Is intraspecific variation in diet and morphology related to environmental gradients? Exploring Liem’s paradox in a cichlid fish

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Abstract
Interspecific studies have demonstrated that trophic morphology and ecology are not always tightly matched: a phenomenon rarely reported at the intraspecific level. In the present study, we explored relationships among diet, morphology and the environment in a widespread cichlid fish, Astatoreochromis alluaudi (Pellegrin 1904), from 6 sites in southern Uganda to test for evidence of eco-morphological matching at the interdemic level. Previous studies of Astatoreochromis alluaudi have demonstrated developmental plasticity in trophic morphology in response to diet: a mollusk diet produces specimens with large pharyngeal jaws and muscles, whereas a soft-food diet produces smaller pharyngeal jaws and corresponding changes in musculature. Sites were chosen to maximize variability in environmental variables that might directly or indirectly affect trophic morphology. We found significant differences in pharyngeal jaw and muscle morphology among populations. Similarly, we found differences in diets among sites: mollusks were found in the stomachs of fish from only 2 populations sampled, despite the presence of mollusks in 5 of the 6 sites. Although trophic morphology did match the observed diet in 2 sites, diet did not correlate with either morphology or environmental variables across sites, nor were environmental variables correlated with morphological variation among sites. These results suggest that mismatch can occur among different populations of a single species for reasons such as seasonality in resources, developmental plasticity and/or complex indirect interactions. Intraspecific mechanisms should be further studied in order to better understand the complex relationships between morphological specialization and ecological generalization.

Key words: ecological specialization, fish diet, Liem’s paradox, population divergence, trophic morphology.

INTRODUCTION
The goal of ecomorphology is to understand the response of organisms to ecological challenges by comparing patterns of variation in morphological characters to patterns of variation in ecological characters across individuals, populations and species (Motta et al. 1995). Several interspecific studies across a range of terrestrial and aquatic taxa have successfully demonstrated the tight correspondence between morphological characters and ecological function. For example, in Darwin’s finches, Neotropical bats, Caribbean labrid fishes and African cichlid fishes, distinct species possess sometimes strik-
ing morphological specializations in their feeding morphology that seem very well adapted to the food resources they most often exploit (Grant 1986; Wainwright 1991; Swartz et al. 2003; Hulsey & De Leon 2005). However, tight associations between ecology and morphology are not ubiquitous in natural systems, and instances of eco-morphological mismatch have been documented in interspecific studies of shrub and grassland birds (Weins & Rotenberg 1980), some trophically specialized guilds of cichlid fishes (McKay & Marsh 1983; Katunzi et al. 2003), and assemblages of Pacific coral reef fishes (Bellwood et al. 2006).

Liem (1980) was among the first to observe that morphological specialists often behave as dietary generalists among fish taxa, an observation often coined “Liem’s paradox.” Liem argued that the aquatic medium offers fish a range of feeding repertoires used to exploit their prey, and this array of prey capture opportunities gives fish a more versatile feeding morphology than other vertebrates (Liem & Osse 1975; Liem 1978; Liem 1980; Liem & Kaufman 1984; Liem 1990). In his view, lack of correspondence between morphology and diet could be a result of phylogenetic constraints and genetic innovations that reflect historic matches between a fish’s ancestral habitat and evolved morphology.

High levels of dietary overlap and food switching in fishes should also lead to ecomorphological mismatch on an intraspecific scale. Certainly, morphological differences in trophic design exist among different populations of a single species, yet the possibility of eco-morphological mismatch occurring at this level of diversity has not yet been fully explored. To address this issue, we ask whether it is possible for eco-morphological mismatch to occur intraspecifically, and, if so, what are the likely explanations for mismatch at the interdemic level?

Studies of interdemic variation across multiple populations offer the opportunity to isolate ecological forces contributing to diversity by minimizing effects of phylogenetic constraints (Futuyma & Moreno 1988). By exploring intraspecific variation in morphology and diet among populations of a widespread species, it is possible to generate a suite of predictor variables across sites, which can then be correlated to differences in diet or morphology among sites. This approach can help disentangle the effect of genetics in determining when and under what abiotic or biotic circumstances Liem’s paradox is most likely to occur.

The cichlid fishes of the African Great Lakes provide an excellent system to study morphological variation in relation to diet. Specialization in diet and feeding morphology of resident fishes has been used to explain the coexistence of a very large number of closely related species (Fryer & Iles 1972). However, these systems are also home to a small number of widespread species that show high levels of plasticity in traits such as diet preference (Huysseune et al. 1994) and various morpho-physiological features (Chapman et al. 2000; Chapman et al. 2006). In particular, the eurytopic cichlid *Astatoreochromis alluaudi* (Pellegrin 1904) inhabits a range of habitats from fast-flowing rivers to the hypoxic vegetated shorelines of lakes. The impressive distribution of this species allows us the opportunity to maximize variation among populations in environmental variables, as well as to improve upon the design of previous intraspecific studies that focus on comparisons between two sites (Huysseune 1995; Robinson et al. 1996; Hulsey et al. 2005).

To test for evidence of ecomorphological matching on an interdemic scale, we studied the diet and morphology of *A. alluaudi* across 6 populations in Uganda, East Africa. *A. alluaudi* has historically been characterized by its pharyngeal jaw structure (Greenwood 1964; Smits et al. 1996b). Hypertrophied morphs have been associated with a molluskivorous diet, whereas non-hypertrophied morphs are believed to feed on softer food items such as insects (Huysseune et al. 1994). Smits (1996) reports that pharyngeal jaw morphology in *A. alluaudi* is a developmental trait that canalizes around 35–40 mm standard length. *Astatoreochromis alluaudi* has been most extensively studied in Lake Victoria, where historic stomach content, isotope data and morphological analyses suggest that it feeds primarily on hard-bodied *Melanoides* snails as well as the softer *Bulinus* and *Biomphalaria* snails, bivalves and other mollusks (Greenwood 1964; Hoogerhoud 1986; Mbabazi 2004). Interspecific competition from other haplochromine cichlids has been suggested as a biotic explanation for why *A. alluaudi* consumes mollusks in some sites, and insects in others (Hoogerhoud 1986; Sloatweg et al. 1994). Thus, it is believed that *A. alluaudi* feeds on mollusks in Lake Victoria because competition from other molluskivores is low, whereas insect resources are exploited by other fishes and are, therefore, scarce (Sloatweg et al. 1994). Sloatweg et al. (1994) even attributes the failure of introduced *A. alluaudi* to control snail (intermediate host for the human disease schistosomiasis) densities when introduced in reservoir ponds to the lack of an intact cichlid fauna outcompeting the molluskivore for more profitable insect prey.

In the present study, we explore the relationship between diet and morphology in populations of *A. alluaudi* across a natural environmental gradient to determine
whether morphology is a good correlate of diet choice in natural systems, or whether fish with specialized morphologies consume generalist diets as predicted by Liem’s paradox. More specifically, we quantify a suite of abiotic (dissolved oxygen (DO), pH, water transparency, conductivity and water depth) and biotic (abundance of mollusks and insects, diet and abundance of haplochromine fish) variables to detect whether alternative explanations for Liem’s paradox, such as competition, resource abundance and environmental variables, are correlated with differences in morphology among populations of *A. alluaudi* (Robinson & Wilson 1998). To achieve this goal we: (i) compare characteristics of the pharyngeal jaw and 2 muscles of *A. alluaudi* among 6 populations; and (ii) relate morphological divergence among sites to diet and environmental variables.

**MATERIALS AND METHODS**

**Study sites**

Specimens for morphological analyses were initially collected from 2 sites in June 2006 that differed markedly in mollusk availability (Lake Saka, mollusks common; Lake Nabugabo, mollusks extremely rare; Efitre et al. 2001). Specimens for morphological analyses from the remaining 4 sites as well as environmental and diet data for all sites were collected between January and February and May through July 2007. These months correspond to the 2 dry seasons in East Africa, when sites are most accessible and where we would predict the greatest potential for ecomorphological matching because fish should be consuming the prey they are morphologically specialized on during these periods of resource limitation (Liem 1980; Liem 1990). Sites were selected to maximize variation in biotic and abiotic variables; in total, three lakes and three river sites were sampled (Fig. 1).

The Napoleon Gulf of Lake Victoria (00°20’N, 33°15’E) was selected as a site, because much historic and contemporary research on *A. alluaudi* has been conducted in this area (Greenwood 1964; Mbabazi 2004). Lake Nabugabo is a satellite of Lake Victoria (24 km², 00°45’S, 31°45’E), isolated from the main basin 5000 years ago by long shore bars (Stager et al. 2005). In this lake, Chapman et al. (2002) found that *A. alluaudi* was restricted to hypoxic wetland ecotones of small bays characterized by dense vegetation and low DO levels. Mollusks are rare in Lake Nabugabo (Efitre et al. 2001), and *A. alluaudi* is thought to be primarily insectivorous. Lake Saka (00°40’N, 31°15’E) is a crater lake that receives input from several wetlands and experiences a high level of eutrophication from extensive agricultural activities throughout its watershed (Crisman et al. 2001). As a result, the lake is characterized by supersaturated DO levels during the peak light of day (15 mg L⁻¹, 180% saturation). A quantitative survey of the fish fauna of Lake Saka in 2000 revealed that *A. alluaudi* was most abundant in wetland ecotones experiencing relatively high DO (10 mg L⁻¹; L. J. Chapman et al. Pers. Obsv.).

Three sites were surveyed from the Mpanga River system that feeds into Lake George, in Western Uganda. Sites were chosen based on variation in environmental variables from a 2006 survey of seven locations within the system and a minimum catch of nine *A. alluaudi* neces-

![Figure 1 Map of Uganda with (a) 3 lake study sites and the location of the Mpanga River indicated with diamonds. (b) Map of the Mpanga River with 3 river sites indicated with diamonds.](image-url)
sary for morphological analyses (Crispo & Chapman 2008). Kamwenge (0°06'N, 30°27'E), the southernmost downstream site, experiences flowing, open waters that are normoxic year round. Kahunge (0°18'N, 30°32'E) is extremely variable in terms of its abiotic parameters because of seasonal flooding and its proximity to the upstream Kairaguru Swamp (Crispo & Chapman 2008). Rwebakwata (0°19'N, 30°32'E), the most upstream site, also experiences seasonal variation in DO and other water quality parameters during heavy rains (L. J. Chapman pers. obsv.).

Environmental variables

At each site, major shoreline habitat types (e.g. Vossia cuspidata (Roxb.) Griff., shrub and brush and forest edge) were identified, and sampling transects were set up along the water edge near the shoreline vegetation. It was important to sample both biotic and abiotic aquatic parameters within each different shoreline habitat to characterize the complete fish assemblage and food base at each site. Within each habitat type, 6 sets of duplicate minnow traps were baited and set 4-m apart for 3 h during the day, at approximately 10.30 hours. For each set of traps, we recorded water temperature, DO, conductivity, pH, water transparency and water depth. In the Mpaanga River system, data were collected between 10.00 and 14.00 hours. Lake sites were sampled comparably, with additional experimental gill nets set between 2 and 5 m from the shoreline to supplement trap captures. Because of diel variation in DO and water temperature in the lake systems, we recorded surface and bottom measures of DO and temperature at 07.00 and 14.00 hours (Crisman et al. 2001). In lakes, we measured water transparency using a Secchi disk. In flowing water, we took water samples, and recorded transparency readings in a PVC Secchi tube (Kasangaki et al. 2008), which we standardized to Secchi disk readings. At all sites, we assessed the abundance of benthic macroinvertebrates using scoop nets at 2 locations within each transect at depths similar to those of the fish captures (frame size 43.2 × 32.4 cm, depth of 18.9 cm, mesh size 0.3 cm) by scooping the bottom substrate. For deeper sites, samples were taken using an Ekman grab (opening 225 cm², depth 17 cm). For both samples, the substrate was poured through a fine mesh Ekman dredge bucket, and samples were sorted directly in the field. Because of the dense, muddy and weedy composition of the lake and river bottoms, both the scoop net and Ekman grab samples were restricted to collecting sediment from the top 3–5 cm of the bottom layer. We assumed that this top sediment layer represents the depth range where A. alluaudi are able to forage. Invertebrates were preserved in 10% formalin and later transferred to 70% ethanol where they were identified to order-level resolution in the laboratory.

All captured cichlid fishes were counted and identified in the field as: A. alluaudi, Pseudocrenilabrus multicolor victoriae Seegers 1990 (a co-occurring widespread species), or unidentified species of haplochromine cichlids (representing the various described and undescribed cichlid species from the Lake Victoria drainage basin). The A. alluaudi fish were killed in MS222, injected (with phosphate-buffered saline buffered paraformaldehyde) into the abdominal cavity and preserved in buffered paraformaldehyde for transport to McGill University, Canada. These methods were reviewed and approved by the McGill University Animal Care Committee (protocol # 5029) prior to fish collections.

Morphology: Pharyngeal muscle and jaw metrics

We were interested in studying both the effect of diet and environmental variables on the pharyngeal jaw and muscles of A. alluaudi. Because of the dual nature of the analysis, we chose pharyngeal jaw and muscle traits that we predicted would vary with a range of prey capture and feeding repertoires as well as potential interactions with the environment. Nine to 10 fish of a representative size range (11) were selected within each population and in situ photographs were taken of the m. geniohyoideus and m. sternohyoideus muscles. These muscles are used in a variety of feeding repertoires, including food intake, suction, grinding and crushing, and differences in size may reflect differences in force, velocity or amplitude during prey capture and processing (Sibbing 1982; Galis 1992; Schaack & Chapman 2003; Carroll 2004; Wainwright & Bellwood 2006). Furthermore, the size of these muscles is known to be influenced by DO in other species of cichlid fish (Chapman et al. 2000; Chapman et al. 2008). The lower pharyngeal jaw apparatus was dissected out and photographed. Traits related to pharyngeal jaw size are useful in prey processing: small pharyngeal jaw traits are required for suction feeding, whereas large jaw traits enhance crushing ability (Barel 1983; Barel et al. 1989). Pharyngeal jaw traits may also be modified by indirect effects from the abiotic environment through architectonic inter-dependency and interaction with the gill arches and muscles of the head (Mallatt 1996; Smits et al. 1996b). Pharyngeal muscle and jaw photos were analyzed using Motic Ver. 2.0 software; metrics included muscle length, muscle width and muscle depth, as well as pharyngeal jaw width, jaw length, horn width, tooth depth and jaw depth (Smits 1996; Fig. 2). These metrics follow the measure-
The m. geniohyoideus runs from the dentary to the hyoid and there are 2 main parts that are usually differentiated: a caudal part and a lateral part separated by a transverse myosept (Anker 1978). We measured the length of both parts (Fig. 2); the width and depth of the m. geniohyoideus were measured at the transverse septum of the muscle, a landmark that could be reliably located and for which we could obtain very repeatable estimates. The m. sternohyoideus connects the hyoids with the shoulder-girdle (Anker 1978). The length of the m. sternohyoideus was measured from the point of attachment to the tendon to the end of the cleithrum. Muscle depth and width were measured at the midpoint of the muscle length (Anker 1978). Muscle cross-sectional area (CSA) was calculated with width and depth measures using the formula for the ellipse: $CSA = \delta \times \frac{1}{2} \text{Depth} \times \frac{1}{2} \text{Width}$ (Chapman et al. 2008).

**Figure 2** Photographs of the m. geniohyoideus, m. sternohyoideus, and lower pharyngeal jaw in *Astatoreochromis alluaudi*. All muscle pictures are taken ventrally, and measurements were taken from the left side of the fish. (a) View of the left m. geniohyoideus and landmarks used for length (GL) and depth (GD) measures. (b) Top view from the right of the fish looking down on the left m. geniohyoideus and landmarks for width (GW) measure. (c) Left side view of the m. sternohyoideus and landmarks for length (SL) and depth (SD) measures. (d) Ventral view of the m. sternohyoideus with width (SW) landmarks. (e) Top view (length, JL; width, JW; horn width, HW measurements) and (f) right side view (tooth depth, TD and jaw depth, JD) of the lower pharyngeal jaw. Landmarks used for muscle and jaw measurements follow Hoogerhoud (1984), Smits et al. (1996a) and Schaak & Chapman (2003).
Because we did not calculate the center of mass of the muscle for our estimate of CSA, we refer to our measures as a CSA index.

Stomach content analysis

Fish standard length (±0.1 mm) was recorded prior to dissection (Table 1). Stomachs were then dissected out, and stomach fullness visually assessed before grouping into 1 of 5 categories: level 1, empty stomach; level 2, stomach ¼ full; level 3, stomach ½ full; level 4, stomach ¾ full; level 5, stomach completely full (Binning & Chapman 2008). Based on the points method reviewed in Hyslop (1980), each prey item was identified to order-level resolution if possible. Similar prey items were grouped together into one of the following categories: insects (Ephemeroptera, Odonata, Plecoptera, Diptera, Hemiptera, Trichoptera, Coleoptera and unidentified insects), plants and algae, detritus (all benthic organic and inorganic material), fish (including scales, larvae, juveniles and eggs), mollusks (Melanoidei, Biomphalaria, Bulinus and Sphaerium), zooplankton (Copepoda, Cladocera and Ostracoda), shrimp (Caridina nilotica, Roux 1833) and other (Acariformes, and worms such as Arhynchobdellid, Tricladida and Tubificida). Each group was assigned a relative percentage that was further converted to a points system rounded to the nearest 10% (Hynes 1950). To adjust for the importance of prey items in stomachs of varying fullness, we multiplied the points by the proportion of stomach fullness. The total number of points per category was calculated and divided by the number of non-empty stomachs in each population. We assume that only a diet comprising of mollusks during development is sufficient to produce hypertrophied pharyngeal jaw morphology based on previous experimental and observational studies of A. alluaudi (Huysseune 1995; Smits 1996).

Statistical analysis

We used multivariate analysis of covariance (ANCOVA) with population as a fixed factor and fish standard length as a covariate to detect differences in morphology among sites (N = 58). Each suite of traits (pharyngeal jaw and muscle metrics) was analyzed separately. Measurements for each trait were first plotted then log_{10}-transformed to improve normality and to linearize the data. In all analyses, interaction terms were first included and then later removed because there was no evidence for heterogeneity among slopes. To explore the degree to which trophic characteristics diverged among sites, we performed discriminant function analyses (DFA); trait values were size-adjusted using the within-groups slope generated from ANCOVA for each trait (N = 58; Hendry & Taylor 2004).

We calculated the standardized abundance of each prey item (insect, mollusk and other) in dredge samples as the number of individuals in a given category expressed as a percentage of the total abundance of prey items at a given site (N = 6). This was done to avoid biases of absolute density estimates associated with different substrates. We used the percentage of A. alluaudi to haplochromine cichlids within each site based on individual counts as an index of potential competitor density (N = 6). The abundances of other fish species were excluded from the analysis because previous studies have shown that haplochromine cichlids share the greatest habitat and dietary overlap with A. alluaudi (Hoogerhoud 1986; Bin-
To correlate environmental variables with fish morphology and diet, principal component analysis (PCA) was used to calculate composite morphological variables of size-adjusted trait values and to obtain multivariate measures of the abiotic environment, the standardized prey abundances and the percentage of major prey items in the stomach of *A. alluaudi* (four PCAs total). We used 11 (6 muscle and five jaw) morphological measures in this analysis to obtain an average population-level measure for the composite morphological traits (*N* = 6). These morphological measures were also size standardized prior to conducting the PCA using the within-groups slopes generated from the ANCOVA on each trait (Hendry & Taylor 2004; Langerhans et al. 2007). Measures of abiotic parameters (water transparency, pH, average surface DO and conductivity) were averaged within a site to produce an overall point-in-time value of the ambient conditions (*N* = 6). We retained axes with eigenvalues greater than 1 for subsequent analysis. Pearson correlation was used to detect correlations between PCA scores among diet, morphology and environment. Because only 6 comparisons were being made, we chose not to correct our *P*-values using Bonferroni correction (Garcia 2004).

## RESULTS

### Environmental variables

Most physico-chemical characters measured exhibited a high level of variation across the 6 sites sampled (Table 1). River sites had slightly lower DO levels than lake sites, with the exception of Lake Nabugabo, where DO is often depleted along its densely vegetated shorelines through swamp seepage (Chapman et al. 2006). Although variation in pH was small, conductivity varied considerably. Water clarity ranged from highly turbid in Lake Saka and Rwabakwata, to moderate clarity in Nabugabo, Kamwenge, Kahunge and the Napoleon Gulf of Lake Victoria. Generally, diel variation was low, with the exception of Lake Saka, where levels of DO increased dramatically over the day, possibly related to its hyper-eutrophied waters (Crisman et al. 2001).

Diurnal fish assemblages differed among sites. Compared to river sites, lake sites harbored a high abundance of haplochromine cichlids and a low abundance of other lentic-water species (*Oreochromis niloticus* (L. 1766), *Lates niloticus* (L., 1758) and *Protopterus aethiopicus* (Heckel, 1851)). Rivers sites typically contained a variety of cyprinids (*Barbus kerstenii* (Peters, 1868), *Barbus cercops* (Whitehead, 1960), *Barbus apleurogramma* (Boulenger 1911), *Barbus “line”*), the killifish *Hypsopanchax deprimozi* (Pellegrin, 1928), a clarid catfish *Clarias liocephalus* (Boulenger 1898), as well as the widespread haplochromine cichlid *P. multicolor victoriae*. *A. alluaudi* was most common in river sites as well as in Lake Saka; however, at all lake sites it comprised only a small percentage of the total haplochromine cichlids encountered (Table 2).

The abundance and richness of potential macroinvertebrate prey were also variable across sites (Table 2). The most common insect orders found were Diptera, Odonata, Coleoptera and Ephemeroptera, with eight insect orders represented in total across sites. Mollusks were found in all sites except Lake Nabugabo. Pearson correlation detected a significant negative relationship between the relative mollusk abundance and relative insect abundance
Napoleon Gulf had the highest richness of prey taxa per dredge sample, over 75% of which were mollusks. The richness of macroinvertebrate taxa was also high in Lake Saka, but most of the abundance was accounted for by mollusks. River sites had, in general, greater invertebrate richness than lake sites, with the exception of Napoleon Gulf.

**Morphology: Pharyngeal muscle and jaw metrics**

Multivariate ANCOVA results on muscle (m. geniohyoideus and m. sternohyoideus length, width and CSA index) and pharyngeal jaw (length, width, depth, tooth depth and horn width) metrics using fish standard length as a covariate showed a strong population effect for both suites of traits (muscles: $F_{5,47} = 4.541, P < 0.001$; jaws: $F_{5,47} = 4.993, P < 0.001$).

Discriminant function analyses based on a representative subset (horn width, jaw width, jaw depth, m. sternohyoideus CSA index, and m. geniohyoideus CSA index) of the size-adjusted pharyngeal jaw and muscle metrics indicated significant morphological differences among the 6 populations and generated three significant functions (2 with eigenvalues greater than 1) accounting for 59.0, 29.3 and 10.0% of the variance, respectively (Wilks’ $\lambda = 0.059, P < 0.001$). The first discriminant function was highly positively correlated with pharyngeal jaw horn width ($r = 0.717$). The second function was highly negatively correlated with the index of m. sternohyoideus cross-sectional area ($r = -0.696$) and more weakly correlated with pharyngeal jaw length ($r = 0.596$) and depth ($r = 0.567$). The DFA classified 75.9% of the original 58 fish into the correct population.

A representative plot of the first 2 DFA factor scores (Fig. 3) was used to explore the degree to which these 2 functions separated the 6 populations based on composite morphological characteristics. Lake Nabugabo and Kamwenge separated from other populations based mostly on Function 1. Lake Saka, Napoleon Gulf, Kahunge and Rwebakwata had similar values for Function 1, but exhibited a degree of separation (although not complete) on Function 2.

**Stomach content analysis**

Of the 224 *A. alluaudi* captured at the 6 sampling sites, 141 had non-empty stomachs and were used for stomach content analysis. There was considerable variation in the diet of *A. alluaudi* among sites (Fig. 4). Fish from Napoleon Gulf consumed a varied selection of prey types, but predominantly fed on mollusks (58%), which is consistent with previous studies of this population (Greenwood 1964; Mbabazi 2004). No other sites showed evidence for consumption of mollusks, with the exception of Kamwenge, where mollusks represented 8% of the total diet across fish (Fig. 4). Insects were consumed in high abundance (20–38%) at all sites, with the exception of Napoleon Gulf and Kamwenge. *A. alluaudi* from Rwebakwata (river site) fed predominantly on fish (47% of total diet).

**Data synthesis: Environmental, morphological and diet variables**

Principal component analysis on 11 morphological measures generated three axes with eigenvalues greater than 1.0, indicating significant variation among populations.
1.0 that cumulatively explained 93.72% of the variance (Table 3). PC1 related most strongly to the five pharyngeal jaw traits, the m. geniohyoideus CSA index and the m. sternohyoideus width, whereas the length of the m. geniohyoideus and the m. sternohyoideus CSA index loaded most heavily on PC2. One principle component was extracted from the physico-chemical data (water transparency, pH, DO and conductivity), and explained 78.55% of the variance (Table 3). Similarly, one component was extracted from the prey availability data (invertebrate richness, relative abundance of mollusks and relative abundance of insects) that explained 83.65% of the variance, and one principle component extracted from the diet data based on stomach content analysis (percentage fish, mollusks and insects in diet) explained 66.94% of the variance. This axis was strongly positively related to the percentage of insects and negatively related to percentage of mollusks in the diet of A. alluaudi.

Pearson correlation on the principle components of morphological and environmental variables revealed no statistically significant results. However, some interesting trends were revealed: the relationship between the prey availability principle component and the diet principle component (degrees of freedom (df) = 5, $r = -0.808$, $P = 0.052$) as well as between the second morphology principle component and both the physico-chemical component (df = 5, $r = 0.765$, $P = 0.076$) and prey availability (df = 5, $r = 0.751$, $P = 0.085$) approached significance. All other results from the correlation matrix were highly non-significant ($P > 0.40$), and no other $r$-values were above 0.4.

**DISCUSSION**

Evidence of eco-morphological mismatch

Although interspecific studies are useful starting points when exploring functional differences in morphology and ecology, conclusions about ecological specialization drawn from them are often confounded by the effects of phylogenetic and genetic constraints (Futuyma & Moreno 1988). Intraspecific comparisons provide a more appropriate level for comparing morphology and testing adaptive hypotheses about ecological specialization and generalization (Robinson et al. 1996). Much of the literature exploring intraspecific differences in fish trophic morphology has focused on pumpkinseed sunfish (*Lepomis gibbosus* L. 1758), which do show eco-morphological matching at the interdemic level (Wainwright et al. 1991; Mittelbach et al. 1992; Osenberg et al. 1992).

The cichlid fishes of East Africa are another popular study system for examining trophic specialization. However, surprisingly little comparative research has been done on widespread species like *A. alluaudi* across more than 2 populations. Our results suggest that, unlike pumpkinseed sunfish, interdemic variation in the morphology of *A. alluaudi* is not tightly correlated with its ecology. Two populations sampled did generate patterns predicted by matching of diet, morphology and resource abundances. The population studied in Lake Nabugabo displayed morphology consistent with its diet: fish from this population were characterized by small pharyngeal

![Figure 4](image.png)

**Figure 4** Percentage contribution of prey items in *Astatoreochromis alluaudi* from 6 sites in Uganda (N = number of fish with non-empty stomachs). Diet was divided into seven major categories: insects (Ephemeroptera, Odonata, Diptera, Trichoptera, Coleoptera, Plecoptera, Hemiptera, and unidentified specimens), mollusks (bivalves and gastropods), fish (eggs and larvae), plant (including algae), detritus, shrimp (*Caridina*) and other (Acariformes, zooplankton, nematode).
jaws, small m. sternohyoideus CSA index and shearing teeth used to feed on the soft-bodied insects and fish fry that comprised 62% of its diet in this lake. Furthermore, Lake Nabugabo is almost completely devoid of mollusks, which supports a link between resource availability and morphology. Napoleon Gulf showed a similar eco-morphological match: a diet comprised mainly of mollusks (58%), enlarged pharyngeal jaws, large sternohyoideus CSA index and a habitat where mollusk resources are very abundant. However, while the pharyngeal jaw and muscle morphologies of *A. alluaudi* collected in Napoleon Gulf are consistent with predicted morphological responses to hard-bodied prey, *A. alluaudi* from Lake Saka and 2 river sites, Rwebakwata and Kahunge, where no mollusk remains could be detected from the stomachs sampled, shared strikingly similar pharyngeal jaw morphologies. Furthermore, in Kamwenge, where the most hypertrophied pharyngeal jaw morphology was observed, stomach contents revealed that only a small proportion of mollusks (8%) were consumed by less than 20% of the fish sampled. These results are not consistent with tight links between morphology and diet across populations for this eurytopic cichlid.

It is possible that the lack of a tight match between morphology and the environment in four of the 6 populations studied is a result of the specific jaw and muscle traits we measured, although most previous studies have considered measures of the pharyngeal jaws to be very informative with respect to diet. Other studies of trophic muscles used by teleost fishes have looked at the levator posterior as a direct measure of snail-crushing force (Mittelbach *et al.* 1992, 1999) and the epaxial muscles as primary generators of suction pressure (Carroll *et al.* 2004; Carroll & Wainwright 2006). Furthermore, morphological differences in the oral jaw apparatus of cichlid fishes have been correlated with food composition (Bouton *et al.* 2002). The approach we took was slightly different. Rather than focus on a set of traits that relate specifically to a particular feeding strategy, we instead incorporated characteristics of the fish head that might show variation related not only to feeding traits, but also to various environmental pressures in order to gain a more complete understanding of Liem’s paradox and why eco-morphological mismatches might occur in natural systems.

How is it that a population can appear phenotypically specialized yet function as an environmental generalist? Liem suggests a number of possibilities related to interspecific level comparisons, but this phenomenon appears to be more general, and is discussed below in light of our interdemic study.

**Plasticity, seasonality and species interactions**

Although the overall contribution of hard-bodied prey in the diet of a morphological specialist may be small, a

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**Table 3** Principle component analysis scores for morphological measures (6 muscle traits, 5 jaw traits, Morph PC1, PC2, PC3), physico-chemical measures (4 variables; Phys-chem PC1), prey availability (3 variables; Prey avail. PC1) and diet (3 variables; Diet PC1)

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<td>Morph PC1</td>
<td>6.842</td>
<td>62.2</td>
<td>0.802† -0.266 0.666</td>
<td>0.662 -0.506 0.805†</td>
<td>0.901† 0.947† 0.945† 0.922†</td>
</tr>
<tr>
<td>Morph PC2</td>
<td>2.510</td>
<td>22.8</td>
<td>-0.310 0.916† -0.321 0.739†</td>
<td>0.693 0.587 0.162 0.031</td>
<td>-0.088 0.180 -0.183</td>
</tr>
<tr>
<td>Morph PC3</td>
<td>1.001</td>
<td>9.1</td>
<td>0.506 0.143 0.641</td>
<td>-0.037 0.338 0.056</td>
<td>-0.294 -0.158 -0.276 0.071 -0.040</td>
</tr>
</tbody>
</table>

|                | Phys-chem PC1 | 3.142 78.55 | Transparency pH DO Conductivity |
|                |              |           | -0.806 0.903 0.927 0.905 |
| Prey avail. PC1 | 2.509        | 83.65     | Invertebrate richness RA mollusks RA insects |
|                |              |           | 0.837 0.947 -0.955 |
| Diet PC1       | 2.008        | 66.94     | % fish in diet % mollusks in diet % insects in diet |
|                |              |           | 0.483 -0.948 0.936 |

Three axes for morphology were extracted (CSA = muscle cross sectional area index). One axis each for the physico-chemical environment, prey availability, and diet components were extracted (RA = Relative abundance). †traits loading heavily on each component (> 0.70). All trait measurements were size-adjusted to a standard length of 65.1 mm.
molluskivore-like morphology may be retained by a population for several reasons: phenotypic plasticity in developmental traits, seasonality and/or interactions among prey resources. Traits that are plastic require a response of the genotype to environmental variation in order to produce the phenotype observed (West-Eberhard 1989). Laboratory-rearing studies on A. alluaudi originating from Lakes Saka and Nabugabo have shown that fish will develop a hypertrophied morphology if fed on a snail diet regardless of their maternal phenotype, suggesting that plasticity remains high in fish from both well-matched and mismatched systems (L. J. Chapman Pers. Obsv.). Because pharyngeal jaw morphology in A. alluaudi is thought to canalize at approximately 35-mm standard length (Smits 1996), a fish exposed to hard-bodied food items during this life stage may develop hypertrophied morphology, even if it routinely feeds on soft prey. Interestingly, rearing experiments on pumpkinseed sunfish have also demonstrated that trophic morphology is plastic in this species (Mittelbach et al. 1999), yet a survey of 6 populations of this species demonstrated positive relationships among the abundance of gastropods in a lake, the amount of gastropods eaten by the pumpkinseeds and the size of an important shell-crushing muscle (Mittelbach et al. 1992). The question that arises is: What is inherently different between these 2 fish species such that A. alluaudi populations are mismatched whereas pumpkinseeds are not?

If the potential to develop hypertrophied morphology exists in all populations given the right juvenile environmental conditions, then there is potential for the scale of environmental fluctuations to be mismatched with the scale of plastic developmental responses. Seasonal diet switching has been documented in many taxa, including several species of birds (Schluter 1982; Tebbich et al. 2004) and freshwater fishes (Katunzi 1983; Adite et al. 2005), and is a potential adaptation to seasonally fluctuating resources. A recent study of the diet of A. alluaudi in Lake Saka over a 30-month period found that although mollusks only contributed minimally to the overall diet of the fish (5%), there was a high degree of variation in the amount of mollusks consumed (0–33%) in any given month (Binning et al. 2009). Furthermore, there was a correlation between the amount of fish consumed per month, and the running rainfall average in the region, suggesting that seasonal fluctuations in environmental variables may have a strong influence on patterns of diet in A. alluaudi (Binning et al. 2009). To our knowledge, it is unknown whether eco-morphological matching occurs constantly throughout the year in pumpkinseed sunfish.

Another interesting component of resource availability is density-dependent interactions between prey species affecting their abundance. In the present study, we detected a significant negative correlation between the abundance of mollusks and insects among sites, suggesting that resource availability might also be related to biotic factors as well as seasonal fluctuations in the abiotic environment. Of course, the absolute abundance of the prey may determine its availability to a fish, rather than the proportional abundance. Future research should further explore seasonality and spatial variation in resource use and resource abundance (both absolute and proportional) at sites both where eco-morphological matching exists and where it does not to explicitly test whether trophic specialization is adaptive year-round or specifically during times of resource scarcity.

Indirect effects and architectonic constraints

Intraspecific mismatch between trophic morphology and the environment may also occur as a result of trait correlations and interactions with other abiotic conditions independent of what food is being consumed. For example, mismatch may occur when a particular morphological character is used for 2 functions such that an organism’s morphology represents a compromise to the pressures of multiple selective regimes. Alternatively, performance in one dimension, such as feeding, may be compromised by changes in a neighboring structure that constrain or alter the targeted character. In fishes, these interactions have been observed between feeding and respiratory structures that may reflect the compact, laterally compressed head morphology characteristic of most teleosts (Barel 1983). Recent work has shown that changes in gill size of hypoxia-adapted fish correlates with a reduction in the size of key trophic muscles, feeding performance and diet (Chapman et al. 2000, 2008; Schaack & Chapman 2003). Recent studies on A. alluaudi suggest that the mismatch between diet and morphology observed in several Ugandan sites may be, at least in part, the result of indirect interactions between the physico-chemical environment and trophic morphology. Binning et al. (2010) found a negative correlation between gill size and pharyngeal jaw size among individuals from the 6 A. alluaudi populations sampled in the current study and a negative correlation between gill size and features of the physico-chemical environment. These findings suggest that even though an organism might appear mismatched with its environment, in actuality, the true relationship between phenotype and the environment may be strong but complex (Langerhans et al. 2007). Indeed, Liem (1980) and Bock (1976) both recognize that distilling biological problems into simple parts and ignoring the integrated nature of an organism’s anatomy may result in misleading conclusions about the
functioning of a trait in nature. Therefore, eco-morphological mismatch might be, at least in part, an artifact of our insights into what morphological and ecological pressures an organism must respond to in natural systems. A multivariate, holistic approach to understanding the complex interactions between the environment and phenotype should be used in the future to determine whether an eco-morphological mismatch truly exists.

Contrary to popular belief at the time, Liem (1984, 1990) dismissed the role of competition as a diversifying force in the trophic morphology of fishes; his explanation for eco-morphological mismatch focused on factors related to phylogenetic constraints and development processes, and he stressed the versatility of fish feeding morphology that may allow specialized morphology to persist because it is adaptively neutral. Robinson & Wilson (1998) suggest an alternative solution to the paradox that retains competition as a diversifying force. They argue that extreme feeding specializations should occur when there is no trade-off between use of preferred and non-preferred resources; that is, a phenotype with a highly specialized morphology can function as a feeding generalist because some resources are easy to use even by a specialist. Interestingly, both explanations rely on the observation that a morphological specialist can use a generalist’s food source. We revisited this earlier debate to ask whether phylogenetic constraints are necessary for the existence of the paradox, and whether resource abundance, potential competitor density and/or physico-chemical variables are good predictors of interdemic variation in trophic morphology and foraging. We have provided evidence suggesting that eco-morphological mismatch can occur intraspecifically, and we suggest that this may reflect complex interactions between ecology (i.e. the variability and the abundance of prey) and developmental flexibility. Future studies exploring the adaptiveness of generalist versus specialist diets and morphological structures should not ignore the complexities of developmental plasticity, seasonality and indirect interactions when drawing conclusions about eco-morphological matching among populations of widespread species.

ACKNOWLEDGMENTS

We thank C.A. Chapman, J. Paterson, V. Campbell, M. Derynck, P. Omeja, D. Twinmusigua, and KFMP field assistants for help with fieldwork in Uganda, V. Tzaneva and J. Dumont for laboratory measurements, F. Crispo for maps of Uganda and F. Galis, A. Gonzalez, B. Leung, D.G. Roche, the Chapman Lab and three anonymous reviewers for helpful comments on the manuscript. Funding was provided by a NSERC discovery grant and Canada Research Chair funds to LJC as well as NSERC and FQRT grants to SAB. This research was carried out with permission from the Ugandan Wildlife Authority and the Government of Uganda.

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Intraspecific variation in a cichlid fish


