Specialized morphology for a generalist diet: spatial and seasonal surveys reveal Liem's Paradox in an African cichlid fish

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Abstract

Ecomorphology is founded on the premise that ecological interactions are reflected in adaptive morphological change across individuals, populations, and communities. Specialization in feeding structures is often used to describe and classify species and is believed to be an important mechanism driving speciation in a number of taxa including Darwin's finches, Caribbean labrid fishes, and African cichlid fishes. However, morphology and ecology are not always tightly linked, and a number of studies have shown species with specialized morphologies consuming primarily generalist diets, a phenomenon known as Liem's Paradox. In this thesis, I use both spatial (interdemic) and seasonal studies to explore Liem's Paradox in natural populations of the specialized "molluscivorous" African cichlid, *Astatoreochromis alluaudi*. In an interdemic study, I quantified relationships among diet, morphology and the environment in *A. alluaudi* from six sites in Uganda. Pharyngeal jaw and muscle morphology differed among populations, with jaw traits explaining most of the variation. Similarly, I found differences in diets among sites; mollusks were rare, found in the stomachs of only two populations sampled. Trophic morphology did match the observed diet in two sites, but diet did not correlate with either morphology or environmental variables across the six sites. The absence of tight links among morphology, diet, and prey abundance among populations of *A. alluaudi* is consistent with Liem's Paradox. To examine whether a mismatch between diet and morphology occurs seasonally or chronically in populations of *A. alluaudi,* I used a seasonal survey of diet at Lake Saka, a site where *A. alluaudi* exhibits a molluscivorous morphology, but an omnivorous diet. Using stomach content and isotope analysis, I found that mollusks form only a small proportion of the overall diet of *A. alluaudi*, and fish and insects are the most important prey items consumed. Rainfall was strongly related to the percentage of fish prey consumed. Consistent with Liem's Paradox, *A.*

alluaudi feeds mostly on a general prey source, fish, during the rainy season when this resource is abundant, and switches its diet to feed on snails and insects when fish prey are potentially less available. This thesis provides evidence to support Liem's Paradox across a spatial and seasonal scale, and suggests that the ecology of a species should not be inferred by its morphology alone.

Résumé

L’écomorphologie est fondée sur le principe que les changements morphologiques adaptatifs observés chez certaines espèces, populations et communautés sont un produit des interactions écologiques en milieu naturel. La spécialisation de structures trophiques est souvent utilisée comme critère pour décrire et classifier des espèces et est également invoquée comme un mécanisme responsable de la spéciation d’une variété d’organismes incluant les pinsons de Darwin, les poissons Labridae des Caraïbes et les cichlidés africains. Cependant, la morphologie et l’écologie d’une espèce ne sont pas toujours liées. Plusieurs études ont démontré que des espèces possédant une morphologie trophique spécialisée s’alimentent souvent de façon généraliste, un phénomène surnommé le paradoxe de Liem. Cette thèse se base sur un échantillonnage spatial et temporel afin d’étudier le paradoxe de Liem en milieu naturel, dans des populations d’un cichlidé africain, *Aststoreochromis alluaudi*, ayant une morphologie adaptée pour se nourrir de mollusques. À travers six sites en Ouganda, j’ai quantifié les relations entre l’alimentation, la morphologie, et certaines variables environnementales importantes pour cette espèce. Mes résultats démontrent que la morphologie de la mâchoire pharyngale et des muscles trophiques de *A .allaudi* est différente entre populations, et que la morphologie de la mâchoire explique un grand pourcentage de la variance. L’alimentation de *A. allaudi* est également différente entre sites et le cichlidé se nourrit de mollusques dans seulement deux des six sites échantillonnés. De plus, la morphologie trophique correspond à l’alimentation de *A. alluaudi* dans uniquement deux sites; la diète n’est pas reliée ni avec la morphologie, ni avec les variables environnementales mesurées dans aucun des six sites. L’absence de liens clairs entre la morphologie, l’alimentation et les variables environnementales supportent le paradoxe de Liem. Afin d’examiner si l’absence de relation entre l’alimentation et la morphologie est due à un

phénomène saisonnier ou chronique dans les populations de *A. alluaudi*, j'ai employé une étude temporelle dans le Lac Saka, où *A. alluaudi* possède une morphologie adaptée pour se nourrir de mollusques, mais s'alimente d'une grande variété de proies. À l'aide de contenus d'estomac et d'analyses d'isotopes, j'ai confirmé que les mollusques ne constituent qu'un petit pourcentage de l'alimentation d'*A. alluaudi* durant l'année et que les insectes et le poisson constituent la majorité de son alimentation. L'accumulation d'eau due aux précipitations est fortement reliée au pourcentage de poisson mangé. En accord avec le paradoxe de Liem, *A. alluaudi* s'alimente surtout de proies abondantes lors de la saison des pluies, comme du poisson, et change son alimentation lors de la saison sèche pour manger des insectes et des mollusques. Cette thèse supporte le paradoxe de Liem et suggère que l'écologie d'une espèce ne devrait jamais être déterminée en se basant uniquement sur la morphologie.

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Contribution of Authors

This Masters thesis contains 2 chapters, both of which are co-authored by my supervisor, Dr. Lauren J. Chapman. Dr. Chapman was integral in developing ideas and the experimental design for the project, providing logistic support both in Uganda and Montreal, and providing useful comments during the development of both manuscripts. Chapter 2 has an additional author, McGill alumni Aurélie Cosandey-Godin, whose independent study project completed in May 2007 (Cosandey-Godin *et al*. 2008) provided the inspiration and preliminary data used to develop this chapter. Her contribution was most extensive in the early stages of the project and in providing comments during the final edits to the manuscript.

Although the help of both co-authors was essential to the successful completion of this thesis, the work presented here is primarily the result of my independent research, for which I carried out the literature review, sampling design, laboratory protocol, statistical analyses and manuscript preparation.

Both chapters have been prepared for publication in peer-reviewed journals. The first has been formatted to meet the requirements of the journal, Oecologia (submitted) and the second has been formatted for publication in the Journal of Fish Biology (submitted).

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General Introduction

In constantly fluctuating environments, organisms sometimes undergo rapid phenotypic change in morphology and behaviour in order to overcome the many ecological challenges faced in nature (Werner and Hall, 1976). The strength or outcome of community-level processes such as facilitation, competition and predation can interact with the physicochemical environment to influence an organisms' response (Breitburg *et al.*, 1997; Callaway and Walker, 1997; Stachowicz, 2001; Lou and Baldwin, 2004; Fordyce, 2006). Thus, in widespread species, environmental gradients of abiotic factors may correlate with phenotypic shifts by modifying the suite of community-level interactions. This phenomenon is readily observed in intertidal regions where gradients of thermal stress and disturbance inversely correlate with the intensity of competitive interactions (Bertness and Leonard, 1997; Bertness *et al.*, 1999; Kawai and Tokeshi, 2004). Elucidating the importance of environmental variation in mediating phenotypic responses in organisms is crucial for our understanding of processes underlying organismal diversification.

Studies of ecomorphology have contributed much to our understanding of relationships between phenotype and the environment, perhaps because of the ease of measuring morphological traits. These studies are founded on the premise that ecological interactions that define the niche of a species are reflected in adaptive morphological variation across individuals, populations, species, and communities (Wainwright, 1991; Motta *et al.*, 1995; Norton *et al.*, 1995; Swartz *et al.*, 2003). Morphological variation in response to ecological factors may arise over evolutionary time if selection leads to change in gene frequencies. On the other hand, developmental plasticity occurs as a result of use-induced changes over the life-span of an individual. In both cases, direct links between ecological and morphological variation have the potential to positively affect fitness-related characters, and lead to adaptive radiation if selection favours morphological variation (Motta *et al.*, 1995; Ferry-Graham *et al.*, 2002).

Although morphology is useful to infer the general ecology of a species or population, it can be misleading to rely too heavily on morphological clues while ignoring the influences of various environmental pressures on organisms in natural systems. Mismatch between an organisms' morphology and its ambient environment may occur when a particular morphological character is used for two functions such that there is a compromise in efficiency or adaptiveness between two divergent activities. Alternatively, eco-morphological mismatch can occur when performance in one dimension such as feeding is compromised by change in a neighboring structure that constrains or alters the targeted character. For example, Langerhans *et al.* (2007) found a strong correlation between water flow and body shape across nine populations of the African cyprinid (*Barbus neumayeri).* Such a correlation seems likely due to adaptation for hydrodynamic efficiency. However, their story was more complex, as there were no net effects of water on body shape. In their system, the direct effect of water flow was equally countered by an indirect effect: increased water flow also increased dissolved oxygen, which led to decreased gill size. This change in gill morphology ultimately impacted body shape.

Eco-morphological mismatch may also occur when specialized anatomical features are functional under a variety of ecological conditions, but have been selected to optimize function on one dimension. For example, specialization in feeding morphology is often used to classify and describe species since feeding on different prey types often requires different attack and processing strategies. However, specialization in feeding morphology may represent a response to a required, but rarely consumed resource. Liem (1980) and others (Katunzi, 1983; McKaye and Marsh, 1983; Lowe-McConnell, 1996; Barnett *et al.*, 2006; Bellwood *et al.*, 2006) have observed that morphologically specialized fish also function as generalist feeders in the wild, a phenomenon known as Liem's Paradox. Although this mismatch between diet and morphology has been documented in both freshwater and marine fishes, most studies do not explore the underlying environmental parameters relating to diet choice in the studied species, and therefore cannot help us understand how common Liem's Paradox may be in nature.

In order to unravel the complex interactions among the environment, diet choice and phenotype, it is useful to study interdemic variation across multiple populations of the same species that cross broad environmental gradients through space and time. This comparison offers the opportunity to isolate ecological forces contributing to diversity by minimizing effects of phylogenetic constraints (Futuyma and Moreno, 1988; Robinson *et al.*, 1996; DeWitt *et al.*, 2000).

The cichlid fish of the Great Lakes in East Africa are an excellent system in which to address the general question of how ecological characteristics work to modify plastic traits such as the morphology and niche space of a species. Specialization in feeding morphology and diets of these fishes has been used to explain the coexistence of such a large number of closely related organisms, and the existence of the multitude of endemic species present in any given lake (Fryer and Iles, 1972; Barlow, 2000). In addition to the endemic species flocks found in the Great Lakes, a small number of eurytopic cichlids can be found inhabiting a broad range of habitats (rivers, streams and swamps) throughout the region. These widespread species show high levels of plasticity in traits such as diet preference (Huysseune, 1995), and other morpho-physiological traits; and this phenotypic flexibility may facilitate their broad habitat range.

To explore the relationships between ecology and specialized morphology, I chose to study a widespread African cichlid, *Astatoreochromis alluaudi*, ubiquitous in the Great Lakes region of

East Africa, and associated with a range of different habitats and feeding behaviours (Witte, 1981). Notably, this species shows two distinct morphs characterized by their pharyngeal jaw structures (Smits *et al.*, 1996b). Hypertrophied jaw morphs have been associated with a molluscivorous diet, whereas non-hypertrophied morphs are believed to feed on softer food items such as insects and plant material (Smits *et al.*, 1996a). Although the ability of *A. alluaudi* to feed on a range of prey items has been known since the 1960's (Greenwood, 1964), many studies, including the first published study of its diet, have focused on Lake Victoria where it is primarily a molluscivore. In Lake Victoria, the species has been predominantly found in the littoral zone (Witte, 1981) feeding primarily on a thick-shelled snail species, *Melanoides tuberculata* (Greenwood, 1964; Hoogerhoud, 1986). However, several field and laboratory experiments have shown that *A. alluaudi* also consumes the softer-shelled snails of the genera *Biomphalaria* and *Bulinus* (McMahon *et al.*, 1977; Slootweg *et al.*, 1994). These results were of great interest, since both these snail genera are important intermediate hosts of parasitic *Schistosoma* worms, which cause a harmful waterborne disease in humans known as bilharzia or schistosomiasis (Oliver and Ansari, 1967). This disease is a widespread human health problem affecting at least 200 million people in over 70 tropical countries, with prevalence of 100 percent in some Nile riparian villages (Farley, 1991). Many affected areas have tried various methods of snail control including environmental management, molluscicides, and biocontrol mechanisms with varying levels of success (De Bondt, 1956; WHO, 1965; Thomas and Tait, 1984; Slootweg, 1989; Chimbari *et al.*, 1997; Mkoji *et al.*, 1999). *Astatoreochromis alluaudi* was recognized early on as a potential biological control candidate, and trials with this species in Cameroon (Bard and Mvogo, 1963; Mvogo and Bard, 1964), Kenya (McMahon *et al.*, 1977), and Rwanda (Slootweg, 1989) reported a decrease in snail abundance following fish introductions. However, Kat and

Kibberenge (1990) found no difference in snail abundances between control ponds and ponds where *A. alluaudi* had been introduced when they returned to McMahon's (1977) study site several years later. Furthermore, fish specimens caught 10 years after the initial stocking of the ponds showed reduced jaw morphologies typical of fish grown on soft-bodied diets (Kat and Kibberenge, 1990). Slootweg *et al*. (1993) also failed to find a decrease in snail abundances after *A. alluaudi* introduction into aquaculture ponds in Northern Cameroon.

Many reasons for the failure of *A. alluaudi* to act as a biocontrol agent have been suggested including low rates of reproduction, lack of habitat overlap, and the presence of prey refuges in the form of aquatic vegetation (Kat and Kibberenge, 1990; Slootweg *et al.*, 1994; Plummer, 2005). However, most studies agree that competition for food is the driving force behind whether *A. alluaudi* feeds on snails or softer items (Hoogerhoud, 1986; Kat and Kibberenge, 1990; Slootweg *et al.*, 1994). For example, it has been argued that *A. alluaudi* feeds on snails in Lake Victoria because interspecific competition from haplochromine cichlids feeding on insects is higher than inter- and intraspecific competition from other molluscivores feeding on snails (Slootweg *et al.*, 1994). Despite the development of these hypotheses, there has been very little research on *A. alluaudi* in natural lake systems, and none in river systems testing competition or describing diet choice in alternate environments.

The overall goal of my thesis was to explore the relationship between diet and morphology in *A. alluaudi* across a natural environmental gradient to determine whether morphology predicts diet choice in a natural system. To achieve this goal I conducted two studies represented in Chapters 1 and 2 of this thesis. Each chapter applied field surveys, one through space and the other through time, to examine the complex relationship among morphology, diet, and the environment. In the first chapter, I explored the link between morphology and diet of *Astatoreochromis alluaudi*, across six sites in Uganda that vary in a number of environmental parameters. The general goals of the chapter were 1) to identify environmental predictors of both diet and morphology and 2) to examine whether morphology is a good indicator of diet choice in a natural system, or whether fish with specialized morphologies consume generalist diets as predicted by Liem's Paradox. In my second chapter, I quantified seasonal variation in the diet of *A. alluaudi* from fish collected every 2 weeks from Lake Saka, Uganda, during the course of 30 months. I also related diet to fluctuations in resource abundance and haplochromine cichlid abundance over 12 months as well as to stable isotopes collected from potential prey items and the fish themselves. This seasonal study was valuable in relating a seasonal profile of diet to the trophic morphology that characterized the population.

This thesis provides important contributions to the field of aquatic ecology, especially pertaining to fish biology. Chapter 1 is the first study to compare diet and morphology in a fish species across several differing habitats, including isolated river sites where little research has been carried out. It also provides the best evidence thus far of Liem's Paradox operating in natural systems. Furthermore, Chapter 2 is unique in using novel techniques for verifying Liem's Paradox operating in nature, as well as evaluating the effect of multiple ecological and environmental factors on diet choice over a seasonal scale.

More generally, I hope the results from this thesis serve as a warning to public health officials searching for biological solutions to human health hazards. Biological control of parasites and parasite hosts is a controversial solution to disease spread that has been implemented with mixed results (Slootweg *et al.*, 1994). Before any decision to introduce a species beyond its native range is made, decision makers should understand that factors underlying diet choice and feeding behaviour in species are complex and that morphology may not be an adequate predictor of an organisms' behaviour in the field.

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Chapter 1: Eco-morphological mismatch in populations of an African cichlid fish

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Running headline: eco-morphological mismatch in a cichlid fish

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Abstract

Trophic morphology is often used to infer the diet and foraging ecology of an organism; however, morphology and ecology are not always tightly matched. Liem's Paradox suggests that specialized phenotypes may be retained to feed on less-preferred prey items and that morphological specialists often act as generalist feeders in nature. We explored relationships among diet, morphology, and the environment in a widespread cichlid fish, *Astatoreochromis alluaudi*, from six sites in southern Uganda. This species exhibits a high level of developmental plasticity in response to diet: a mollusk diet produces specimens with large pharyngeal jaws and hypertrophied trophic muscles, whereas a soft-food diet produces smaller jaws and associated musculature. Sites were chosen to maximize variability in environmental traits that may directly or indirectly affect trophic morphology. We found significant differences in pharyngeal jaw and muscle morphology among populations, with jaw traits explaining most of the variation among sites. Similarly, we found differences in diets among sites: mollusks were rare and found in the stomachs of fish from only two populations sampled. Environmental variables were only weakly correlated with morphological variation among sites. Although trophic morphology did match the observed diet in two sites, diet did not correlate with either morphology or environmental variables across sites. The absence of tight links among morphology, diet, and prey abundance observed in populations of *A. alluaudi* is consistent with Liem's paradox and suggests caution when interpreting the ecology of a species based on morphological analyses.

Key Words: Fish diet, population divergence, Liem's Paradox, specialization, trophic morphology

Introduction

In natural systems, organisms face a multitude of ecological challenges and often respond phenotypically with morphological shifts and/or niche shifts (Werner and Hall 1976). The goal of ecomorphology is to understand these responses by comparing patterns of variation in morphological characters to patterns of variation in ecological characters across individuals, populations, and species (Motta et al. 1995; Wainwright 1991). Ecomorphological studies lend important insights into the field of evolution, since strong selection on morphological variation may lead to adaptive radiation (Ferry-Graham et al. 2002; Motta et al. 1995). For example, in Darwin's finches, Caribbean labrid fishes, and African cichlid fishes, specialization in feeding structures is believed to be an important mechanism driving diversification of these species flocks (Grant 1986; Hulsey and De Leon 2005; Liem 1973; Wainwright 1991). However, tight associations between ecology and morphology are not ubiquitous in natural systems, and it can be misleading to infer ecological characteristics of a species based solely on morphological measures. For example, in a study of shrub and grassland bird species, Weins and Rotenberry (1980) found no clear interspecific associations between diet and beak morphology, which they attributed to the substantial inter- and intraspecific variability in both morphology and ecology.

Eco-morphological mismatch typically occurs when specialized anatomical features are functional under a variety of ecological conditions such that the presence of a feature does not predict its use in a given ecological situation (Liem 1980). In fishes, specializations in feeding morphology, including adaptations of jaws, trophic muscles and teeth, are often used as a way of describing and classifying species, since particular anatomical features are required for effective processing of different prey items. However, Liem (1980) and others (Bellwood et al. 2006; Katunzi 1983; Katunzi et al. 2003; McKaye and Marsh 1983) have observed that seemingly specialized feeders can also function as generalists, a phenomenon known as Liem's Paradox. Although this mismatch has been documented in nature, most studies focus on a species within a restricted habitat or limit interdemic comparisons to two populations. As such, we still do not fully understand factors influencing diet choice in specialized feeders or the circumstances under which retaining a specialized morphology is adaptive.

Studies of interdemic variation across multiple populations offer the opportunity to isolate ecological forces contributing to diversity by minimizing effects of phylogenetic constraints (Futuyma and Moreno 1988). By exploring intraspecific variation in morphology and diet among several 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 answer the question of under what abiotic and 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 the resident fishes has been used to explain the coexistence of a very large number of closely related species (Fryer and Iles 1972). However, these systems are also home to a small number of cichlids inhabiting a broad range of habitats. These widespread species show high levels of plasticity in traits such as diet preference (Huysseune et al. 1994) and various morpho-physiological traits (Chapman et al. 2006; Chapman et al. 2000). If species are well-adapted to their environments, and the traits of interest are plastic, we would predict tight associations between the environment and both diet and morphology across the species range. However, if organisms with a specialized morphology are able to function as dietary generalists, trophic morphology may not be a useful indicator of feeding ecology. To test these predictions, we studied the diet and morphology of a eurytopic cichlid, *Astatoreochromis alluaudi* (Pellegrin, 1904), in Uganda, East Africa. *A. alluaudi* has historically been described as a polymorphic species characterized by its pharyngeal jaw structure (Greenwood 1964; Smits et al. 1996). Hypertrophied morphs have been associated with a molluscivorous diet, whereas non-hypertrophied morphs are believed to feed on softer food items such as insects (Huysseune et al. 1994). However, most studies are restricted to comparisons between 2 populations, and it is unknown whether jaw morphology is continuous across populations rather than discrete, as is the case in other molluscivorous cichlids (Hulsey et al. 2005). Although the ability of *A. alluaudi* to feed on a range of prey items has been known since the 1960's, this cichlid is generally assumed to be a molluscivore specialized for consuming the hard-bodied *Melanoides* snails common in Lake Victoria, as well as the softer *Bulinus* and *Biomphalaria* snails (Greenwood 1964; Hoogerhoud 1986). Yet, in the absence of interspecific competition from other haplochromine cichlids, *A. alluaudi* has been observed consuming soft food items such as soft-bodied insects (Hoogerhoud 1986; Slootweg et al. 1994). Thus, it has been argued that *A. alluaudi* feeds on snails in Lake Victoria because interspecific competition from haplochromine cichlids feeding on insects is higher than inter- and intraspecific competition from other molluscivores feeding on snails (Slootweg et al. 1994). Slootweg et al. (1994) even attributed the failure of introduced *A. alluaudi* to control snail densities when introduced in reservoir ponds to the lack of an intact cichlid fauna out-competing the molluscivore for more profitable insect prey.

Interestingly, populations of *A. alluaudi* are known to persist in Ugandan river systems where mollusks can be rare but haplochromine cichlids co-occur (Binning and Chapman 2008). Binning and Chapman (2008) found relatively high dietary overlap between *A. alluaudi* and an unidentified riverine haplochromine species in one site within the Mpanga River basin of

Uganda. These results suggest that the range of *A. alluaudi* encompasses a broad diversity of habitats and wide variation in both the food base and competitor density.

In this study, we explored the relationship between diet and morphology in *A. alluaudi* across a natural environmental gradient to determine whether morphology is a good indicator of diet choice in a natural system, or whether fish with specialized morphologies consume generalist diets as predicted by Liem's Paradox. More specifically, we quantified a suite of abiotic (DO, pH, water transparency, conductivity, water depth) and biotic (diet and abundance of haplochromine fish, snails, and insects) parameters to detect predictors of morphology across six sites. To achieve this goal we a) compared the jaw and muscle morphology of *A. alluaudi* among sites and b) related morphological divergence among sites to diet and environmental variables.

Methods

Study sites

Specimens for this study were initially collected from two sites in June 2006 for morphological analyses that differ markedly in snail availability (Lake Saka, snails common; Lake Nabugabo, snails extremely rare; Efitre et al. 2001). Specimens for morphological analyses from the remaining four sites as well as environmental and diet data for all sites were collected between January and February and May through July 2007 (Fig. 1). These months correspond to the dry season in East Africa, when sites are most accessible. Sites were selected to maximize variation in ecological and environmental variables, particularly the abundance of snails and other haplochromine cichlids. In total, three lake and three river sites were sampled (Fig. 1).

The Napoleon Gulf of Lake Victoria (0°20'N; 33° 15'E) was selected as a lacustrine site, since much historic and contemporary research on *A. alluaudi* has been conducted in this area

(Greenwood 1964; Mbabazi 2004). Furthermore, it is the only site where both stomach content and isotopes analysis have confirmed a primarily molluscivorous diet of *Melanoides* in this species (Mbabazi 2004). The second lake site, Lake Nabugabo, is a satellite of Lake Victoria (24 km^2, 0°45'S; 31°45'E), isolated from the main basin 5,000 years ago by long shore bars (Stager et al. 2005). In this lake, Chapman et al. (2002) found that *A. alluaudi* was restricted to the hypoxic wetland ecotones of small bays characterized by dense vegetation and low levels of dissolved oxygen (DO). Snails are rare in Lake Nabugabo (Efitre et al. 2001), and *A. alluaudi* are thought to be primarily insectivorous. The third lacustrine site, Lake Saka (0°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}; Chapman et al., unpubl. data).

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 data from a 2006 survey of 7 locations within the system and a minimum catch of 10 *A. alluaudi* necessary for morphological analyses (Crispo and Chapman 2008). Kamwenge, the southern-most downstream site, experiences flowing, open waters that are likely to be normoxic year round. Kahunge, the second site, is extremely variable in terms of its abiotic parameters due to seasonal flooding and its proximity to the upstream Kairaguru Swamp (Crispo and Chapman 2008). Rwebakwata also experiences seasonal variation in DO and other water quality parameters during heavy rains (L.J. Chapman, unpubl. data).

Environmental data

At each site, major habitat types (*Vossia cuspidata*, shrub and brush, forest edge) were sampled to characterize the fish assemblage and food base. Within each dominant habitat type, six sets of duplicate minnow traps were baited and set 4 m apart for 3 hours during the day, at approximately 10h30. For each set of traps, we recorded water temperature, DO, conductivity, pH, water transparency, and water depth. In the Mpanga River system, data were collected between 10h00 and 14h00. Lake sites were sampled comparably, with additional experimental gill nets set between 2 and 5m from the shoreline to supplement minimum trap captures. Due to diel variation in DO and water temperature in the lake systems, particularly Lake Saka, we recorded surface and bottom measures of DO and temperature at 07h00 and 14h00 (Crisman et al. 2001). In lake systems, we measured water transparency using a Secchi disk. In flowing water, we took water samples, and recorded transparency readings in a PVC Secchi tube, which we standardized to Secchi disk readings (Kasangaki et al. 2008). We assessed the abundance of benthic macroinvertebrates at two sites within each transect using scoop nets (frame size 43.2 cm x 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 cm^2, depth 17 cm). For both samplers, the substrate was poured through a fine mesh Eckman dredge bucket, and samples were sorted directly in the field. Invertebrates were preserved in 10% formalin and later transferred to 70% ethanol.

All captured cichlid fishes were counted and identified in the field as: *A. alluaudi, Pseudocrenilabrus multicolor victoriae* Seegers (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). *A. alluaudi* were euthanized in MS222, injected into the abdominal cavity with paraformaldehyde and preserved in buffered paraformaldehyde for transport to McGill University, Canada.

Morphology- Muscle and jaw metrics

We were interested in studying the effect of diet on the pharyngeal jaw and muscles that play a role in feeding. Since this cichlid was known to feed primarily on insects in at least one site sampled (Lake Nabugabo), we choose to measure muscles that are useful in both suction feeding (m. sternohyoideus) as well as the handling of large prey within the pharyngeal jaws (m. geniohyoideus) (Galis 1992). The traits measured on the pharyngeal jaw were specifically chosen to reflect jaw crushing force (Smits 1996). Approximately 10 fish of a representative size range (Table 1) were selected within each population, and in situ photographs were taken of the m. geniohyoideus and m. sternohyoideus muscles. Additionally, the lower pharyngeal jaw was dissected out and photographed. Muscle and jaw photos were analyzed using Motic Ver. 2.0 software; metrics included muscle length, muscle width, muscle depth, and muscle cross-sectional area, as well as jaw width, jaw length, tooth depth, horn width, and jaw area (Smits 1996) (Fig. 2). Muscle cross-sectional area (CSA) was calculated with width and depth measures using the formula for the ellipse: $CSA = \pi * \frac{1}{2} Depth * \frac{1}{2} Width$.

Stomach content analysis

Fish standard length (± 0.01mm) was recorded prior to dissection (Table 1). Stomachs were then dissected-out, and stomach fullness visually assessed using a modification of methods in Bwanika et al. (2006). Stomachs were grouped into one of five 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. 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), plant and algae, detritus (all benthic organic and inorganic material), fish (including scales, larvae, juveniles and eggs), mollusk (*Melanoides*, *Biomphalaria*, *Bulinus*, and *Sphaerium*), zooplankton (Copepoda, Cladocera, Ostracoda), shrimp (*Caridina nilotica*), and other (Acariformes, and worms such as Arhynchobdellidae, Tricladida, and Tubificida). Each group was assigned a relative percentage that was further converted to a points system rounded to the nearest 10 percent (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.

Statistical analysis

MANCOVA was used to detect differences in muscle and jaw metrics among sites. Muscle, jaw, and body length measurements were log transformed to improve normality and minimize heteroscedasticity. Each suite of traits was first analyzed separately; then jaw and muscle metrics were combined as is commonly done in morphological analyses. We used standard length as a covariate, and population as a fixed factor. In all analyses, interaction terms were first included, then later removed because there was no evidence for heterogeneity among slopes. We performed Discriminant Function Analyses (DFA) to explore the degree to which trophic characteristics diverged among sites; trait values were size-adjusted using the within-groups slope generated from ANCOVA for each trait (Hendry and Taylor 2004).

Diet similarity between pairs of sites was analyzed using the Percent Similarity Index (PSI), or Schoener's Index (1970). PSI was calculated for each species' pair as follows:

$$PSI = 1-1/2(\Sigma\,|p_{xi} - p_{yi}|)$$

where p is the proportion of prey i in predators x and y. The amount of diet overlap was categorized as low (0-24%), moderate (25-49%), high (50-74%), or very high (75-100%) (Buckley et al. 1999).

We calculated the relative abundance of each prey item (insect, mollusk, 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. We used the percentage of *A. alluaudi* to haplochromine cichlids within each site based on individual counts as an index of competitor density, but excluded *Pseudocrenilabrus multicolor victoriae*, since this species exhibits less dietary overlap with *A. alluaudi* than do other haplochromine species (Binning and Chapman 2008). However, results were similar when *P. multicolor* were included. The abundance of other fish species was also excluded from the analysis since previous studies have shown that haplochromine cichlids share the greatest dietary overlap with *A. alluaudi* (Binning and Chapman 2008; Hoogerhoud 1986).

To correlate environmental data 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 relative prey abundances, and the percentage of major prey items in the stomach of *A. alluaudi* (4 PCAs total). We used 11 (six muscle and five jaw) morphological measures in this analysis to obtain an average population-level measure for the composite morphological traits (N=6 populations). 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. 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.

Due to the small number of comparisons being made (6 variables), we chose not to correct our p-values using Bonferroni correction, but to interpret our data with caution (Garcia 2004).

Results

Environmental data

Most physico-chemical characters measured exhibited a high level of variation across the six sites sampled (Table 2). 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 via 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 Rwebakwata, 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 harboured a high abundance of haplochromine cichlids and a low abundance of other lentic-water species (*Oreochromis niloticus*, *Lates niloticus*, *Protopterus aethiopicus).* River sites typically contained a variety of cyprinids (*Barbus kerstenii, B. cercops, B. apleurogramma, Barbus "line"*), killifish (*Hypsopanchax deprimozi*), a clariid catfish (*Clarius liocephalus*) 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, in all lake sites it comprised only a small percentage of the total haplochromine cichlids encountered (Table 3).

The abundance and richness of potential macroinvertebrate prey was also variable across sites (Table 3). The most common insect orders found were Diptera, Odonata, Coleoptera, and

Ephemeroptera with 8 insect orders represented in total across sites. Snails and clams were found in all sites except Lake Nabugabo.

Napoleon Gulf had the highest richness of prey taxa per dredge sample, over 75% of which were clams or snails. The richness of macroinvertebrate taxa was also high in Lake Saka, but most of the abundance was accounted for by snails. River sites had, in general, greater invertebrate species richness than lake sites, with the exception of Napoleon Gulf.

Morphology- Muscle and jaw metrics

MANCOVA results on muscle and jaw metrics using fish standard length as a covariate showed a strong population effect both when each suite of traits (muscles, jaws) was considered separately (muscles: N= 61, F= 4.238, P<0.001; jaws: N=55, F= 6.878, P<0.001), and when muscle and jaw metrics were pooled within sites (N=60, F= 6.791, P<0.001).

Discriminant function analyses based on a representative subset (horn width, jaw width, jaw depth, sternohyoideus CSA, geniohyoideus CSA) of the size-adjusted jaw and muscle metrics indicated significant morphological differences among the six populations and generated four significant functions accounting for 63.5 %, 20.1 %, 11.9 % and 3.4 % of the variance, respectively (Wilks' $\lambda = 0.063$, P<0.0001). The first Discriminant Function was highly positively correlated with jaw measures (horn width r= 0.745, jaw depth r= 0.734, jaw width r= 0.599) and more weakly correlated with the cross-sectional area of the m. sternohyoideus (r= 0.366); whereas the second function related more strongly to muscle metrics (m. geniohyoideus cross-sectional area r=0.557). The DFA classified 71.7% of the original 60 fish into the correct population.

A frequency distribution of the first DFA factor scores across all 6 sites showed a normal distribution suggesting trophic morohology is a continuous rather than a polymorphic trait, as would have been represented by a bimodal distribution. A representative plot of the first two DFA factor scores (Fig. 3) was used to explore the degree to which these two functions separated out the six populations based on composite morphological characteristics. Lake Nabugabo and Kamwenge separated from other populations based mostly on function 1, (63.5 % of the variance). Lake Saka, Napoleon Gulf, Kahunge, and Rwebakwata had similar values for Function 1, but exhibited a degree of separation (though not complete) on Function 2 (20.1 % of the variance).

Stomach content analysis

Of the 224 *A. alluaudi* captured at the six 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 wide 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 only 7% 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).

Napoleon Gulf fish had the most distinct diet exhibiting low overlap values with other sites (33 to 42% similarity). Lake Nabugabo, on the other hand, showed high similarity in diet to the 4 other sites sampled excluding Napoleon Gulf (between 57 to 85% similarity, Table 4).

Data synthesis: environmental, morphological and diet variables

PCA on 11 morphological measures generated 3 axes with eigenvalues greater than 1.0, which cumulatively explained 93.72% of the variance (Table 5a). PC1 related most strongly to jaw traits, whereas the length of the geniohyoideus and sternohyoideus muscles loaded most heavily on PC2. One principle component was extracted from the physico-chemical data (water transparency, pH, DO and conductivity), and explained 79.18% of the variance (Table 5b). Similarly, one component was extracted from the prey abundance data (invertebrate richness, relative abundance of mollusks, 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, molluscs and insects in diet) explained 67.43% of the variance. This axis was strongly positively related to the percentage insects and negatively related to percentage molluscs in the diet of *A. alluaudi.*

Pearson correlation on the principle components of morphological and environmental variables revealed a significant relationship between the prey availability principle component and the second principle component for morphology (r=0.814, P=0.049, Table 6a), as well as with the principle component for diet (r=-0.821, P=0.045 Table 6b). Although these p-values are close to the threshold of rejection, this result is most likely due to low sample size (6) and lack of power to detect a statistically significant relationship. However, the high r values of these relationships are notable. No other results from the correlation matrix were statistically significant, nor were other r values above 0.80 (Table 6 a,b).

Discussion

Morphology and mismatch

Understanding how morphology relates to diet and the environment is crucial for elucidating the general principles driving evolutionary mechanisms such as speciation, as well as physiological and behavioural adaptations to fluctuating resources (Ferry-Graham et al. 2002). The cichlid fishes of East Africa are classic study systems for examining trophic specialization as it relates to the evolution of these species flocks. However, surprisingly little comparative research has been done on widespread species like *A. alluaudi* across more than two populations. As such, studies on *A. alluaudi* have suggested that jaw morphology is a polymorphic trait. By quantifying variation in jaw morphology across multiple populations and environmental gradients, we observe more continuous variation than has been reported in other studies.

Although *A. alluaudi* has been traditionally described as a molluskivore, we found only one population, in Napoleon Gulf, Lake Victoria, where snails and other hard-bodied prey comprised an important part of the diet according to our point in time sample. While the jaw and muscle morphologies in this population are consistent with predicted morphological responses to hard-bodied prey, fish from Napoleon Gulf in Lake Victoria shared a surprisingly similar morphology to populations such as Lake Saka, where molluscs comprised a small component of the diet, and two river sites, Rwebakwata and Kahunge, where no mollusc remains could be detected from the stomachs sampled. Furthermore, in Kamwenge, where the most hypertrophied jaw morphology was observed, stomach contents revealed that only a small proportion of snails and clams (7%) were consumed by less than 20% of the fish sampled. These results are not consistent with a tight link between morphology and diet. Yet, evidence of a diet-morphology mismatch was not found at all sites. In addition to the population studied in Lake Victoria, *A. alluaudi* from Lake Nabugabo displayed a morphology consistent with their diet: fish from this population were characterized by small jaws, and fed omnivorously, with soft-bodied insects comprising 29% of our point-in-time diet sample. Overall, however, the integration of morphology, diet, and food base across sites suggests that trophic morphology in *A. alluaudi* is not a consistent predictor of diet across the range of this species. One possible explanation is that a molluscivore-like morphology may be retained if populations feed occasionally on hard-bodied resources. The potential for plasticity in trophic traits observed in many cichlid species, including *A. alluaudi*, may also account for the large variety of diets and morphologies found among populations, particularly if gene flow is high across sites as has been demonstrated in the widespread cichlid *P. multicolor* that co-occurs with *A. alluaudi* (Crispo and Chapman 2008).

It is also possible for mismatch to occur when environmental conditions change before a species is able to respond such that an organism's morphology reflects a historic food source rather than what is currently being consumed. Although the lake and river systems studied have undergone rapid environmental change in the past several decades (Ogutu-Ohwayo et al. 1997), the enormous potential for plasticity in African cichlids makes it unlikely that the eco-morphological mismatch observed is a product of past environmental conditions. Lab-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, unpubl. data).

Eco-morphological mismatch between trophic elements 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 two functions such that an organism's morphology represents a compromise to the pressures of two activities. Alternatively, performance in one dimension, such as feeding, may be compromised by change in a neighboring structure that constrains or alters the targeted character. In fishes, these interactions have been observed between feeding and respiratory structures because of the compact, laterally compressed head morphology characteristic of most teleosts (Barel 1983; Cech and Massingill 1995). 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. 2008; Chapman et al. 2000; Schaack and Chapman 2003). Complex indirect interactions and trade-offs between morphological and other (ie. respiratory) structures could also produce the patterns observed, and should not be ignored in future studies.

Liem's Paradox

Resource acquisition is among the most important ecological challenges faced by individuals in natural communities. Access to resources is dependant not only on what food items are present, but also on the particular energetic and anatomical demands required to efficiently process prey (Ferry-Graham et al. 2002). Since different prey items are presumed to require particular anatomical specializations in order to be processed effectively, divergence in jaw and trophic muscle morphology among species has long been considered an important driver of evolutionary change, especially in birds, bats, and fish (Grant 1986; Purnell et al. 2007; Swartz et al. 2003). However, this study exemplifies the complexity of applying morphological traits as predictors of ecological function. The coupling of trophic specialization with a generalist diet, known as Liem's Paradox was derived from the observation that some species of morphologically-specialized cichlid fishes could also function as generalist feeders (Liem 1980; Robinson and Wilson 1998). Liem proposed that specialized morphology is retained to feed on less-preferred prey items rather than prey consumed most frequently. Theoretically, this should only be possible if a specialized morphology permits efficient feeding on other prey sources (Van Wassenbergh et al. 2007). Several Ugandan populations of *A. alluaudi* (Lake Saka, Kamwenge, Kahunge, Rwebakwata) seem to confirm Liem's predictions: mollusks were consumed infrequently and in small quantities despite the enlarged jaw morphology observed.

According to the paradox, the maintenance of large jaw morphologies in *A. alluaudi* would be an adaptive strategy for ensuring a supply of unexploited resources during times of resource scarcity. This scenario is in agreement with the theory presented by Futuyma and Moreno (1988): in times of food shortage, it is advantageous for an individual to exploit several resources; thus in a changing environment, some opportunity must exist at all times for selection to broaden a species' niche. However, interspecific competition should act to oppose overlap between diets of competitors, and thus restrict the niche occupied by any given species (Dayan and Simberloff 2005). Specialization in diet should therefore be a consequence of intense competition. The lakes and rivers sampled in this study were host to varying abundances of haplochromine cichlids, some of which consume a diet highly similar to that of *A. alluaudi* (Binning and Chapman 2008). The effects of these two opposing factors, competition acting to reduce niche breadth and fluctuating resource availability acting to increase it, could be operating in synchrony in this system. Our index of competitor density was not a significant predictor of either diet or morphology across the six sites sampled, but these results should be interpreted with caution because of the small number of sampling sites. Future studies that include additional sites may be required to detect the interaction between competition and resource availability.

Seasonality in resource abundances

The availability of food resources in tropical waters may be related to environmental factors such as water depth, water temperature, DO, and turbidity, all of which can be influenced by rainfall and seasonal flooding. Reardon and Chapman (2008) found that peaks in the timing of spawning of an African cichlid correlated with peaks in rainfall. This finding suggests that increases in rainfall are associated with an increased abundance of resources (Denlinger 1980) not only for juvenile fish, but also for piscivorous and paedophageous fish waiting to consume new broods. Seasonal diet switching has been documented in many taxa including several species of birds (Schluter 1982; Tebbich et al. 2004) and freshwater fishes (Adite et al. 2005; Katunzi 1983), and is posed as an adaptation to seasonally fluctuating and /or seasonally scarce resources. Since 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 fed on soft prey. Similarly, muscle and jaw morphology may be retained throughout adult life if, at certain periods of the year, mollusks form an important component of *A. alluaudi's* diet. In a year-long 2006 study of the diet of *A. alluaudi* in Lake Saka, Cosandey-Godin et al. (2008) found that, while molluscs only contributed minimally to the diet of the fish (5%), there was a high degree of variation in the amount of molluscs consumed (1.4- 35%). Future research should explore the potential seasonality in resource use and abundance at additional sites such as Rwebakwata, Kahunge, and Kamwenge, where a Lake Victoria-like morphology exists, but where molluscs were consumed infrequently during the timeframe sampled in this study.

Conclusions

Although morphology is a useful tool for understanding an organism's development and ancestry, ecological properties should not be inferred in isolation of the environment, or other populations. This study is the first inderdemic survey across a number of sites to document Liem's Paradox occurring in nature, and emphasizes the importance of quantifying both diet and trophic morphology to describe the feeding ecology of a species. Our study was restricted to characterizing diet over a limited time period and sampling early- adult and mature life stages. Future studies should explore the potential seasonal aspects of this Paradox as well as the development of trophic morphology in juveniles. Our demonstration that phenotypic specialization is not necessarily accompanied by ecological specialization has important implications for our understanding of coexistence, competition, and the role of phenotype in influencing foraging ecology and species diversification.

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Table 1: Sample sizes and mean standard lengths of fish used for stomach content and morphological analyses from each site (maximum and minimum standard lengths of fish in parentheses).

	Stomachs		**Morphology**	
Site	N	SL mm (min-max)	N	SL mm (min-max)
Kamwenge	33	58.54 (24-93)	11	59.64 (27-98)
Kahunge	53	56.25 (39-81)	10	59.58 (43.72-79.48)
Rwebakwata	19	64 (43-105)	10	66 (43-105)
Napoleon Gulf (Lake Victoria)	34	115.02 (86-143)	10	104.8 (86-116)
Lake Saka	27	57.13 (38-66)	9	64.18 (41.21-74.8)
Lake Nabugabo	45	52.27 (36-90)	10	51.41 (35.66-69.87)

Table 2: Point-in-time measures of various environmental characteristics for 6 sites sampled (with standard errors) in Uganda. All data were recorded during the dry season between mid-May and mid-July, 2007.

	Max depth	**Dissolved Oxygen (mg l^{-1})**				**pH**	**Conductivity**	**Temperature (°C)**		**Transparency**
		AM top	**PM top**	**AM Bottom**	**PM Bottom**		**μS/cm**	**AM Top**	**AM Bottom**	**Secchi Depth**
Site	**(cm)**	**(*N*=78)**	**(*N*=30)**	**(*N*=39)**	**(*N*=29)**	**(*N*=26)**	**(*N*=26)**	**(*N*=78)**	**(*N*=29)**	**(cm; *N*=26)**
Kamwenge	100	5.60 ± 0.10	-	6.89 ± 0.06	-	7.61 ± 0.05	311.10 ± 2.99	22.08 ± 0.02	-	82.4 ± 7.7
Kahunge	100	6.03 ± 0.02	-	6.03 ± 0.03	-	6.95 ± 0.04	337.00 ± 1.27	21.10 ± 0.02	-	87.1 ± 4.5
Rwebakwata	50	4.38 ± 0.29	-	-	-	7.25 ± 0.03	223.48 ± 4.70	19.43 ± 0.05	-	33.8 ± 6.1
Napoleon Gulf (Lake Victoria)	140	6.63 ± 0.16	6.65 ± 0.16	4.51 ± 0.28	5.54 ± 0.26	6.62 ± 0.09	155.76 ± 6.93	24.63 ± 0.02	24.52 ± 0.03	115.0 ± 4.7
Lake Saka	120	9.48 ± 0.13	14.15 ± 0.29	8.70 ± 0.18	9.98 ± 0.34	7.97 ± 0.05	596.51 ± 7.50	23.79 ± 0.03	23.83 ± 0.03	23.4 ± 0.9
Lake Nabugabo	185	5.91 ± 0.03	5.39 ± 0.34	4.23 ± 0.29	4.69 ± 0.22	7.12 ± 0.28	30.78 ± 3.69	22.96 ± 0.08	22.95 ± 0.08	78.5 ± 3.9

Table 3: Cichlid and macroinvertebrate abundances in river and lake sites. The percentage of *A. alluaudi* versus other cichlid species excluding counts of *Pseudocrenilabrus multicolor victoriae*. Cichlid fish included were potentially 2 undescribed species from river sites, 2-3 species from Lake Saka, 2-4 species from Lake Nabugabo, and an unknown number from Lake Victoria. Invertebrate abundances sampled at each site were standardized to the number of dredge/dipnet samples collected per sampling site. Mollusks include *Melanoides*, *Biomphalaria*, *Bulinus*, and *Sphaerium*, insects include Ephemeroptera, Odonata, Plecoptera, Diptera, Hemiptera, Trichoptera, Coleoptera, and unidentified insects, other includes Nematoda and Acariformes.

	Kahunge	**Kamwenge**	**Rwebakwata**	**Napoleon Gulf (Lake Victoria)**	**Lake Saka**	**Lake Nabugabo**
Percent *A. alluaudi* of cichlids	45.5	14.4	40.0	<1.0	1.5	<1.0
Percent mollusks in dredges	20.5	6.5	3.1	75.8	80.8	0
Percent insects in dredges	69.2	84.6	58.6	16.4	19.2	100
Percent other invertebrates in dredges	10.2	9.0	38.6	7.6	0	0
Relative macroinvertebrate prey abundance	7.9	19.4	18.1	151.0	26.0	5.5

Table 4: Percent Similarity Index between the diets of *A. alluaudi* at pairs of sites. Diets overlap is considered high if PSI exceeds 50% .

	Percent similiarity Index					
	Lake Nabugabo	Napoleon Gulf (Lake Victoria)	Lake Saka	Rwebakwata	Kahunge	Kamwenge
Lake Nabugabo	100	-	-	-	-	-
Napoleon Gulf (Lake Victoria)	42	100	-	-	-	-
Lake Saka	57	21	100	-	-	-
Rwebakwata	85	34	55	100	-	-
Kahunge	80	33	52	76	100	-
Kamwenge	65	41	40	63	58	100

Table 5: Principle Component Analysis scores for A) morphological measures (6 muscle traits, 5 jaw traits) and B) physico-chemical measures (4 variables), prey availability (3 variables) and diet (3 variables). Axes were retained if eigenvalues were greater than 1.0. Three axes for morphology were extracted cumulatively explaining 93.72% of the variance. One axis each for the physico-chemical environment, prey availability, and diet components were extracted. Numbers in **bold** represent traits loading heavily on each component (>0.60). All trait measurements were size-adjusted to a standard length of 63.7 mm.

A)			**Geniohyoideus**			**Sternohyoideus**			**Pharyngeal Jaw**				
Morphology	Eigenvalue	% variance	Cross-sectional area	Length	Width	Cross-sectional area	Length	Width	Width	Length	Depth	Horn width	Tooth size
PC1	6.776	61.6	**0.751**	-0.198	0.576	**0.732**	-0.531	**0.852**	**0.919**	**0.968**	**0.952**	**0.900**	**0.862**
PC2	2.463	22.39	-0.429	**0.927**	-0.432	**0.675**	**0.697**	0.497	0.129	-0.05	-0.049	0.100	0.051
PC3	1.07	9.73	0.488	0.265	**0.687**	0.027	0.28	0.129	-0.089	-0.03	-0.299	0.100	-0.305

B)	Eigenvalue	% variance	Transparency	pH	DO	Conductivity
Physico-chemical	3.167	79.18	**-0.820**	**0.909**	**0.923**	**0.903**
			Invertebrate richness	RA Molluscs	RA Insects	
Prey availability	2.509	83.646	**0.837**	**0.947**	**-0.955**	
			% fish in diet	% molluscs in diet	% insects in diet	
Diet	2.023	67.428	0.483	**-0.95**	**0.941**	

Table 6: Pearson correlation coefficients between principle components of size-adjusted morphological variables and environmental variables (α=0.05, all environmental variables are composite variables extracted from PCA except the proportion haplochromines). B) Pearson correlation coefficients between environmental variables (α=0.05).

A)

Morphology	**Environment**	**r**	**p**
PC1	Physico-chemical	0.055	0.918
	Prey availability	0.069	0.897
	Diet	-0.181	0.731
	Proportion haplochromines	-0.520	0.290
PC2	Physico-chemical	0.682	0.136
	Prey availability	**0.814**	**0.049**
	Diet	-0.432	0.393
	Proportion haplochromines	0.699	0.122

B)

Other variables		**r**	**p**
Physico-chemical	Prey availability	0.151	0.776
	Diet	0.238	0.649
	Proportion haplochromines	0.360	0.483
Prey availability	Diet	**-0.821**	**0.045**
	Proportion haplochromines	0.663	0.151
Diet	Proportion haplochromines	-0.499	0.314

Figure 1: Map of Uganda with A) three lake study sites and the location of the Mpanga River indicated with diamonds. B) Map of the Mpanga river with three river sites indicated with diamonds.

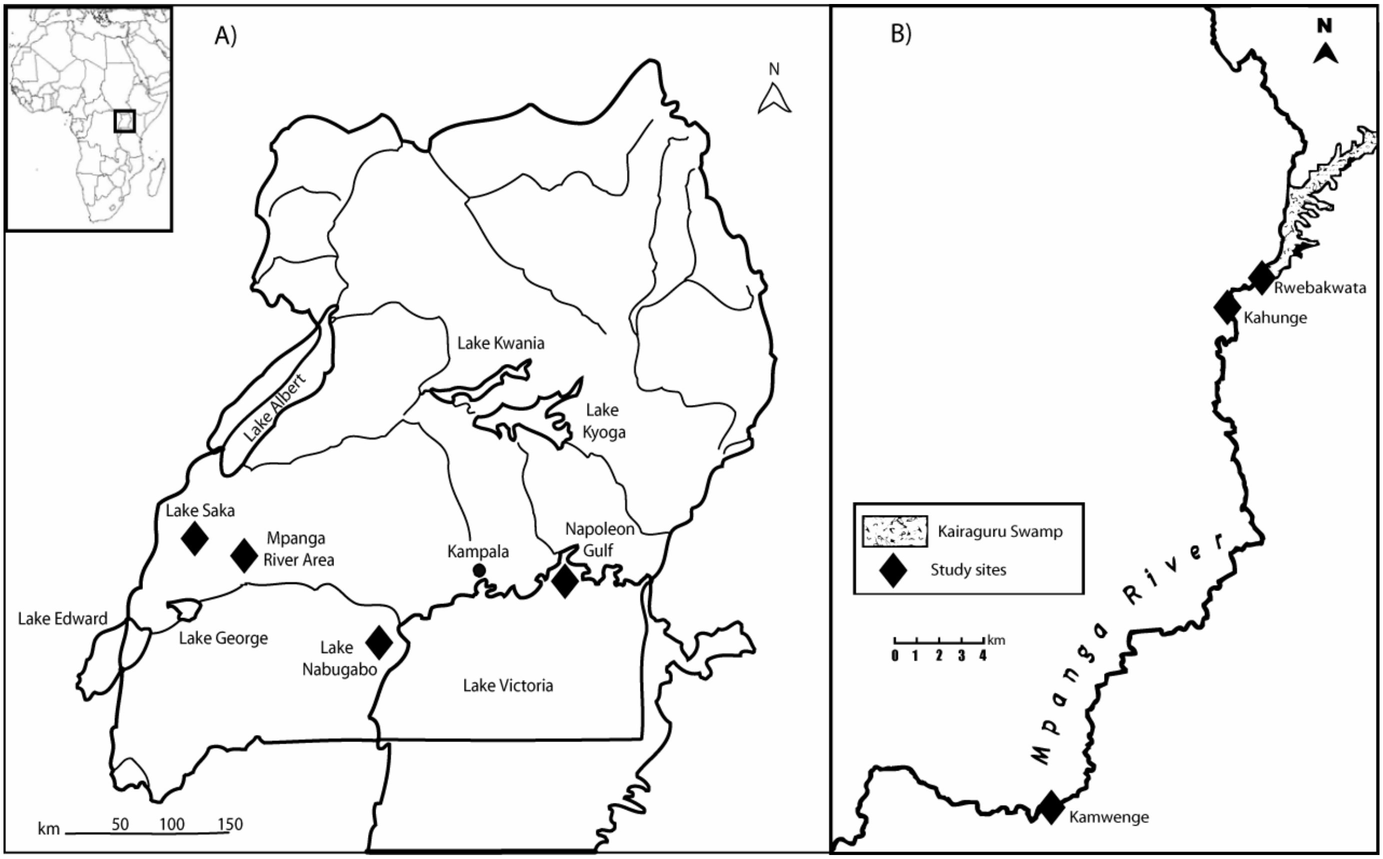
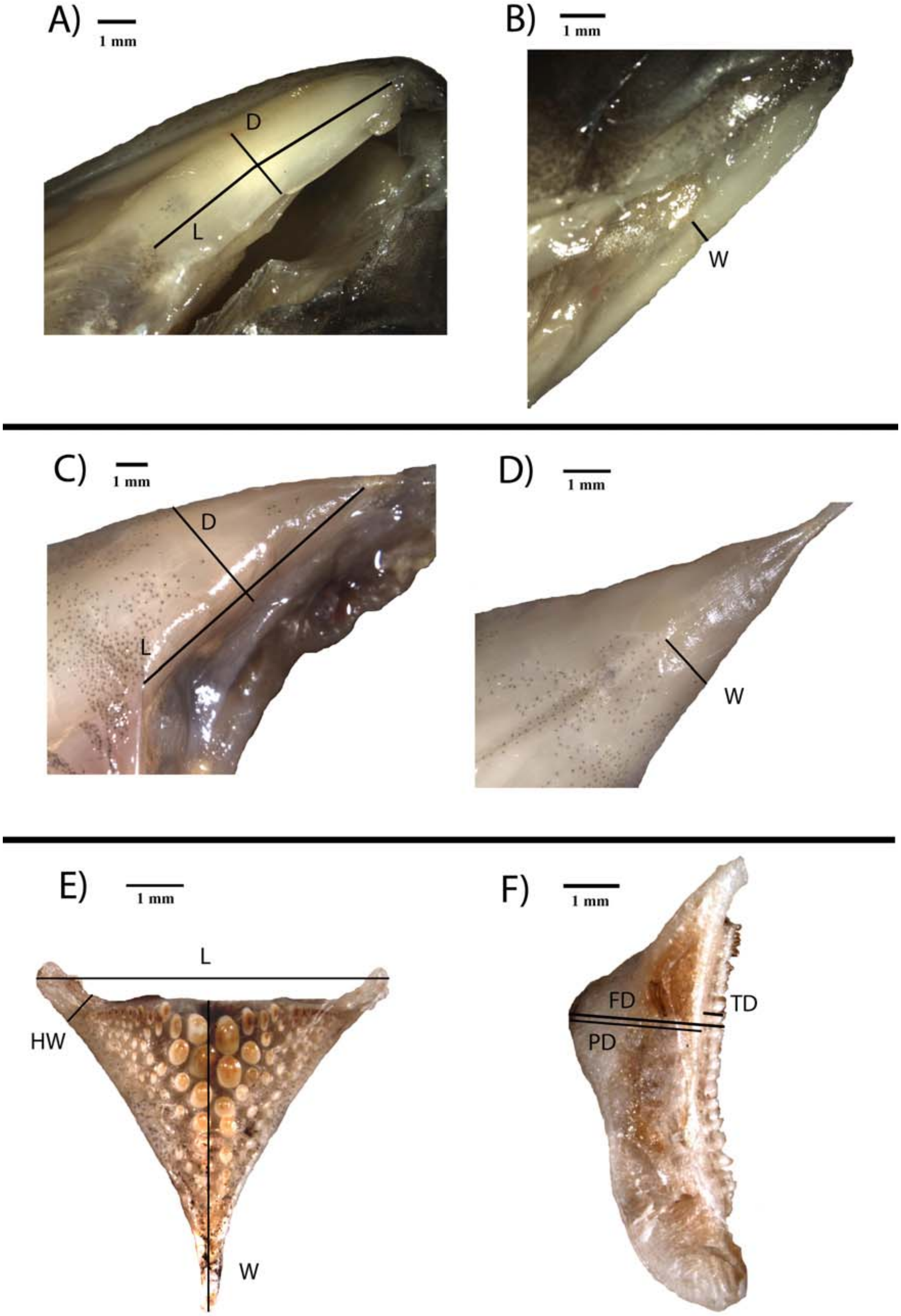


Figure 2: Photographs of the musculus geniohyoideus, musculus sternohyoideus, and lower pharynegal jaw in *A. alluaudi*. All muscle pictures were taken ventrally, and measurements were derived from the left side of the fish. A) View of the left geniohyoideus muscle used for length (L) and depth (D) measures. B) View looking down from the right of the fish on the left geniohyoideus muscle used for width (W) measure. C) Left side view of the sternohyoideus muscle used for length (L) and depth (D) measures. D) Ventral view of the sternohyoideus muscle used for width (W) measure. E) Top view (length, L; width, W; horn width, HW measurements) and F) right side view (tooth depth, TD; full jaw depth, FD; and partial depth, PD measurements) of the lower pharyngeal jaw.


A)
1 mm
D
L
B)
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W
C)
1 mm
D
L
D)
1 mm
W
E)
1 mm
L
HW
W
F)
1 mm
FD
TD
PD

Figure 3: Plot of the factor scores of the first two functions from a Discriminant Function analysis (DFA) of 5 morphological measures of pharyngeal muscles and jaws in *Astatoreochromis alluaudi* from 6 sites in Uganda (Lake Saka= Saka, Lake Nabugabo= Nab, Napoleon Gulf (Lake Victoria)= Vic, Kahunge= Kah'ge, Rwebakwata= Rweb, Kamwenge= Kam). DF1 related strongly to jaw traits, whereas DF2 relates more strongly to muscle cross-sectional area. 71.9% of individuals were correctly classed based on the DFA. All measurements were size corrected to a standard length of 63.7 mm prior to analysis.

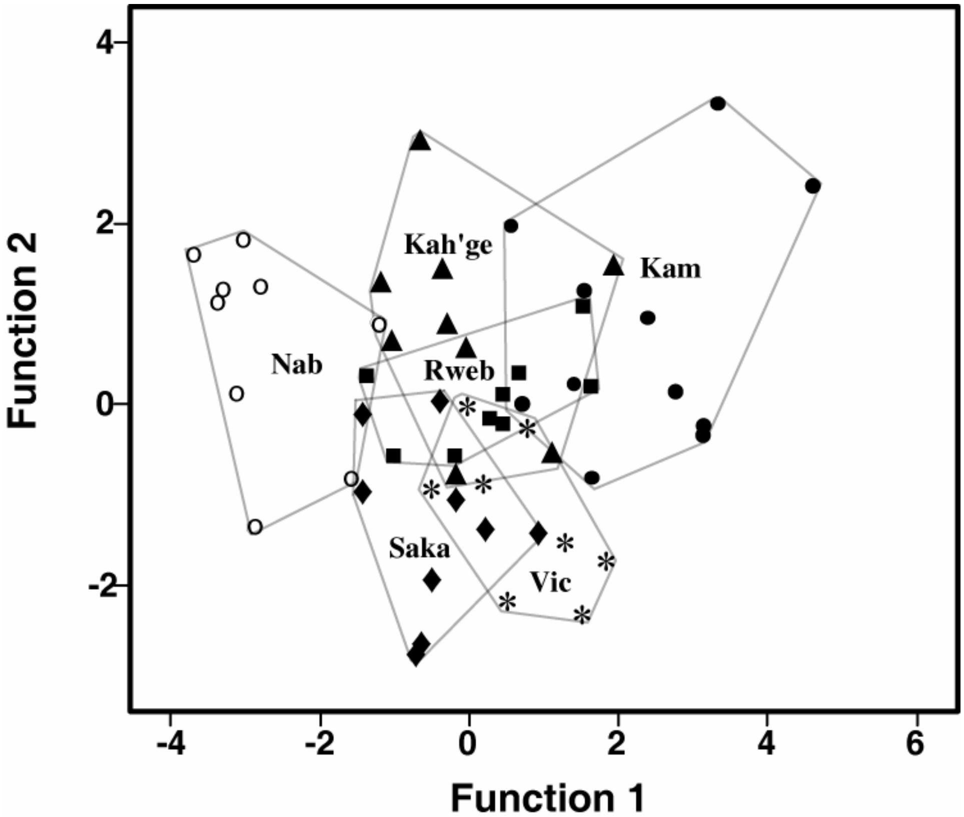
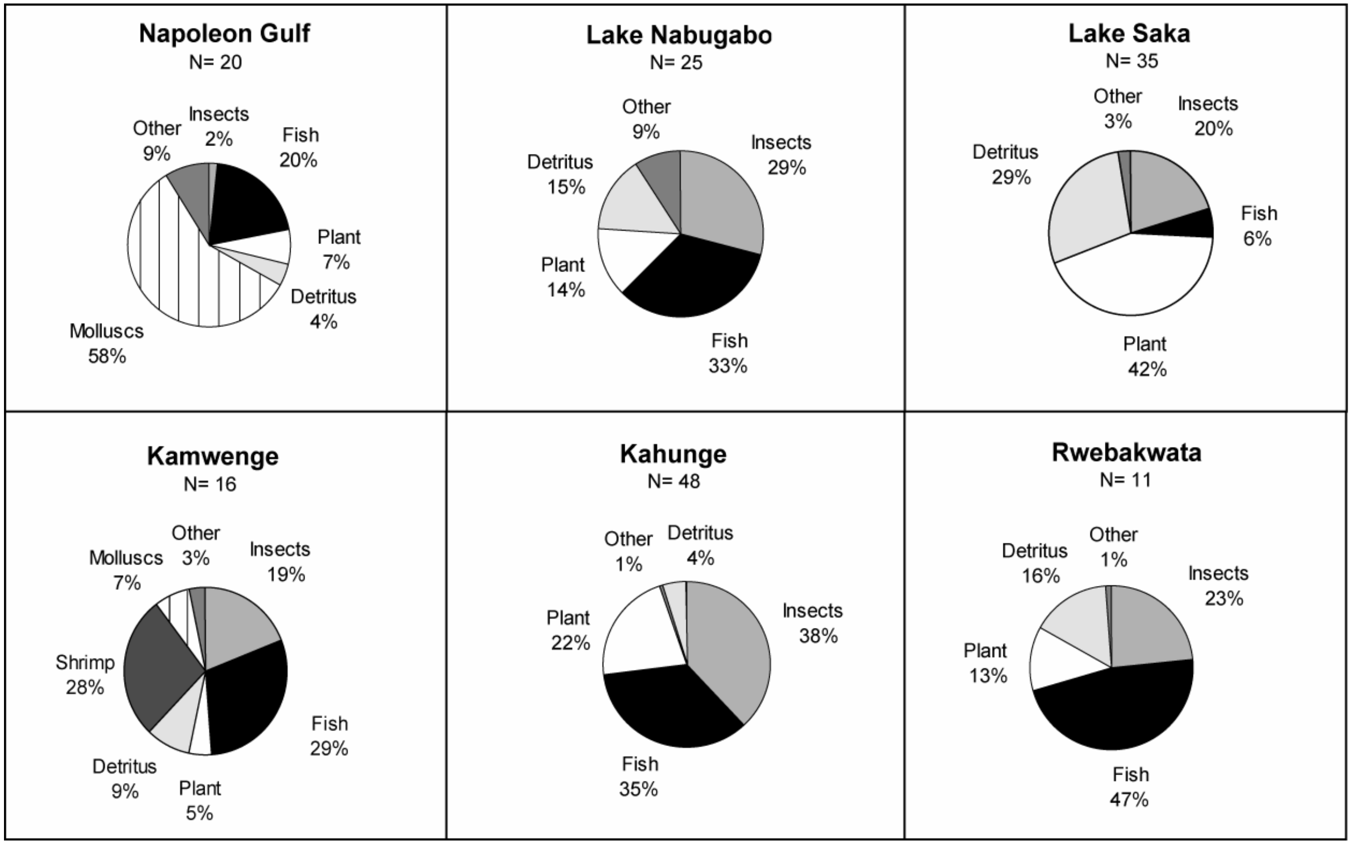


Figure 4: Percentage contribution of prey items in *Astatoreochromis alluaudi* from 6 sites in Uganda (N= number of fish with non-empty stomachs). Diet was divided into 7 major categories; insects (Ephemeroptera, Odonata, Diptera, Trichoptera, Coleoptera, Plecoptera, Hemiptera and unidentified specimens), mollusks (clams and snails), fish (eggs and whole individuals), plant (including algae), detritus, shrimp and other (Acariformes, zooplankton, nematodes).



Connecting Statement

In the previous chapter, I used a spatial survey across 6 sites in Uganda to show that morphological differences in the trophic anatomy of *Astatoreochromis alluaudi* are not accompanied by predictable differences in diet, nor in environmental gradients among sites. I interpreted this result as evidence supporting Liem's Paradox, wherein morphological specialists sometimes function as generalist feeders in the field. In the following chapter, I focus on one of these sites, Lake Saka, where I found this paradox to exist, and use a year long survey and stable isotope analysis to explore changes in diet and food abundances over time as a way of understanding how Liem's Paradox operates in natural systems and the effect of multiple ecological and environmental factors on diet choice over a seasonal scale.

Chapter 2: Specialized morphology for a generalist diet: evidence for Liem's Paradox in a cichlid fish

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Abstract

Morphologically specialized fish often function as generalist feeders in the field, a phenomenon known as Liem's Paradox. This study describes the isotopic signature and seasonal changes in diet of the African cichlid *Astatoreochromis alluaudi*, a fish with massive pharyngeal jaws well known for its ability to process hard-bodied prey. The diet of *A. alluaudi* was quantified in Lake Saka, Uganda over a period of 30 months. Variation in physico-chemical variables (mean monthly rainfall, water temperature, turbidity, dissolved oxygen) as well as potential competitor density and food abundance were measured throughout the second half of the study (14 months). Stomach contents and isotope analysis revealed a diet comprised mainly of fish and insects, with a low contribution of mollusks (0-33%) in any given month. No correlation was detected between diet, and either macro-invertebrate abundance or competitor abundance. Mean running rainfall was positively related to the percentage of fish consumed per month. Although *A. alluaudi* exhibits a molluscivorous trophic morphology in Lake Saka, mollusks did not appear to compose a major portion of its diet. Gradients of rainfall seemed to be the most important environmental predictor of diet choice in Lake Saka, which may reflect seasonal peaks in the abundance of juvenile fish and eggs in the rainy season. Consistent with Liem's Paradox, *A. alluaudi* in Lake Saka exhibit a specialized morphology but a generalist diet, feeding more heavily on fish and insects than on mollusks, the hard-bodied prey that may shape its trophic morphology.

Keywords: Seasonality, isotope analysis, IsoSource, stomach content analysis, trophic specialization, molluscivory.

Introduction

Of all freshwater fish species in the world, the cichlid fishes of East Africa are arguably the most diverse, well-known, and well-studied group (McKaye and Marsh, 1983; Alphen *et al.*, 2003). Over 600 species, half of which are endemic to the area, are found in the Lake Victoria basin alone (Alphen *et al.*, 2003). It has long been hypothesized that specialization in diets and feeding (trophic) morphology of these fishes is an important factor contributing to their radiation and coexistence within the Great Lakes (Greenwood, 1959; Liem, 1991; Barlow, 2000). However, there is increasing evidence that some morphologically specialized cichlids will feed on a wide range of prey items in natural systems (McKaye and Marsh, 1983; Ribbink, 1991; Katunzi *et al.*, 2003), suggesting that these fishes may be more flexible in their feeding behaviours than their morphology would predict.

The observation that specialized feeders frequently prey on non-specialized resources is coined Liem's Paradox (Liem, 1980; Robinson and Wilson, 1998), and calls into question some of the key diversifying factors, notably the role of competition, in shaping the African cichlid species flocks. Liem's Paradox highlights the inconsistencies between morphological descriptions and ecological observations. Studies providing support for this paradox have mainly focused on documenting the existence of a mismatch between trophic morphology and diet (McKaye and Marsh, 1983; Katunzi *et al.*, 2003), but further research is required to understand the factors driving diet choice and the degree to which fishes with specialized trophic morphology consume alternate prey in the wild through time.

Fluctuations in resource abundance, both spatially and seasonally, may be an important driver of dietary variation in fishes (Deus and Petrere-Junior, 2003) and may explain why some specialized feeders readily consume a variety of prey. It has been argued that when resources are abundant, fish should feed opportunistically on whichever prey they encounter most frequently, assuming there is no significant anatomical trade-off to feeding omnivorously (Van Wassenbergh *et al.*, 2007). In times of resource scarcity, however, morphological specializations may be adaptive, since individuals exploiting resources unavailable to competitors may gain a fitness advantage (McKaye and Marsh, 1983). Thus, specialized morphology may not reflect prey consumed preferentially or frequently by a fish, but rather food that can be exploited when resources are scarce. Consistent with this idea, the goal of this study was to quantify seasonal relationships among three environmental variables (food resources, potential competitor abundance and the physico-chemical environment) and diet in the morphologically specialized African cichlid, *Astatoreochromis alluaudi* (Pellegrin, 1904), to better understand the factors that may result in Liem's Paradox.

Although widespread in East Africa, *A. alluaudi* has been most extensively studied in Lake Victoria, where it feeds primarily on snails (Witte, 1981) and is largely assumed to be molluscivorous (Greenwood, 1964). However, *A. alluaudi* is also known to feed on various prey items (Mbabazi, 2004) and to prefer insect prey over mollusks (Slootweg *et al.*, 1994). Jaw morphology in *A. alluaudi* is a developmental trait that is thought to canalize at approximately 35 mm standard length: fish exposed to hard-bodied food items during early life stages develop a molariform morphology even if they routinely feed on softer prey items (Smits, 1996). Seasonal fluctuations in resource abundances may therefore influence diet and jaw morphology in *A. alluaudi,* particularly if the cichlid must sometimes consume snails in order to survive.

In Lake Saka, western Uganda, morphological analyses performed on *A. alluaudi* specimens (Chapter 1) revealed that this population has relatively well-developed hypertrophied jaws that are similar to those of the well-known mollusk-feeding population in Lake Victoria.

Interestingly, diet analyses by Cosandey-Godin et al. (2008) suggested that only a small percentage of molluscivory is necessary to produce this morphology: in a year-long sampling study of *A. alluaudi* in Lake Saka, mollusks comprised only 5% of the annual diet of the fish, and the percentage of other prey consumed fluctuated throughout the year.

These results are important since the majority of studies examining effects of seasonality in resource abundance on fish behaviour and diet focus on temperate waters where strong seasonal variation in temperature drives seasonal changes in the prey base (Sumpter, 1990). However, tropical freshwaters may also experience seasonal variation, which can structure aquatic communities. In particular, seasonal flooding affects patterns of habitat availability, food resources, water quality and predation risk (Chapman and Kramer, 1991; Chapman and Chapman, 1993). Behavioural changes such as altered foraging strategies, and seasonal spawning may result as a consequence of these fluctuations. As such, ecological surveys in tropical aquatic systems conducted over a seasonal scale should be sensitive to changes in abiotic gradients (ie., dissolved oxygen, water depth, turbidity) as well as biotic gradients (ie. prey availability, competition) in order to better understand the role of environmental variation in structuring communities and shaping the morphology and behaviour of organisms.

A common method for detecting seasonality in fish diet is stomach content analysis; however, this technique provides only a snapshot of food intake, and may not present an accurate picture of dietary contributions to energy assimilation (Perga and Gerdeaux, 2005). Stable isotope analysis allows a more in depth understanding of food web structure and diet by providing information on an organism’s likely food sources and trophic position. This technique involves obtaining a tissue sample from a consumer, and from all potential prey items, and analyzing the tissues for the relative proportions of two heavy isotopes, ^{13}C and ^{15}N (Fry, 2006).

The combination of stomach contents (point-in-time) and stable isotope (time-averaged) analyses allows more accurate inferences regarding which prey items provide most of the energy for an organism's growth, maintenance, and reproduction (Fry, 2006).

This study explores seasonal fluctuations in the diet of the "molluscivorous" African cichlid, *Astatoreochromis alluaudi,* in Lake Saka, Uganda by relating fluctuations in diet to seasonal variation in environmental parameters in order to detect predictors of diet shifts. Using stomach content and stable isotope analysis, the goal of this study was to verify when, if ever, mollusks are being consumed in high frequencies, and whether these dietary patterns are related to seasonal variation in environmental parameters. To achieve this goal, (a) seasonal variation in physico-chemical parameters (water temperature, dissolved oxygen, rainfall, turbidity), macroinvertebrate abundances, and potential competitor density was quantified over the course of 14 months to detect seasonal fluctuations; (b) these fluctuations were related to variation in diet over time; and (c) the results from the point-in-time stomach samples were corroborated with a time-averaged isotopic signal obtained from likely prey of *A. alluaudi.*

Materials and Methods

Study site

Lake Saka is a crater lake in western Uganda, East Africa (0°40'N; 31°15'E, Figure.1 in Campbell *et al*., 2006) that experiences hyper-eutrophication of its waters due to extensive clearing of surrounding forest and wetland (Crisman *et al*., 2001). Enhanced phytoplankton productivity results in dissolved oxygen (DO) levels often reaching supersaturation (>12 mg l^{-1}), and exceeding 15 mg l^{-1} at the surface. The fish fauna and introduction history of Lake Saka are similar to that of Lake Victoria. The lake harbors a flock of native haplochromine cichlids including two undescribed endemic cichlids and the widespread, *Pseudocrenilabrus multicolor* *victoriae ,* as well as the cyprinid, *Barbus neumayeri.* It was also stocked with Nile tilapia (*Oreochromis niloticus*) and Nile perch (*Lates niloticus*).

Fish collection

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 (L.J. Chapman, unpubl. data), and these areas were targeted for fish collection. Specimens of *A. alluaudi*, and other haplochromine cichlids were collected from three vegetated shoreline sites in the northern end of Lake Saka every 2 weeks between January 2007 and June 2008 (see Campbell et al., 2006). Baited minnow traps were set between 10h00 and 14h00 in densely vegetated nearshore sites, and gill nets were set 2 m offshore for 1 hr to supplement catches. All fishes caught were counted, but haplochromine cichlids were recorded as a single species complex, due to the high levels of hybridization occurring in the lake (Seehausen and Chapman, unpubl. data). Since *Pseudocrenilabrus multicolor* is not known to hybridize with the other haplochromine cichlid species, it was identified separately.

Astatoreochromis alluaudi specimens (maximum 15 per sampling day) were euthanized with buffered MS-222. The stomach of each fish was injected with 10% formalin to prevent further digestion prior to preserving the entire specimen in 10% formalin. Fish were transported to the Makerere University Biological Field Station (MUBFS) laboratory in Kibale National Park, Uganda for stomach content analysis.

Physico-chemical parameters and invertebrate abundances

During the last 14 months of the study, a sampling protocol was implemented to evaluate seasonal changes in environmental parameters likely to influence diet choice. Two sites representing the major ecotonal habitat types were selected for invertebrate sampling (shoreline dominated by *Phragmites* and shoreline dominated by forest and *Cladium spp*.). At each site, two locations were marked 8 m apart to insure that samples would be taken from the same patches throughout the year. An Ekman grab (opening 225 cm^2, depth 17 cm) was used to sample these 4 locations (ie. 2 per site). A scoop net (frame size 43.2 cm x 32.4 cm, depth of 18.9 cm, mesh size 0.3 cm) sample was also taken from a nearby bay by disturbing and scooping the bottom substrate. The substrate for both sampling techniques was poured through a fine mesh (541 μm) Ekman dredge bucket to capture a comparable size-range of organisms from each habitat. Scoop net and dredge samples were sorted directly in the field, and all invertebrates preserved in 10% formalin and later transferred to 70% ethanol.

Physico-chemical data (water temperature, DO, Secchi depth) were collected from the same sampling sites in the early morning (07h00) and afternoon (14h00) to capture a range of diel variation in these parameters. Rainfall was measured daily between 2006 and 2008 at the nearby MUBFS, summed for each month.

Stomach content analysis

Stomach content analyses followed methods outlined in Binning and Chapman (2008). Fish stomachs were dissected out, and stomach fullness was then visually assessed using a modification of methods found in Ball (1961): stomachs were grouped into one of five categories as follows: level 1, empty stomach; level 2, stomach ¼ full; level 3, stomach ½ full; level 4, stomach ¾ full; level 5, completely full stomach. Following the points method reviewed by Hyslop (1980), each prey item was identified to order-level resolution when possible. Similar prey items were grouped together into one of the following categories: insects (including the orders Ephemeroptera, Plecoptera, Diptera, Hemiptera, Trichoptera, Coleoptera, Megaloptera and unidentified insects), plants and detritus (includes all benthic organic material), fish (including scales, juvenile fish, larval fish and eggs), mollusks (*Biomphalaria* and *Bulinus* snails) zooplankton (Copepoda, Cladocera, Ostracoda) and other (Acariformes, and worms such as Arhynchobdellidae, Tricladida and Tubificida). Prey groups were then visually assigned a relative percentage, which was converted to a points system rounded to the nearest 10 percent. To adjust for the importance of prey items in stomachs of varying fullness, the points were multiplied by the proportion of stomach fullness for each specimen. The total number of points per category was calculated and divided by the total number of non-empty stomachs in each population.

Stable isotope analysis

Specimens used for stable isotope analysis were collected in June 2007. They included mature *A. alluaudi*, haplochromine cichlid yolk-sac broods, haplochromine cichlid late-stage broods, *A. alluaudi* late-stage broods, mollusks *(Bulinus* and *Biomphalaria* snails), invertebrates (Diptera, Coleoptera, Plecoptera, Ephemeroptera, Odonata) and aquatic vegetation (*Cladium*). Yolk sac and late-stage broods were obtained from brooding haplochromine and *A. alluaudi* females. Most invertebrates were sorted in the field and pooled by order such that samples represented homogenized tissue from several individuals. Since some orders, including Odonata, contain species with distinct feeding habits, and therefore distinct isotopic signatures, this group was initially separated and analyzed by suborder (Anisoptera and Zygoptera). All samples were preserved in 70% ethanol until arrival at McGill University at which point they were placed in a -20 ºC freezer, and then transferred to -80 ºC prior to freeze-drying. Although there is disagreement over whether ethanol preservation significantly alters the ^{13}C signature of tissues due to lipid hydrolysis, some authors argue that this form of preservation is appropriate for isotope analysis (Sarakinos *et al.*, 2002), and careful comparisons with previously published studies on Lake Saka suggest the results of this study were within the range expected of *A. alluaudi* despite differences in preservation techniques (Campbell *et al.*, 2006). Fish were dissected by removing the skin, and excising a filet from the dorsal anterior muscle. Tissue from each fish was analyzed separately after being freeze-dried at -50 ºC in a SuperModulyo (ThermoSavant) freeze drier for 24 hr and hand-ground into a fine powder.

Invertebrate samples with high amounts of carbonates (Diptera, Coleoptera, Anisoptera, Zygoptera, mollusks and vegetation) were acidified (Cloern *et al.*, 2002; Carabel *et al.*, 2006) to avoid biases associated with differences in the origin of carbon in tissue versus exoskeleton (Jacob *et al.*, 2005). Fish muscles were not acidified (Carabel *et al.*, 2006). Mollusk tissues were removed from their shells prior to acidification. Samples were acidified by placing them in a dessicator with an open beaker containing 20ml 1M HCL for 24 hr; they were then dried in an oven at 60 ºC for 24 hr and 1 mg (± 0.05 mg) was placed into sealed tin capsules (5X 3.5 mm) for analysis. The Plecoptera and Ephemeroptera samples were not acidified since there was insufficient material for this procedure.

Stable carbon and nitrogen isotope analyses were performed using a Delta Plus continuous flow Finnigan mass spectrometer coupled to a Carlo Erba Elemental analyzer at the Environmental Isotope Laboratory at the University of Waterloo, Canada. Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material;

$$\delta^{13}C \text{ or } \delta^{15}N = ([R_{\text{sample}}/R_{\text{standard}}]-1) \text{ X } 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The standard material used in these calculations is Pee Dee belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Results were corrected to the International Atomic Energy Agency (IAEA) standards N1 and N2 (ammonium sulfate) for nitrogen, and EIL-72 (cellulose) for carbon. Replicate runs were inserted every 10 samples to determine between-run variation. The relative standard deviation was ±0.13 for δ^{13}C and ±0.21 for δ^{15}N. The relative analytical precision of the analysis is ±0.2‰ for carbon and ±0.3 ‰ for nitrogen. This error can be expected to increase depending on the homogeneity, type and amount of sample used in each analysis. Since exposure to hydrochloric acid may affect the N values measured (Carabel *et al.*, 2006), the acidified δ^{13}C value and the non-acidified δ^{15}N were used in subsequent analyses.

Statistical analysis

Diet and environmental data collected within a given month were averaged between habitats and sampling dates to achieve a representative monthly value of the ambient conditions (diet, abiotic parameters, haplochromine density, invertebrate abundance). Principle Component Analysis (PCA) was used to condense the data set into composite variables first for the physico-chemical data: parameters included were morning DO, afternoon DO, morning temperature, afternoon temperature, monthly rainfall and Secchi depth. The percentage fish, mollusks, zooplankton, plants and insects in the diet of *A. alluaudi* were grouped using a second PCA into a composite diet measure. Finally, the relative abundance of insects and mollusks, and their richness per month were combined into a third prey availability principle component. The proportion of *A. alluaudi* to other cichlids was also compared among months, and a Pearson correlation matrix was used to detect relationships among the PCA scores.

Linear regression analysis was used to test for a relationship between fish diet and rainfall. The running rainfall average rather than the monthly average was used in the analysis since water accumulation is continuous: therefore trends observed at one point in time may reflect a response to the rainfall one or two months prior. A two-month running rainfall average was calculated from the average rainfall during the month of interest and the previous.

To estimate the proportional contribution of each prey to the isotopic signature of *A. alluaudi*, the computer-based mixing model, IsoSource version 1.3 (Phillips and Gregg, 2003; Phillips *et al.*, 2005) was used. This program generates all possible combinations of each source contribution to an isotopic mixture from 0-100% within a given tolerance range. The stable ^{13}C and ^{15}N data obtained from isotope analysis were used to generate maximum and minimum contributions from a range of prey items with similar isotopic signatures to that of the predicted diet of *A. alluaudi* assuming carbon fractionation rates of 1‰ and nitrogen fractionation rates of 3.6‰ between trophic levels.

Results

Physico-chemical parameters and invertebrate abundances

Dissolved oxygen (DO) showed fluctuations between months: morning DO ranged from 4.56 mg l^{-1} in December 2007 to 9.15 mg l^{-1} in May 2007 (Table I). Afternoon levels experienced similar fluctuations throughout the year ranging from 8.95 mg l^{-1} in January 2008 to 13.44 mg l^{-1} in June 2007. Fluctuations in water temperature were less marked throughout the year. Afternoon temperatures varied between 22.08 °C in December 2007 and 25.29 °C in June 2008. Morning temperatures varied between 20.23 °C in January 3008 and 23.80 °C in May 2007. Secchi depth averaged 27.58 cm, but ranged from 23.38 cm in May 2007 to 33.68 cm in December 2007. Rainfall fluctuated throughout the 30-month sampling period with the wettest months occurring from September to November and March and April (Table I).

Mollusks made up the largest proportion of invertebrates sampled between June 2007 and May 2008 accounting for 55.5 - 95% of the prey base in a given month. Insects from 7 different orders (Diptera, Coleoptera, Odonata, Emphemeroptera, Trichoptera, Hemiptera, Megaloptera) were collected in the dredge samples; the most common were Diptera, Odonata, Ephemeroptera and Hemiptera. Other invertebrates including Acariformes and Hirudinea were encountered rarely in the dredge samples. The relative abundance of invertebrates sampled each month varied considerably throughout the year (Table II). June 2007 had the highest abundance with an average of 224 invertebrate prey items collected per sampling day. April 2008 had the fewest number of invertebrates sampled with only 25 prey items collected per sampling day.

The proportion of *A. alluaudi* relative to other haplochromine cichlids varied among months; however, on average, *A. alluaudi* comprised only 6.8% of the cichlid fauna sampled ranging from 1.4% in March 2008 to 20% in October 2007 (Table II).

Stomach content analysis

Of the 357 *A. alluaudi* collected during this study, 149 had non-empty stomachs. An additional 133 stomachs collected between January and December 2006 (Cosandey-Godin *et al.*, 2008) were also included in the analysis bringing the total number of non-empty stomachs analyzed to 282. *Astatoreochromis alluaudi* fed on a wide range of food types. Insects were consumed in all months, as were fish and plants, although the relative importance of each prey item varied considerably (Figure 1). The percent importance of insects ranged from 2.8% in March 2008 to 43% in August 2006. Fish ranged in importance from 3.3% in May 2007 to 74% in September 2006. Mollusk remains were found in fish in 15 of the 27 months used in the analysis; however, they never formed more than 33% of the diet in any month. Overall, mollusks formed approximately 5% of the diet of *A. alluaudi* during the sampling period (Figure 2).

Isotope analysis

A total of six fish (43.22 – 55.72 mm standard length) were used for isotope analysis. Based on the average ^{13}C and ^{15}N signatures of the fish collected (d^{13}C= -18.18 ± 0.22 ‰, d^{15}N= 5.46 ± 0.16 ‰), the isotopic range of *A. alluaudi*'s diet was estimated to be approximately -19.18 for ^{13}C and 1.86 ‰ for ^{15}N assuming isotopic fractionation of 3.6‰ for nitrogen and 1‰ for carbon (Campbell *et al.*, 2006). These values most closely correspond to the following insect prey items: Diptera, Anisoptera, Zygoptera, and Coleoptera (Figure 3). Nitrogen signatures of *A. alluaudi*, which relate to the trophic position of an organism, were as expected of fish feeding one trophic level down with a 3-4 ‰ difference between predator and prey. Based on the carbon isotopic signature of *A. alluaudi*, alternative prey such as mollusks and cichlid broods do not appear to be important food sources for growth. Mollusks and late-stage broods had a heavier ^{13}C /^{12}C signature than *A. alluaudi,* (-17.46‰, and -17.48‰ respectively), whereas yolk-sac brood signatures were lighter (-20.19‰). The vegetation and Plecoptera signals diverged greatly from that expected of *A. alluaudi* prey items; vegetation was highly depleted in carbon and nitrogen (d^{13}C= -27.42‰, d^{15}N= -1.04), and Plecoptera was highly enriched in both isotopes (d^{13}C= -14.96‰, d^{15}N= 5.16) suggesting that neither potential prey item contributes to the growth of *A. alluaudi.*

IsoSource (Phillips and Gregg, 2003; Phillips *et al.*, 2005), is a useful tool for generating probable combinations of isotopic sources contributing to a mixture, and was used to compare the isotope and diet data. Due to the large number of potential sources being tested, the signatures of several insect prey (Zygoptera, Anisoptera, Ephemeroptera, Coleoptera, Diptera) that had similar isotopic values were combined (Phillips *et al.*, 2005). Insects, mollusks, late-stage brood, and yolk-sac brood were included in this analysis. Isosource generated 23 potential combinations of the source signatures to the mixture signal representing the diet of *A. alluaudi* (Table III). Insects appear to constitute an important part of the diet, (1-99th percentile; 34-72%) whereas mollusks contribute less to the overall prey signal (0-26%). Yolk-sac broods could potentially contribute between 15 % and 40 % to the diet of *A. alluaudi* (mean 30.3%) whereas late-stage brood ranged from between 0% and 13% of the diet (mean 0.05%).

Data ordination using PCA

The first principle component analysis on physico-chemical parameters generated three functions with eigenvalues above 1.0, which cumulatively explained 78.31% of the variance (Table IV). The second PCA on diet measures extracted one component and explained 62.41% of the variance. Similarly, one component was extracted from the third macro-invertebrate abundance PCA and explained 72.95% of the variance.

Six environmental and diet variables (three composite physico-chemical variables, one composite diet variable, one composite macro-invertebrate variable, and the proportion of *A. alluaudi* to haplochromine cichlids) were compared using Pearson correlation to look for significant trends explaining the patterns of variation observed. There was a significant correlation between diet and the second physico-chemical principle component (r=0.634, p=0.03), however, no other significant relationships were found. Although six variables were being compared using this data set, we did not control for multiple comparisons to minimize the risk of rejecting a significant, biologically meaningful relationship between diet and the physico-chemical environment due to conservative correction procedures (Garcia, 2004). Instead, the composite diet and physico-chemical variables were further examined in order to understand which components were responsible for driving this relationship. The diet principle component related strongly to all variables except insects, but fish weighed most heavily on this trait

(-0.951). The second physico-chemical component related strongly to total rainfall (-0.694). This relationship was explored in more depth.

Linear regression was used to detect a relationship between rainfall and the degree of piscivory in *A. alluaudi* as indicated by the percentage of fish consumed. We used rainfall data collected daily from December 2005 to May 2007, and diet data from January 2006. There was a significant relationship between the running rainfall average and the percentage piscivory observed in *A. alluaudi* (R^2= 0.24, p=0.013).

Discussion

Isotopic confirmation of Liem's Paradox

Stable isotope analysis is increasingly used as an ecological tool to corroborate dietary studies and describe food web interactions. By providing a time-averaged measure of the food sources assimilated by an organism, stable isotopes make up for some of the shortfalls of traditional stomach content analysis, which provide only a snapshot of diet at a given time (Peterson and Fry, 1987). When isotope analysis is used in conjunction with stomach contents sampled at regular intervals, an integrative picture of an organism's diet can be obtained.

More than 350 fish stomachs collected over 30 months were analyzed in this study to characterize the diet of *A. alluaudi* and determine whether or not mollusks are consumed regularly. Results indicated that mollusks compose only a small proportion of the diet of *A. alluaudi* from Lake Saka, which is not consistent with the hypertrophied pharyngeal jaws characteristic of this population (Chapter 1). In addition to extensive sampling of *A. alluaudi* stomachs over an entire seasonal cycle, stable isotope analysis was used to assess the importance of mollusks in *A. alluaudi*'s diet. Results from this analysis suggest that insects comprise the most important component of *A. alluaudi's* diet, with mollusks and fish contributing only marginally.

It is important to note, however, that the laboratory and mixing model (IsoSource) results in this study provide an estimate of the contribution of isotopes inputted, but do not identify unknown prey items, or items missing from the analysis. Therefore, IsoSource model results are not an absolute measure of diet, but rather a means of comparing the relative contribution of the sources included in the analysis. Additionally, the accuracy of isotope analysis is dependent upon the sources of prey collected. For instance, IsoSource and isotope results did not reveal fish remains as an important component of *A. alluaudi's* diet, although stomach contents suggest otherwise. This discrepancy may have resulted from the source of fish used in the analysis: only fish fry restricted to the buccal cavity of their mothers were sampled, whereas fish present in *A. alluaudi* stomachs may be juveniles feeding exogenously in the water column. These juveniles would likely have a different isotopic signature compared to late-stage brood feeding off of a yolk sack. The difference in the isotopic signature between the yolk-sac and late-stage brood (2.71 for ^{13}C, 1.07 for ^{15}N) clearly illustrates the discrepancy in signatures that might result from sampling fish prey at different life-stages.

Regardless of these caveats, the results from the isotope analysis and IsoSource model agree with the stomach data in showing that mollusks do not contribute significantly to the overall diet of *A. alluaudi*. Even in the most extreme example computed by the IsoSource model, *A. alluaudi*'s diet comprised a maximum of only 26% of mollusks. Similarly, the greatest percentage of mollusks found in the stomachs of *A. alluaudi* throughout the study was 33% (November 2006). On average, stomach content analysis revealed that approximately 5% of the diet comprised mollusks over the 30 months sampled. Although IsoSource averages were somewhat higher (15.7%), both analyses strongly suggest that mollusks are not the dominant food source consumed by *A. alluaudi* in Lake Saka in any given month. These findings are consistent with the predictions of Liem's Paradox, and provide evidence that a morphologically-specialized fish regularly behaves as a generalist consumer in nature.

Plants and detritus were regularly found in the stomachs of *A. alluaudi*, sometimes in great abundance. However, the isotopic signature from the vegetation collected suggests that plants do not contribute to the assimilated carbon or nitrogen in the tissues of *A. alluaudi*. It is possible that these materials are ingested incidentally rather than purposely by *A. alluaudi* as a consequence of feeding on the muddy lake bottom, or along the densely vegetated shoreline. However, the ubiquity and quantity of both plant and detritus consumed suggests that *A. alluaudi* is potentially feeding on these resources in addition to other food items.

Alternatively, plant material and detritus may be difficult to digest without the appropriate alimentary tract. Most herbivorous fish possess elongated intestines relative to omnivorous or carnivorous fish of a comparable size in order to increase the efficiency of digestion (Kramer and Bryant, 1995). A fish lacking the digestive enzymes and system to efficiently process plant material may retain these items in their stomach for longer than easily digestible material such as zooplankton or other small, soft-bodied prey (Gannon, 1976; Hyslop, 1980). As a result, the amount of plants and detritus ingested by *A. alluaudi* may be overrepresented in our study, an important shortcoming of stomach content analysis (Appendix 2).

Environmental fluctuations and seasonality in diet

While there was some variation in all the physico-chemical parameters throughout the sampling year, rainfall was the only significant predictor of diet shifts in *A. alluaudi*. Equatorial regions of East Africa are typically characterized by two wet (September to November, March and April) and two dry (December to February and May to August) seasons, with seasonal flooding occurring during periods of heavy rain. Since flooding increases the available habitat for spawning, some species of both riverine and lacustrine fishes experience peaks in reproduction during the rainy months (Munro, 1990; Reardon and Chapman, 2008) leading to increased spawning activity and abundance of fish fry (Kramer, 1978; Chapman and Frankl, 2000). This phenomenon may explain the positive relationship between the running rainfall average and the percentage of fish consumed by *A. alluaudi*. It is possible that *A. alluaudi* feeds opportunistically on abundant fish prey when readily available, but switches to an alternative diet when this resource becomes scarce.

When feeding on juvenile fish becomes inefficient, *A. alluaudi* may switch its feeding strategy to exploit bottom-dwelling macro-invertebrates such as insects and mollusks. Although *Bulinus* and *Biomphalaria* snails are pulmonates (air breathers) they typically associate with shallow muddy bottoms rich in organic detritus (Dillon Jr, 2000), similar habitat to that of aquatic insect larvae (McCafferty, 1981). The relationship between the percentage fish and the percentage of mollusks and insects found in the stomach of *A. alluaudi* was marginally significant ($r = -0.39$, $p = 0.054$), suggesting the possibility of a tradeoff between insect/mollusk feeding, and piscivory.

Astatoreochromis alluaudi is known to feed on insects at sites in East Africa (Mbabazi *et al.*, 2004, Chapter 1), and has been shown to prefer this prey over snails in laboratory prey-choice experiments (Slootweg *et al.*, 1994). However, the availability and consumption of fish prey may be a significant driver of diet choice for *A. alluaudi* in Lake Saka. Piscivory has rarely been recorded in this species (but see Binning and Chapman, 2008); however, results from this study are consistent with Liem's Paradox in that prey requiring specialized morphology (mollusks) should be consumed at times of resource scarcity, or when preferred prey items (insects/fish) are unavailable. Since fish form the most abundant prey group consumed, (37% of the overall diet of *A. alluaudi)*, it is presumed that fish are the preferred prey of this population. During months with less rainfall, *A. alluaudi* appears to switch its prey base to feed on mollusks and insects. Although some fluctuations in the relative abundance of macro-invertebrate prey were observed, no relationships between macro-invertebrate abundance and the contribution of this prey type to the diet of *A. alluaudi* were detected. Benthic macro-invertebrate resources may be sufficiently available at all times such that fluctuations in their abundance do not affect their rate of consumption by *A. alluaudi*.

If insect resources are not limiting in Lake Saka, fluctuations in the abundance of other haplochromine cichlids should also have no effect on diet since potential competitive interactions would be weakened. Stomach content analysis of haplochromines captured in the same habitat as *A. alluaudi* in Lake Saka revealed a diet consisting primarily of plant material and detritus, with modest amounts of fish and insects consumed during the three months sampled (fish= 21 %, insects= 8 %; Appendix 1). Assuming that this diet is representative of the entire year, dietary overlap does not appear to be high between *A. alluaudi* and other haplochromines. While competitive interactions between these taxa cannot be ruled out, there is no evidence suggesting that shifts in the abundance of haplochromines correlates with dietary shifts in *A. alluaudi.*

Conclusions

Despite a molariform trophic morphology characteristic of specialized mollusk-crushers, *A. alluaudi* in Lake Saka rarely consumes mollusks, and routinely feeds on a variety of prey types including fish, insects and zooplankton throughout the year. This type of ecomorphological mismatch supports Liem's Paradox - tight couplings between ecology and morphology are not ubiquitous in nature. Rainfall was a significant predictor of the percent piscivory in this cichlid's diet. However, fish did not appear to contribute extensively to the isotopic signature of *A. alluaudi*. Future studies should explore the importance of piscivory and paedophagy, a feeding strategy never before recorded in *A. alluaudi*, to its overall diet. Specialized molluscivore morphology in *A. alluaudi* appears to be maintained by low levels of snail consumption throughout the year, and therefore does not reflect the preferred prey, nor the most frequently consumed prey of this species.

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Table I: Average measures of physico-chemical variables (with standard error) from *A. alluaudi* habitat in Lake Saka, Uganda between May 2007 and June 2008. Rainfall data were collected at the Makerere University Biological Field Station, approximately 35 km from Lake Saka.

	AM dissolved oxygen (mgl^{-1})	PM dissolved oxygen (mgl^{-1})	AM Temperature (ºC)	PM Temperature (ºC)	Secchi Depth (cm)	Total rainfall (mm)
May-07	9.15 ± 0.16	11.77 ± 1.61	23.80 ± 0.03	25.41 ± 0.51	23.38 ± 0.88	61.6
Jun-07	8.87 ± 0.84	13.44 ± 1.51	22.49 ± 0.04	24.29 ± 0.28	31.30 ± 1.70	144
Jul-07	5.31 ± 0.49	10.96 ± 0.86	22.01 ± 0.05	23.31 ± 0.22	29.3 ± 0.73	47.3
Aug-07	4.68 ± 0.56	11.63 ± 0.83	21.63 ± 0.07	22.44 ± 0.19	24.09 ± 0.71	95.9
Sep-07	5.54 ± 0.17	9.77 ± 1.15	22.16 ± 0.06	23.43 ± 0.24	26.05 ± 0.42	166.2
Oct-07	5.32 ± 0.32	10.65 ± 0.97	21.94 ± 0.03	23.40 ± 0.27	25.58 ± 0.61	269.5
Nov-07	6.51 ± 0.48	10.86 ± 0.92	20.86 ± 0.15	24.17 ± 0.35	28.66 ± 0.49	213.4
Dec-07	4.58 ± 0.06	7.87 ± 0.19	21.61 ± 0.06	22.08 ± 0.08	33.68 ± 0.73	56.2
Jan-08	5.58 ± 0.44	8.95 ± 1.06	20.23 ± 0.13	24.31 ± 0.31	25.00 ± 1.37	70.34
Feb-08	5.54 ± 0.15	8.76 ± 0.50	21.90 ± 0.06	22.90 ± 0.22	29.50 ± 3.24	47.3
Mar-08	6.38 ± 0.52	9.91 ± 0.13	23.15 ± 0.07	25.39 ± 0.48	26.36 ± 0.76	224.9
Apr-08	8.03 ± 0.45	11.31 ± 1.23	23.42 ± 0.37	24.65 ± 0.16	29.75 ± 1.27	114.8
May-08	7.33 ± 0.53	12.65 ± 0.78	23.30 ± 0.07	24.75 ± 0.15	27.38 ± 1.40	148.1
Jun-08	5.07 ± 0.54	8.96 ± 2.03	23.40 ± 0.08	25.29 ± 0.74	26.13 ± 0.59	N/A

Table II: Point in time index of macro-invertebrate abundances and the proportion of *A. alluaudi* caught relative to haplochromine cichlids. Macro-invertebrate abundances are represented as the average number of individuals caught per sampling day per month. Percentage *A. alluaudi* is calculated as a percentage of all other cichlid species caught in similar habitat in Lake Saka.

	Percentage *A. alluaudi*	Mollusk index of abundance	Insect index of abundance
May-07	0.02	N/A	N/A
Jun-07	0.03	175	48.5
Jul-07	0.07	120.5	44.5
Aug-07	0.09	37.5	29.5
Sep-07	0.06	135	18.5
Oct-07	0.20	147	21
Nov-07	0.08	102.5	18
Dec-07	0.11	N/A	N/A
Jan-08	0.06	47	24
Feb-08	0.08	106	5
Mar-08	0.01	48.5	16.5
Apr-08	0.02	19	5.57
May-08	0.09	120	20
Jun-08	0.03	N/A	N/A

Table III: Mean, minimum and maximum proportional contributions of four prey sources to the diet of *A. alluaudi* as generated by the IsoSource mixing model. Insects included: Diptera, Odonata, Ephemeroptera and Coleoptera.

	Insects	Mollusks	Yolk-sac brood	Late-stage brood
Mean	0.49	0.157	0.303	0.05
Minimum	0.34	0	0.15	0
Maximum	0.72	0.26	0.4	0.13
Standard deviation	0.114	0.078	0.076	0.04

Table IV: Principle Component Analysis scores for environmental measures and diet. Numbers in **bold** represent variables loading heavily on each component (>0.60). Six variables were condensed into three principle components for the physico-chemical environment, five variables condensed into one component for diet, and two traits condensed into two principle components for macroinvertebrate abundances for eigenvalues greater than 1.0.

Physico-chemical	Eigenvalue	% variance	Morning DO	Afternoon DO	Morning Temperature	Afternoon Temperature	Secchi Depth	Total rainfall
PC1	2.433	40.55	**0.884**	**0.684**	**0.774**	**0.608**	-0.323	0.330
PC2	1.212	20.20	0.353	-0.235	0.303	0.099	**0.670**	**-0.694**
PC3	1.054	17.56	-0.033	**0.617**	0.199	**-0.764**	0.218	-0.037

Diet	Eigenvalue	% variance	% mollusks	% fish	% insects	% plants	% zooplankton
PC1	3.120	62.41	**0.753**	**-0.951**	0.419	**0.878**	**0.839**

Macroinvertebrate Abundance	Eigenvalue	% variance	Mollusks	Insects
PC1	1.459	72.95	**0.854**	**0.854**

Figure 1: Percent importance of major food categories in the diet of *A. alluaudi* sampled across 30 months between January 2006 and June 2008 (N= number of non-empty stomachs per month). The insect category includes the following orders: Diptera, Coleoptera, Ephemeroptera, Odonata, Hemiptera, Trichoptera and unidentified insects. The other category includes Nematoda, Acariformes and Hirudinea.

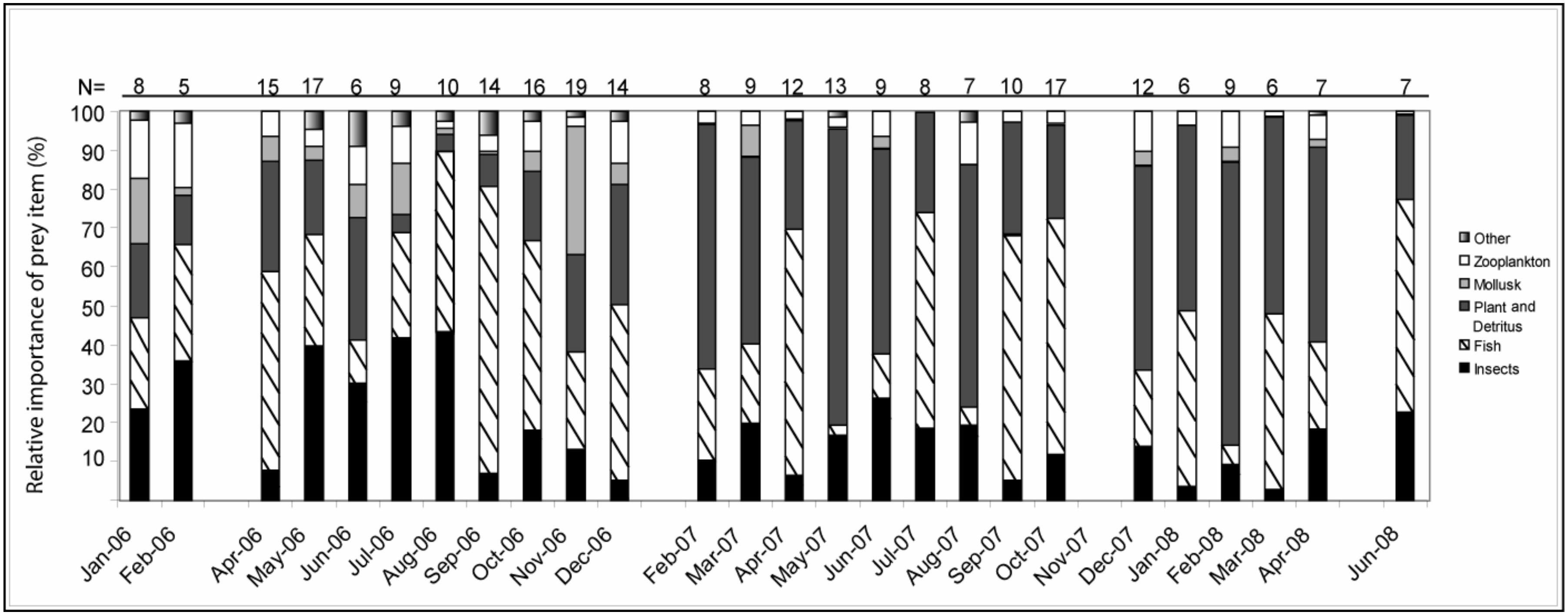


Figure 2: Percent importance of major food categories in Lake Saka averaged over 30 months. The insect category includes Diptera, Coleoptera, Ephemeroptera, Odonata, Hemiptera, Trichoptera and unidentified insects. The "other" category includes Nematoda, Acariformes, and Hirudinea.

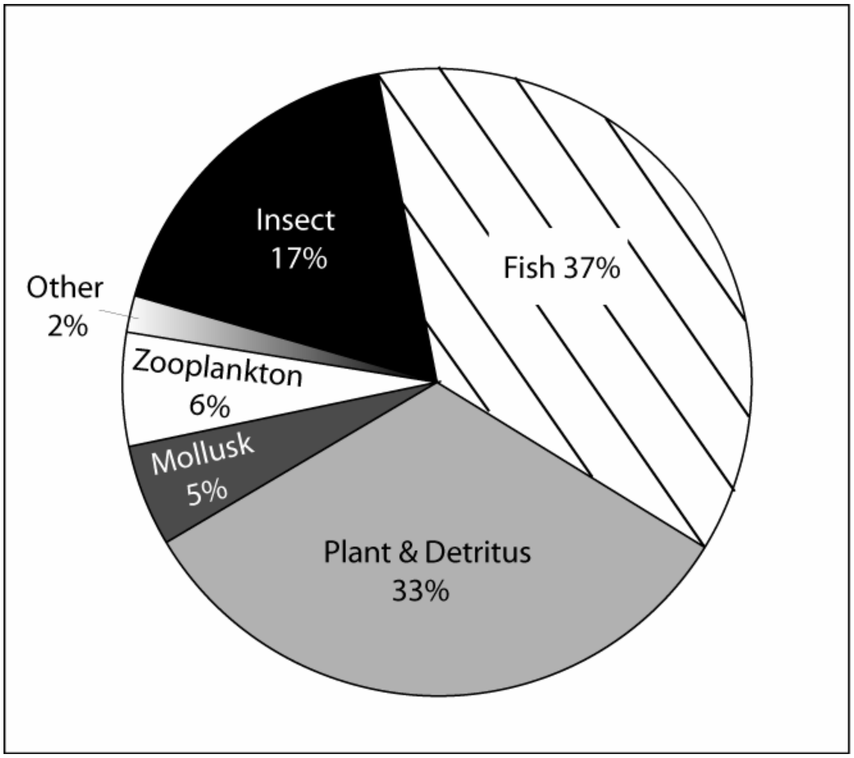
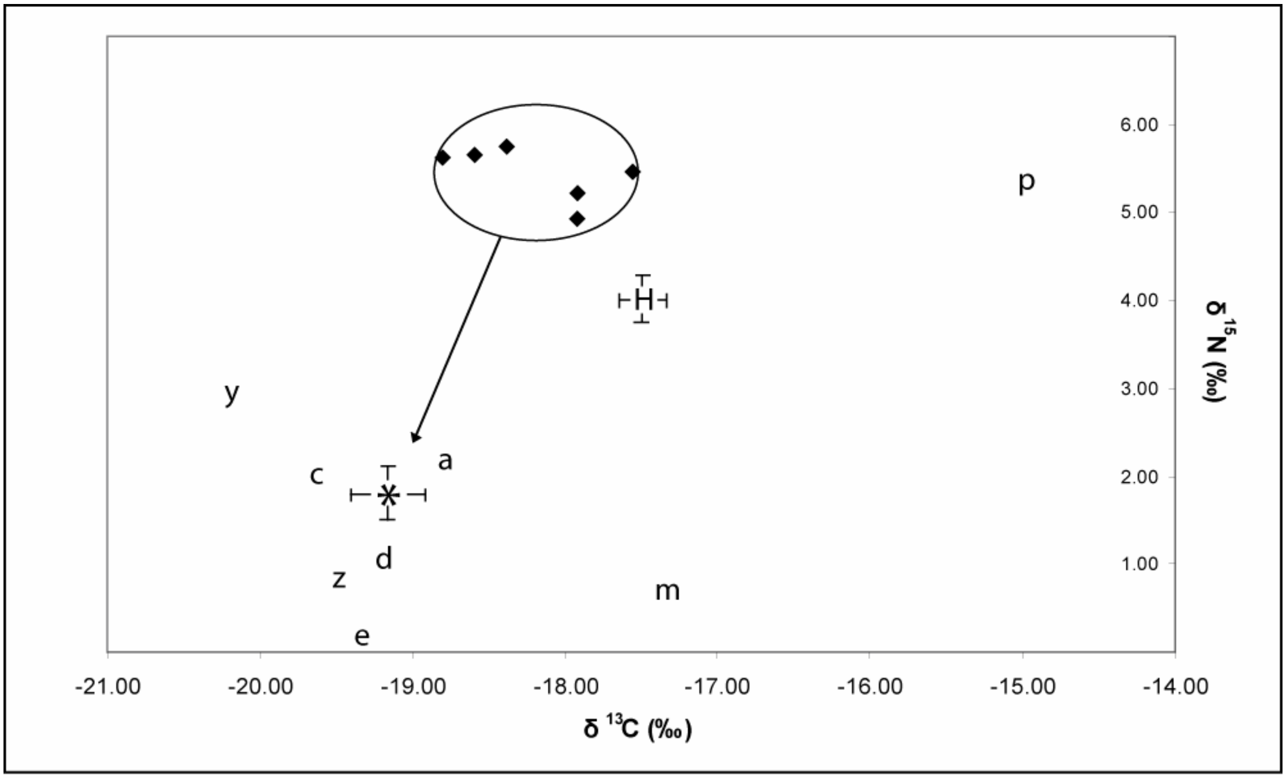


Figure 3: The relationship between $\delta^{15}N$ (trophic position) and $\delta^{13}C$ (dietary carbon) for *A. alluaudi* (diamonds surrounded by ellipse) and potential prey items in Lake Saka (y= yolk sack cichlids, c= Coleopteran, z= Zygoptera, a= Anisoptera, e= Ephemeroptera, d= Diptera, m= mollusks, p= Plecoptera, H= late-stage cichlid brood). The asterisk (*) represents the estimated isotopic range of *A. alluaudi*'s diet.



General Conclusions

Although cichlids are among the best studied families of freshwater fishes in the world, much remains unknown about their ecology. Trophic morphology is commonly used to distinguish species, and infer ecological functionality in this group. However, the results of this thesis on diet-morphology relationships in *A. alluaudi* suggest that caution be used when inferring an organisms' diet based solely on morphology, and highlight the importance of combining dietary analyses with morphological studies in order to fully understand the feeding ecology of a species. Liem's Paradox has been documented in other natural populations of freshwater fish (Bouton *et al.*, 1998; Genner *et al.*, 1999); however, few studies have included across-population surveys and isotope analysis of fish tissues as a basis for diet comparison between observed morphology and stomach contents. This study is unique in using novel techniques for verifying Liem's Paradox operating in nature, as well as evaluating the effect of multiple ecological and environmental factors on diet choice over a temporal and spatial scale. The integration of these different techniques has revealed a case of Liem's Paradox in the morphologically specialized cichlid, *A. alluaudi*, and highlights the possibility of seasonal changes in rainfall patterns acting as an important driver of fluctuations in resource consumption over time. Although trophic specialization and niche segregation are widely accepted as mechanisms for diversification in the cichlid species flock, the results from this study suggest that some contemporary species are more flexible in their resource use than their morphology implies, and that divergence among widespread species may not be driven by competition for resources.

From a practical perspective, it can be risky to infer the ecology of a species based on morphology. Although preventing the spread of harmful pathogens is a cost-effective way of managing diseases in developing countries (Fenwick, 2006), biological control of parasites and parasite hosts is a controversial solution to disease spread that has been implemented with mixed results (Slootweg *et al.*, 1994). Often, species are not adequately studied prior to introduction into a new range, and the interaction between the two organisms does not result in the appropriate control (Kat and Kibberenge, 1990). Although laboratory experiments suggest that *A. alluaudi* is an effective feeder of schistosoma-carrying snails (Slootweg, 1987), field studies have proven otherwise (Kat and Kibberenge, 1990). The results from both our spatial and temporal survey of *A. alluaudi* in its native range suggest that factors underlying diet choice in this cichlid are complex, and that very few populations reliably consume mollusks. Biological control initiatives against schistosomiasis should not rule out the possibility of using fish as a natural enemy, but rather should be wary of generalizing the ecology and diet of a species in one site across divergent habitats.

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Appendix 1: Analysis of the stomach contents of the haplochromine cichlids in lakes and rivers of southern Uganda

Although cichlids of the haplochromine lineage are often cited as the primary competitors of *Astatoreochromis alluaudi* (Kat and Kibberenge, 1990; Slootweg *et al.*, 1994), little research has been done to either quantify the diet of these two groups in their area of sympatry or to record the degree of resource overlap shared by them (but see Binning and Chapman, 2008). This realizaton is surprising given that competition from haplochromine cichlids is among the primary explanations for the observed polymorphism in the pharyngeal jaw apparatus of *A. alluaudi*. It is believed that in the absence of competition from morphologically specialized insectivorous cichlids, *A. alluaudi* will preferentially switch its diet from mollusks to insects, and develop less hypertrophied pharyngeal jaws in subsequent generations (Slootweg *et al.*, 1993; Slootweg *et al.*, 1994). This explanation for why some populations of *A. alluaudi* do not feed on snails even when these resources are abundant was most notably used to explain the failure of *A. alluaudi* to provide effective biological control for schistosomiasis-hosting snails in various regions of East Africa (Kat and Kibberenge, 1990; Slootweg *et al.*, 1994). Despite several studies underlining the potential of *A. alluaudi* to successfully control snail densities in affected water sources (De Bondt, 1956; Bard and Mvogo, 1963; Mvogo and Bard, 1964; McMahon *et al.*, 1977; Slootweg, 1989), the long-term results of such introductions documented a switch in diet of *A. alluaudi* to an insect prey base that was attributed, at least in part, to the lack of an intact cichlid fish fauna in these water bodies, and therefore a lack of competitors for soft-bodied prey (Kat and Kibberenge, 1990).

Although competition between *A. alluaudi* and other haplochromine cichlids seems widely accepted in the literature as a mechanism explaining diet choice and polymorphism in this species, there are few studies documenting food resource overlap in communities that host both *A. alluaudi* and high densities of haplochromine cichlids (Hoogerhoud, 1986). Quantifying the diet of *A. alluaudi* is of particular interest in populations persisting in systems densely populated with other cichlids, but where mollusk prey are rare (pers. observations, Efitre *et al.*, 2001). This appendix provides much needed background information regarding the diets of haplochromine cichlids from the 6 sites sampled in Chapter 1 (Lake Victoria, Lake Saka, Lake Nabugabo, Kahunge, Kamwenge, Rwebakwata) as well as comparisons with the diets of *A. alluaudi* coexisting within each site. We present a measure of dietary overlap between *A. alluaudi* and co-occurring haplochromine cichlids (i.e. cichlids captured at the same time and location as *A. alluaudi*, and with the same gear). The major limitation of the study is that many of the haplochromines are unidentified species and were grouped together as "haplochromines", so it is probable that more than one species is represented in the lake sites, but likely only 1 species in the river sites (see Results below).

Methods

Haplochromine cichlids were caught in gill nets and baited minnow traps and were processed according to the procedure as documented for *A. alluaudi* (for details refer to Chapter 1, pg. 32). Stomach content analysis was carried out in the same manner as that of *A. alluaudi*, and analyzed in the same way to facilitate measures of dietary overlap. In addition, the frequency of occurrence (FOC) of all food items was also noted for each site and was estimated as a percentage of the number of non-empty stomachs in a sample containing a particular food item (Hyslop, 1980). Dietary similarity between *A. alluaudi* and the haplochromine cichlids within each site was calculated using the Percent Similarity Index (Schoener, 1970; Buckley *et al.*, 1999, see Chapter 1 pg. 37)

Results and Discussion

The haplochromine cichlid lineage contains a diverse array of species, some of which have diverged extensively within the Great Lakes of East Africa (Lowe-McConnell, 1996; Kaufman *et al.*, 1997). Although detailed taxonomic descriptions of the cichlid fauna have been carried out in well-studied lakes such as Malawi, Victoria and Tanganika (Fryer and Iles, 1972; Lowe-McConnell, 1993; Barlow, 2000), little information exists on the species diversity or taxonomy of many smaller lake and river systems in East Africa. Because many of the sites we sampled were home to a number of undescribed species, we treated all haplochromine cichlids captured as one group, and did not try to distinguish between species. In the river sites, we likely sampled 1 species; however, more than 1 species comprised the samples from the lake sites.

A total of 217 fish were collected from all six sites sampled (Lake Victoria N=33, Lake Nabugabo N=44, Lake Saka N=45, Kamwenge N=47, Kahunge N= 24, Rwebakwata N=24). Of these fish, 127 had non-empty stomachs (Lake Victoria N=16, Lake Nabugabo N=22, Lake Saka N=30, Kamwenge N=22, Kahunge N=22, Rwebakwata N=14).

There was considerable variation in the diet of the haplochromine cichlids both among and within sites (Figure 1). However, Lake Nabugabo fish exhibited a narrower diet breath with zooplankton dominating the diet (74%). Lake Nabugabo is home to 5 haplochromine cichlids endemic to the Nabugabo region (Greenwood, 1965; Ogutu-Ohwayo, 1993); however, only three of these species are currently abundant in the lake, and two persist in *A. alluaudi* habitat (*Haplochromis annectidens* and *Astatotilapia velifer*, L.J. Chapman unpubl. data). Zooplankton were present in the stomachs of 77% of the haplochromines sampled in Lake Nabugabo (Table 1), therefore it is reasonable to assume that the cichlids co-occuring in habitat with *A. alluaudi* at this site are predominantly zooplanktivores. However, one must keep in mind that our survey was a point-in-time exploration of diet at these sites.

In Lake Victoria, it is likely that our sample of haplochromine cichlids was comprised of more than one species given that several species can still be found in Napoleon Gulf, our focal collection site in Lake Victoria. There was little overlap between the diets of *A. alluaudi* and haplochromine cichlids (Table 2) in Lake Victoria (12.6%) and only moderate overlap in Lake Nabugabo (35.8%). This low overlap is a result of niche specialization by either *A. alluaudi* or the haplochromine cichlids at each site. For example, *A. alluaudi* has a narrow diet breath in Lake Victoria feeding primarily on mollusks (59%, see Chapter 1, Figure 4) whereas the haplochromine cichlids sampled consume a wider range of food items including plant material, detritus, insects and shrimp (Figure 1). In Lake Nabugabo, however, it is the haplochromine cichlids that exhibit a more specialized diet of zooplankton, while *A. alluaudi* consumes a wide range of food items including fish, plants, detritus, insects and small quantities of other prey types including zooplankton (see Chapter 1 Figure 4, pg.64).

The three river sites and Lake Saka had much higher level of diet overlap between haplochromine cichlids and *A. alluaudi.* At the Kamwenge site, these two groups showed 64.1% dietary similarity, or moderate overlap while at Kahunge and Rwebakwata, haplochromines and *A. alluaudi* overlapped by 76.4% and 77.1% respectively. Lake Saka had the highest level of overlap of all the sites sampled; 83.5% of the diet of *A. alluaudi* and haplochromine cichlids is shared between the two groups. At these 4 sites, the diet of both fish groups consisted mainly of fish, detritus, plants and insects, with only Kamwenge fish found to consume mollusks (Figure 1; Chapter 1, Figure 4). It is surprising that the high levels of overlap at these sites have not resulted in a shift to molluscivory in *A. alluaudi*.

With the exception of fish from Lake Nabugabo, most individuals had 2 or more prey types (insects, plants or fish) in their stomachs when they were caught indicating possible omnivory. Insects never formed the majority of the stomach contents in any population of haplochromine cichlid sampled, and at only 1 site, insects were the dominant prey type encountered (Rwebakwata, 34%).

These data provide evidence to suggest that overlap in food resource use is minimal between *A. alluaudi* and co-occurring haplochromines in lakes Nabugabo and Victoria (Napoleon Gulf). This diet segregation may be a result of the niche partitioning driven by competition in the past, or reflect the co-occurrence of species pre-adapted to a different prey base. Conversely, dietary overlap in between *A. alluaudi* and co-occurring haplochromine cichlids was relatively high in the three river sites and in Lake Saka. Competition for resources at these sites may not be severe enough to select for niche partitioning, or there may be seasonal fluctuations in resource abundance that result in different levels of overlap at different times of the year depending on food availability.

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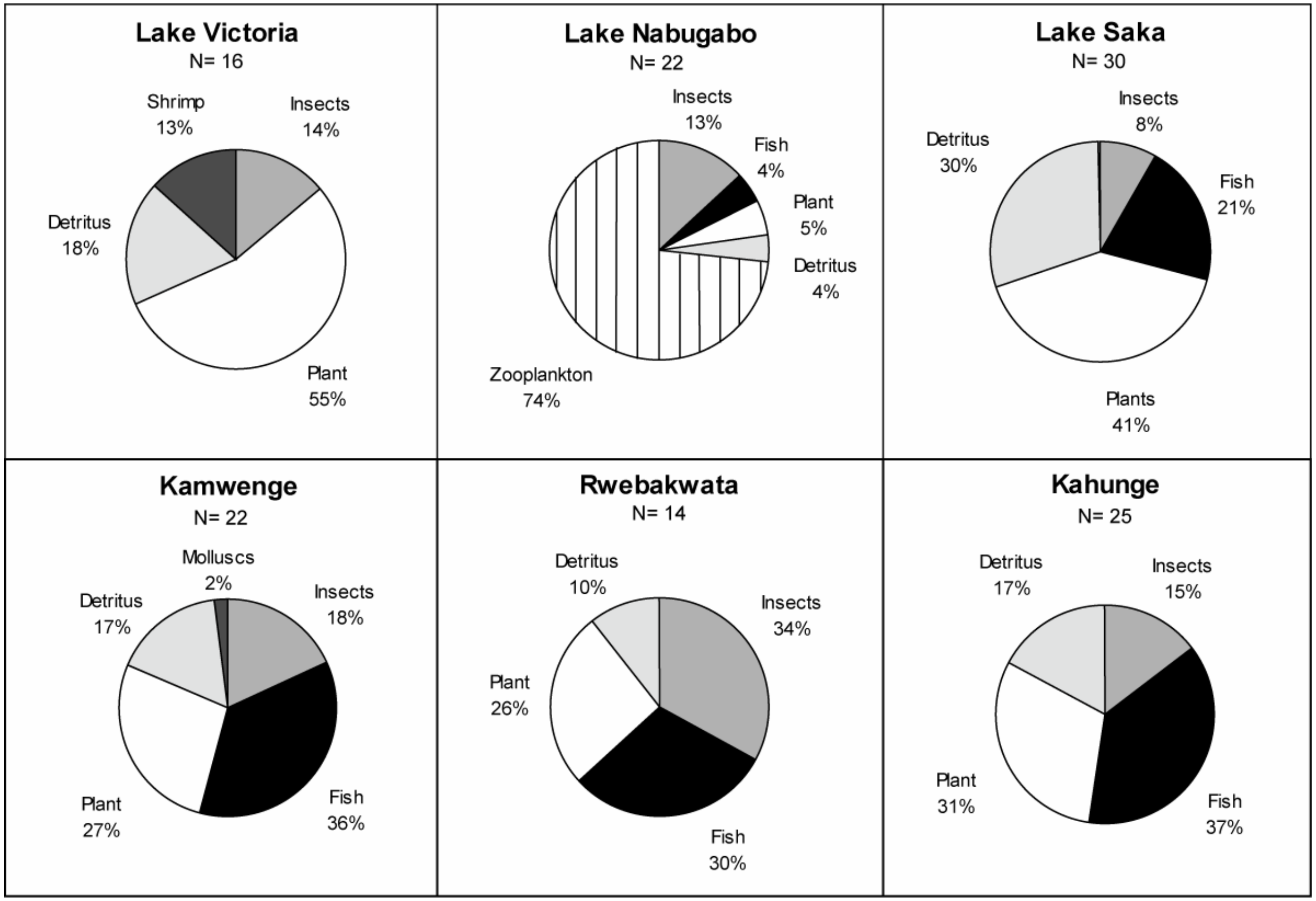
Table 1: Frequency of occurrence (FOC) of major prey items in the diet of haplochromine cichlids co-occurring with *Astatoreochromis alluaudi* from 6 sites in southern Uganda. FOC is represented as percentages of the number of non-empty stomachs within each site.

Prey Item	**Lake Victoria**	**Lake Nabugabo**	**Lake Saka**	**Kahunge**	**Kamwenge**	**Rwebakwata**
Insects	0.56	0.18	0.20	0.68	0.41	0.79
Fish	0	0.09	0.27	0.50	0.32	0.50
Plants	0.88	0.23	0.87	0.86	0.59	0.71
Detritus	0.69	0.23	0.80	0.73	0.59	0.36
Zooplankton	0	0.77	0.03	0	0	0
Shrimp	0.13	0	0	0	0	0
Mollusks	0	0	0	0	0.05	0

Table 2: Percent similarity (PSI) between the diets of haplochromine cichlid species and *A. alluaudi* within each site sampled. PSI was calculated following procedures by Krebs (1999) and Buckley and colleagues (1999).

Site	**Percent diet overlap (%)**
Lake Victoria	12.6
Lake Nabugabo	35.8
Lake Saka	83.5
Kamwenge	64.1
Kahunge	76.4
Rwebakwata	77.1

Figure 1: Diet of haplochromine cichlids that co-occurred with *Astatoreochromis alluaudi* in 6 sites in southern Uganda. Volumes are represented as percentages of the number of non-empty stomachs. Volume was converted to percentages following points and stomach-fullness adjustments as explained in the methods. All endemic haplochromine cichlids were grouped together within a site for analyses.



Appendix 2: Advantages and disadvantages of dietary studies

Diet studies are often limited in their ability to accurately describe the feeding ecology of the taxa under study due to the highly subjective nature of some methods of quantification, difficulty in standardizing collection techniques and environmental conditions, lack of resolution when identifying partially-digested prey items, and differential digestibility of some prey items versus others (Hyslop, 1980; Wootton, 1990; Marrero and Lopezrojas, 1995; Finstad, 2005; Sreeraj *et al.*, 2006). In this study, careful measures were taken to reduce the amount of bias associated with stomach content analyses. First, all cages were pulled after they had been set for similar amounts of time, and all fish were collected within 30 minutes of each other to avoid differences in diets due to feeding times, and minimize the frequency of empty-stomachs. Second, fish were injected with a preservative immediately after euthanization in order to neutralize stomach acids and prevent further digestion of the contents post-mortem. This technique increases the resolution of the prey items identified, and reduces the likelihood of underestimating the importance of prey items that are more easily digested.

Techniques for estimating the percentage contribution of individual prey items in stomach content analyses vary widely, with some techniques being more appropriate for some organisms than others. In cichlids, the pharyngeal jaws act as shredding teeth used for ripping apart prey items. As such, delicate items such as insects are often not intact when they reach the stomach making numerical methods of diet study inappropriate (Hyslop, 1980). Volumetric methods of assessing the relative importance of a particular food category in stomachs provide a more complete assessment of an organism's diet (Hyslop, 1980). Measures of percentage volume of a particular prey group may be easy to quantify in some larger organisms using water displacement or similar techniques. However, mass or volume analyses of organisms consuming small prey types such as plankton and insect larvae are best performed using visual techniques of estimating percentage cover. Although this technique is intrinsically more subjective, conversions and adjustments can be made to remove the illusion of accuracy when deciding on relative percentages. In this study, volumetric data were converted into a points system such that the error associated with an individual prey category is ± 5% (Hynes, 1950; Hyslop, 1980). Furthermore, an adjustment was made to take into consideration the fullness of the stomach such that a single item occupying 100% of the volume of a nearly empty stomach was not given more weight in the analysis than an item occupying 50% of the volume of a full stomach. These adjustments help to standardize the data, and strengthen the conclusions that can be drawn from the results.

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Appendix 3: Is there evidence for inter-annual variation in the trophic morphology of *Astatoreochromis alluaudi?*

A premise of the field of ecomorphology is that ecological inferences can be drawn from morphological data (Motta *et al.*, 1995; Norton *et al.*, 1995). When organisms exhibit strong patterns of developmental plasticity in their morphology, it is important to recognize that morphology may vary from one year to the next depending on the life span of the organism, its developmental rate, and the degree of interannual variation in the environmental drivers of the morphological traits.

This appendix presents an evaluation of inter-annual variation in the trophic morphology from two populations of *Astatoreochromis alluaudi* studied in this thesis. Fish from Kamwenge (N=3) and Rwebakwata (N= 7, Chapter 1, Figure 1) were collected during a summer expedition in June 2006, and the trophic morphology of each population was compared with specimens collected in 2007 (Kamwenge N= 11, Rwebakwata N=10). Measurements of the musculus geniohyoideus cross-sectional area, musculus sternohyoideus cross-sectional area (Anker, 1978) and the lower pharyngeal jaw (length, width, horn width; Smits *et al.*, 1996a) were used to explore differences in trophic morphology within a population through time (see Chapter 1 p. 60 for description of morphological measurements).

We used a 1-way MANCOVA to test for significant differences between years for each population independently. All muscle, jaw and body length measurements were log transformed to meet the assumptions of normality. Homogeneity of slopes was tested by examining the interaction between the covariate (standard length) and the main effect (year). Interaction terms were removed since there was no evidence for heterogeneity between slopes. We performed Principle Component Analyses (PCA) on the same 5 morphological measures to explore the degree to which trophic characteristics diverged between years for these 2 sites. For the PCA, trait values were size-adjusted using the common within-groups slope generated from ANCOVA for each trait (Hendry and Taylor, 2004; Crampton *et al.*, In Press).

Results from MANCOVA for the Rwebakwata population indicated no significant difference in morphological traits between sampling years (Table 1). Principle component analysis based on the 5 size-adjusted trophic traits generated 2 functions with eigenvalues greater than 1.0, which cumulatively explained 81.3% of the variance (Table 2a). PC1 related most strongly to jaw morphology whereas PC2 related most strongly to muscle measures. A representative plot of the first 2 factor scores (Figure 1) was used to explore the degree of overlap between the 2 sampling years based on composite morphological characteristics. The 2 years sampled overlap to a large extent. Overlap between the years appears to be greater along the first function, which explains a greater proportion of the variance (61%) in the system.

Our sample size for the Kamwenge population was lower than for Rwebakwata, therefore these results should be interpreted with caution. Nonetheless, MANCOVA did not detect significant differences between the sampling years (Table 1). Principle component analysis generated 2 functions with eigenvalues greater than 1.0, which cumulatively explained 75.9% of the variance (Table 2b). Both principle components related to muscle and jaw characteristics, with jaws weighing more heavily on PC1. Overlap between the years was greatest along the first function, which accounted for 49.3% of the variance.

These results suggest that trophic morphology in *A. alluaudi* is conserved between years, and that remodeling of the pharyngeal jaw apparatus and associated musculature is not likely over a short period of time (Smits, 1996). These findings give strength to our inter-population comparison, where morphology was compared among fish populations that were sampled in either 2007 or 2005, and suggests that an interaction between time and site did not drive the differences observed in Chapter 1.

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Table 1: Summary of multivariate analyses of covariance (MANCOVA) for effect of year (2006, 2007) on morphological traits for *Astatoreochromis alluaudi*. Slopes were homogenous, so interaction terms were removed. Wilks lambda was used to approximate the F-values for the population term. Standard length was used as the covariate for morphological traits.

Population	**N**	**F (Wilk's Lambda)**	**df**	**p**
Kamwenge	14	0.404	7	0.186
Rwebakwata	17	0.641	10	0.410

Table 2: Principle Component Analysis scores for morphological measures of two muscle traits (m. geniohyoideus cross-sectional area- GenioCSA; m. sternohyoideus cross-sectional area= SternoCSA) and 3 jaw traits. A) PCA scores for Rwebakwata. Two components with eigenvalues greater then 1.0 were extracted cumulatively explaining 81.36% of the variance. All trait measurements were size-adjusted to a standard length of 63.5 mm. B) PCA scores for Kamwenge; two components with eigenvalues greater then 1.0 were extracted cumulatively explaining 75.95% of the variance. All trait measurements were size-adjusted to a standard length of 56.7 mm.

Note: Numbers in **bold** represent traits loading heavily on each component (>0.60).

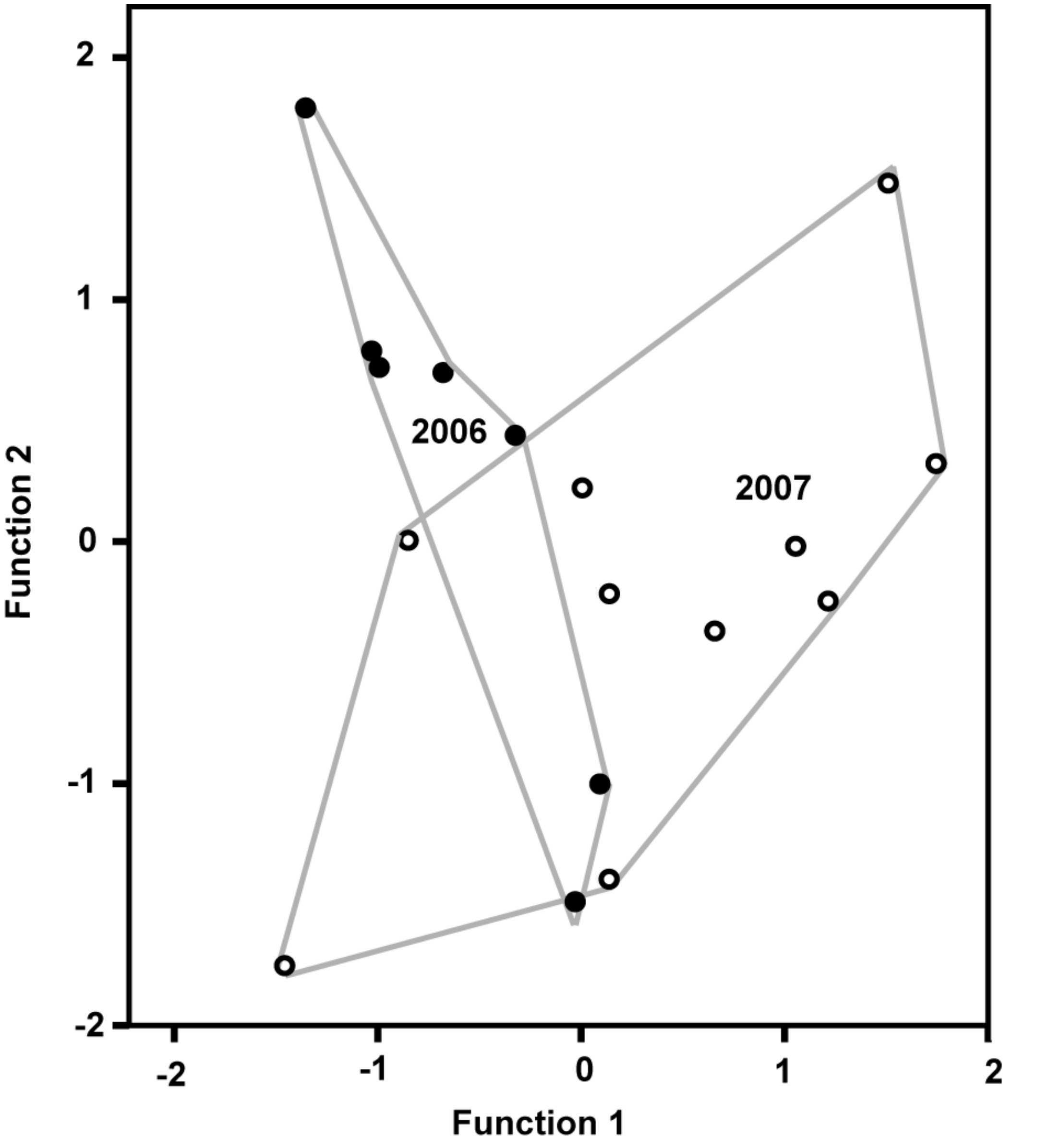
A)

			Muscles		**Jaws**		
Morphology	Eigenvalue	% variance	GenioCSA	SternoCSA	Length	Width	Depth
PC1	3.053	61.069	**0.623**	-0.562	**0.853**	**0.925**	**0.875**
PC2	1.015	20.294	0.578	**0.744**	0.167	0.163	-0.270

B)

			Muscles		**Jaws**		
Morphology	Eigenvalue	% variance	GenioCSA	SternoCSA	Length	Width	Depth
PC1	2.465	49.294	0.502	0.541	**0.959**	**0.847**	0.532
PC2	1.333	26.655	0.520	**0.710**	-0.149	-0.144	**-0.717**

Figure 1: Plot of the factor scores of the first two functions from a Principle component analysis of 5 morphological measures of pharyngeal muscles and jaws in *Astatoreochromis alluaudi* from Rwebakwata in 2006 and 2007. PC1 related strongly to jaw traits, whereas PC2 relates more strongly to muscle cross sectional area. All measurements were size adjusted according to fish standard length of 63.5 mm prior to analysis.



Appendix 4: McGill University Animal Use Protocol Approval

Please refer to the following attached documents issued by the McGill University Animal Care Committee for confirmation of Animal Use Protocol approval.

ACTION	✓	DATE
CCs		
DB	[illegible]	
APPROVED		

www.mcgill.ca/research/compliance/animal/forms/ COPY

✓

McGill University Animal Care Committee
RENEWAL of Animal Use Protocol

For: Research ☒ Teaching ☐ project

For Office Use Only:
Protocol #: 5029
Approval end date: April 30, 2008
Facility Committee: MAC
Renewal#: 1st [✓ 2nd]

Principal Investigator: Lauren J. Chapman **Protocol #** 5029

Protocol Title: Hypoxia & faunal diversification: evolutionary & ecological consequences of respiratory strategies in fishes - RGPIN 312201 **Category:** C

Unit, Dept. & Address: Faculty of Science, Dept. of Biology, N6/4 Stewart Biology Building, 1205 Dr. Penfield Ave., Montreal, H3A 1B1

Email: lauren.chapman@mcgill.ca **Phone:** 514 398 6431 **Fax:** 514 398 5069

Funding source: NSERC/CRC Research Funds 31470 31622

Start of Funding: April 1, 2005 **End of Funding:** March 31, 2010

Emergency contact #1 + work AND home phone #s: Lauren Chapman work #: 398 6431 home #: 378 5788

Emergency contact #2 + work AND home phone #s: Erin Reardon work #: 398 8199 home #: 578 4964

1. Personnel and Qualifications

List the names of the Principal Investigator and of all individuals who will be in contact with animals in this study and their employment classification (investigator, technician, research assistant, undergraduate/ graduate student, fellow). Indicate if the Principal Investigator is not handling animals. If an undergraduate student is involved, the role of the student and the supervision received must be described. Training is mandatory for all personnel listed here. Refer to *www.animalcare.mcgill.ca* **for details. Each person listed in this section must sign.** *(Space will expand as needed)*

Name	Classification	Animal Related Training Information		Occupational Health Program *	Signature *"Has read the original full protocol"*
		UACC on-line Theory course	Workshops + others		

Me Rec 26/04/07

26 AVR 2007

1. Lauren Chapman	Investigator	20+ years of research, completed advanced, basic, & wildlife training modules	registered (for another AUP)
2. Colin Chapman	Investigator	20+ years of research, completed basic & wildlife modules.	registered (for another AUP)
3. Erin Reardon	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered
4. Erika Crispo	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered
5. Jaclyn Paterson	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered
6. Sandra Binning	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered
7. Laurence Piche	Lab assistant	Trained by PI, completed basic & wildlife training modules	not registered
8. Velislava Tzenava	Undergraduate (work study)	Trained by PI, completed basic training module.	not registered
9. Cassandra McEwan	Undergraduate	Trained by PI, completed basic training module.	not registered
10. Ruoqi Wang	Undergraduate (work study)	Trained by PI, completed basic training module.	not registered
11. Etienne Low-Decarie	Undergraduate (summer USRA)	Will be trained by PI, will complete basic training module before April 18th (I will forward the results)	not registered

All checked Apr 3

Please note that the undergraduates will be supervised by myself and/or one of my graduate students. The work study students are generally involved in the maintenance of our aquaria (e.g., water qualify, fish feeding, etc.) and/or morphologicl analyses.

* Indicate for each person, if participating in the local Occupational Health Program, see http://www.mcgill.ca/research/compliance/animal/occupational/ for details.

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director	(see next page) S	Date:
Chair, Facility Animal Care Committee	Linda Wykes	Date: April 18, 07
UACC Veterinarian		Date: 24/04/07
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*		Date:
Approved Animal Use Period	Start:	End:

5029

1. Lauren Chapman	Investigator	20+ years of research, completed advanced, basic, & wildlife training modules	registered (for another AUP)	
2. Colin Chapman	Investigator	20+ years of research, completed basic & wildlife modules.	registered (for another AUP)	E.A. Chap
3. Erin Reardon	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered	Erin Reardon
4. Erika Crispo	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered	
5. Jaclyn Paterson	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered	Please see attached
6. Sandra Binning	Graduate Student	Trained by PI, completed basic & wildlife training modules.	not registered	Please see attached
7. Laurence Piche	Lab assistant	Trained by PI, completed basic & wildlife training modules	not registered	Laurence Piché
8. Velislava Tzenava	Undergraduate (work study)	Trained by PI, completed basic training module.	not registered	Vi Tzanev
9. Cassandra McEwan	Undergraduate	Trained by PI, completed basic training module.	not registered	Cassandra McEwan
10. Ruoqi Wang	Undergraduate (work study)	Trained by PI, completed basic training module.	not registered	Ruoqi Wang
11. Etienne Low-Decarie	Undergraduate (summer USRA)	Will be trained by PI, will complete basic training module before April 18th (I will forward the results)	not registered	Etienne

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*** Indicate for each person, if participating in the local Occupational Health Program, see http://www.mcgill.ca/research/compliance/animal/occupational/ for details.**

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director		**Date:**	Apr 24/2007
Chair, Facility Animal Care Committee	(see previous page) S.	**Date:**	
UACC Veterinarian		**Date:**	see other page
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*		**Date:**	
Approved Animal Use Period	**Start:** MAY 1, 2007	**End:**	April 30, 2008

1. Lauren Chapman	Investigator	20+ years of research, completed advanced, basic, & wildlife training modules	registered (for another AUP)
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11. Etienne Low-Decarie	Undergraduate (summer USRA)	Will be trained by PI, will complete basic training module before April 18th (I will forward the results)	not registered

Please note that the undergraduates will be supervised by myself and/or one of my graduate students. The work study students are generally involved in the maintenance of our aquaria (e.g., water qualify, fish feeding, etc.) and/or morphologicl analyses.

* Indicate for each person, if participating in the local Occupational Health Program, see http://www.mcgill.ca/research/compliance/animal/occupational/ for details.

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director		**Date:**
Chair, Facility Animal Care Committee		**Date:**
UACC Veterinarian		**Date:**
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*		**Date:**
Approved Animal Use Period	**Start:**	**End:**