or the trophic apparatus, depth (Malmquist et al. 1992), flow rate (Fivelstad et al. 1999), and transparency (Schellart and Prins 1993) may exert selection pressure on the morphology.
of *B. neumayeri* in different habitats. Support for this hypothesis is based on adjusted means for individual sites (not presented here), which revealed that high–east was consistently different from the other three groups. Of the three sites, high–east is deepest and has the fastest flow rate (not represented by the mode data in Table 2), which may have contributed to the observed morphological patterns.

Overall, morphological variation in *B. neumayeri* correlates with differences in DO and gill size. The correlation between large gills and reduced muscle size has also been documented in experiments with the African cichlid *Pseudocrenilabrus multicolor victoriae* (Chapman et al. 2000) in which food and environmental characteristics were controlled. Although the morphological differences evident in this study may result from several selection pressures, the correlations here suggest that dissolved oxygen may be an important variable for understanding morphological variation and genetic differentiation in this species (Chapman et al. 1999). Future studies examining the specific role of genes versus environment in generating and maintaining variation in gill size and trophic morphology among populations will further increase our understanding of the role of hypoxic swamps in faunal diversification.

**Links to performance and diversification in papyrus swamps**

Fish originating from the swamp (low–west) spent more time moving around, looking at food, taking in food, and chewing compared with stream (high–west) fish of the same size. Increased time foraging did not result in higher gross intake for swamp fish. This suggests that fish from the swamp (large-gilled fish) are less efficient at foraging on a novel prey item than conspecifics collected from the stream (small-gilled fish). Although reduced foraging efficiency may simply reflect differences in diet of *B. neumayeri* in their natural habitat (Schaack 2002), it could be a reflection of differences in size of trophic muscles between small- and large-gilled fishes described earlier.

The morphological and behavioral data presented here suggest several potentially important ecological scenarios for *B. neumayeri* distributed throughout papyrus swamps. First, the reduced feeding efficiency of the large-gilled fish in behavioral trials might suggest that in natural habitats, swamp-dwelling fish may be at a competitive disadvantage if they moved into nearby well-oxygenated sites with small-gilled competitors. The second possibility is that the differential success of these two morphotypes on a single, novel prey item reflects mechanistic feeding differences that correlate with different selectivity and diets of these populations in natural habitats. The trade-off between efficiently processing different prey items may not, however, have a fitness consequence if both morphotypes encounter enough food for which they can successfully compete. If trade-offs exist, however, over evolutionary time they may lead to trophic specialization in each population (Lauder 1983; Schluter 1996; for review, see Futuyma and Moreno 1988). The differences in TGFL and trophic morphology among populations of *B. neumayeri* may lead towards increasingly specialized, locally adapted populations in and around papyrus swamps.

The extensive distribution of papyrus swamps throughout East and Central Africa make them a habitat of great ecological importance (Beadle and Lind 1960; Beadle 1981; Thompson and Hamilton 1984). In addition, the results of this study and previous work provide several lines of evidence to suggest that the structure of these swamps may create genetic barriers for aquatic species (Chapman et al. 1999). *Barbus neumayeri* belongs to a genus demonstrating high levels of morphological diversification in other systems throughout Africa (Nagelkerke and Sibbing 1996; Nagelkerke et al. 1994, 1995; De Graaf et al. 2000). Investigating patterns of interdemic variation may improve our understanding of zoogeographical distributions and faunal diversification in this region.

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**References**


