FOOD WEB STRUCTURE OF A SUBTROPICAL SOUTH AMERICAN STREAM WITH
PARTICULAR FOCUS ON THE CO-EVOLUTION OF FORM AND FUNCTION IN AN
ENDEMIC SPECIES FLOCK

A Thesis
by
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FOREWORD

Chapters 2, 3, 4, and 5 are either in review, in revision or will be submitted, and thus are formatted according to specific journal formats: Ecology (Chapter 1), Hydrobiologia (Chapter 2), Ecology of Freshwater Fish (Chapter 3), Freshwater Science (Chapter 4) and Ecology Letters (Chapter 5).

ABSTRACT

FOOD WEB STRUCTURE OF A SUBTROPICAL SOUTH AMERICAN STREAM WITH PARTICULAR FOCUS ON THE CO-EVOLUTION OF FORM AND FUNCTION IN AN ENDEMIC SPECIES FLOCK. (May 2012)

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Food webs and aquatic ecology of subtropical South American streams are poorly understood. I investigated the trophic structure of the upper Rio Cuareim (Uruguay, South America). I analyzed the stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of 23 fish and 8 invertebrate taxa in March and November 2010. Additionally, I analyzed the stomach contents and ecomorphology of six endemic pike cichlids (*Crenicichla*). Predatory water bugs (Belostomatidae) and caddisflies (Hydropsychidae) displayed the highest trophic positions of invertebrates and bivalves (*Neocrobicula limosa* and *Corbicula fluminea*) and crabs (*Aegla uruguayana* and *Trichodactylus panoplus*) occupied the lowest trophic positions. Fishes generally occupied higher trophic positions than invertebrates. *Prochilodus lineatus* displayed the lowest trophic position and *Hoplias* spp., *Serrasalmus spilopleura,* and *Oligsarcus robustus* are the top trophic predators. Trophic guilds were successfully classified using stable isotope ratios and almost half the species sampled were omnivorous. Food web structure shared traits with temperate (e.g., few detritivores) and tropical (e.g., many omnivores) systems and also displayed characteristics that may be unique to subtropical South American streams, such as high proportions of mesopredatory species (e.g.,

Crenicichla). An endemic species flock of pike cichlids strongly partition food-resources and most species display trophic specialization by preferentially exploiting a single suite of prey items. External (skull) and internal (pharyngeal jaw) morphology were associated with dietary patterns.

ACKNOWLEDGEMENTS

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CHAPTER 1

GENERAL INTRODUCTION

The food webs and aquatic ecology of subtropical South American systems are poorly

studied. The Rio de la Plata drainage basin in subtropical ("sub-Amazonian") South America

is derived from the Mesozoic breakup of Gondwana (Cox 1989; Potter 1997; Ribeiro 2006).

This basin is the fifth largest river basin in the world and second largest in South America

with a drainage area of more than 3 million km^2 (Brea and Zucol 2011). The Rio de la Plata

basin consists of three major tributaries: the Rio Paraguay, Rio Parana, and Rio Uruguay

(Carvalho and Albert 2011). These drainages are supported by an extensive groundwater

reservoir (Favetto et al. 2005; Brea and Zucol 2011) and their ichtyofauna are derived from

headwater migration from the southern Amazon (Pearson 1937; Carvalho and Albert 2011).

The divide between the Rio Guapore (Bolivia) and the Rio Paraguay (Paraguay) likely served

as a major dispersal route via stream capture (Reclus 1895; Eigenmann 1906; Eigenmann et

al. 1907) and thus, subtropical systems share approximately one-third of their ichthyofauna

with the Amazon basin (Carvalho and Albert 2011). Although the subtropical drainages do

not support fish diversity comparable to Amazonian systems (Albert et al. 2011), they often

support considerable diversity and comparatively high levels of endemism (Lucena and

Kullander 1992; Kullander et al. 2010; Pialek et al. 2011). For example, the Rio Parana

supports more than 330 species, of which approximately 35% are endemic (Reis et al. 2003

and references therein).

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The Uruguay and Paraña basins each support some strictly endemic faunas including several highly successful lineages (e.g., *Crenicichla*; Pialek et al. 2011) yet share others (e.g., *Hypostomus*; Reis et al. 1990) and also exhibit some fauna with varying degrees of endemism (e.g., *Australoheros*; Rican et al. 2011). The complex faunal distributions in southeastern South America are likely the result of complex biogeographic history (Albert and Carvalho 2011), particularly considering that most fish lineages of the La Plata basin are non-monophyletic (Albert and Carvalho 2011). The current basin configuration has been present for approximately 7-8 Mya (Pialek et al. 2011) and the Rio Uruguay is considered a closed system both physically and genetically (Kullander et al. 2010). Thus, endemic assemblages are relatively derived (<6 My; Rican et al. 2011; Pialek et al. 2011). Moconá Falls separates the upper Rio Uruguay drainage from the middle and lower drainages and has been an important dispersal barrier in several highly successful lineages (Rican et al. 2011; Pialek et al. 2011). However, many taxa are conserved across the entire Rio Uruguay drainage (Lucena and Kullander 1992; Zarucki et al. 2010; Serra et al. 2011), indicating that several taxa have either dispersed prior to the formation of Moconá Falls or have managed to disperse downstream of the falls (Kullander et al. 2010).

Subtropical South American freshwater fauna is characterized by several unique lineages of both invertebrates and fishes. Crayfishes dominate most of the temperate zones in the Northern Hemisphere (Cambaridae, Astacidae) but have a very limited distribution in the southern hemisphere (Parastacidae; Crandall and Buhay 2008). The distribution of crayfish in the southern hemisphere is also largely temperate-subtropical with high diversities in Australia (Crandall and Buhay 2008) and poor diversity on the Atlantic coast of South America, where their distribution is confined to eastern Uruguay, north to the Brazil-Uruguay

border and coastal Chile (Morrone and Lopretto 1994). This distribution suggests an association with the breakup of Gondwana (Toon et al. 2010). In subtropical South America, two freshwater crab lineages dominate invertebrate communities. Freshwater crab lineages arose post-Gondwanan breakup (Klaus et al. 2011) and are thus comparatively derived compared to crayfishes. Trichodactylid crabs (Brachyura: Trichodactylidae) are confined entirely to freshwater and have a Neotropical distribution (Yeo et al. 2008), where they often dominate invertebrate biomass (Collins et al. 2006). The aeglid 'crabs' (Anomura: Aeglidae) are also exclusively freshwater and restricted to Southern South America (Bond-Buckup et al. 2008). Thus trichodactylid and aeglid crabs co-occur in subtropical South America. Interestingly, aeglid crabs share similar morphology to crayfishes and overlap the distribution of parastacid crayfish along only a narrow region of the Atlantic coast of Uruguay (W.S. Serra, personal communication). Because crabs and crayfishes rarely co-occur (Rodriquez 1986), the range overlap of crab- (Trichodactylidae) and crayfish-morphologies (Aeglidae) in the Rio Uruguay affords an interesting and unique opportunity to investigate trophic interactions of these two groups and is the major focus of Chapter 2.

Subtropical South America also supports several endemic fish lineages (e.g., *Australoheros*; Rican et al. 2011; *Gymnogeophagus*; Reis and Malabarba 1988). These groups are widespread but are not particularly speciose (Reis & Malabarba 1988; Rican et al. 2011). Several lineages that are shared with the Amazon basin have been highly successful and large endemic species flocks can be found in both the Rio Paranã and Rio Uruguay basins (Pialek et al. 2011). The endemic lineages of pike cichlids (*Crenicichla*) are one notable group. *Crenicichla* are important components of an evolutionary model system in Trinidad, South America, where *Crenicichla* predation pressure has influenced guppy

(Poecilidae) evolution via selection for several natural history characteristics, including schooling behavior (Seghers 1974; Magurran and Seghers 2004a), body size and coloration (Winemiller 1990), reproductive allotment (Reznick and Endler 1992), and time budget allocation (Magurran and Seghers 2004b). Although these studies underscore the ecological and evolutionary importance of *Crencichla* in South American systems, little is known about *Crenicichla* ecology apart from this model system.

The diets of *Crenicichla* have been analyzed in several studies (Lowe-McConnell 1969; Knoppel 1970; Gibran et al. 2001; Layman et al. 2005; Beathea 2007; Monatana and Winemiller 2009), which collectively support their mesopredatory role in South American food webs. However, most, if not all, of these studies address systems of comparatively low *Crenicichla* diversity. Subtropical South America supports 24 species of *Crenicichla*, of which 22 are endemic (Lucena and Kullander 1992; Kullander et al. 2010; Pialek et al. 2011), and *Crenicichla* often co-occur in diverse assemblages of 6-8 species in Argentina, Brazil and Urugay (Lucena an Kullander 1992; Pialek et al. 2011; Serra et al. 2011). If *Crenicichla* predation affects subtropical rivers similarly to tropical systems, these mesopredators may be highly important in subtropical South American community structure, dynamics and evolution.

In this thesis, I investigate the aquatic ecology of the Rio Cuareim. The Rio Cuareim basin forms the border between Brazil and Uruguay and is a major tributary to the middle Rio Uruguay (Serra et al. 2011). The Rio Cuareim fauna is representative of the diverse and endemic-rich fish communities found in the Rio Uruguay drainage (Zarucki et al. 2010; Serra et al. 2011). This tributary also supports numerous species that are of conservation concern in Uruguay (Soutullo et al. 2009) and is considered to have high fish diversity (Zaruck et al.

2010). Basic understanding of the food web structure and the community composition is elementary to the investigation of complex ecological processes. Over the last three years, including three expeditions to the Uruguay/Brazil border, I have studied the food web structure of the Rio Cuareim. My research has increased the understanding of the community composition of this basin (see Serra et al. 2011). In this thesis, my research includes functional comparisons of endemic macrocrustaceans (Chapter 2), broad comparisons of tropical and temperate food web structure (Chapter 3), and the co-evolution of form (morphology) and function (trophic roles) and ecological speciation of a species flock of rheophilic cichlids (Chapters 4 and 5).

HISTORY

The central aim of my thesis is to investigate the relationships between functionally similar species within a resource-based context. Fishes are model organisms for studying competition (e.g., Mittelbach 1984; Mittelbach 1988). The ecological role of species is often influenced by the presence (or absence) of functionally similar species that compete for mutual resources (Osenberg et al. 1992; Robinson et al. 1993). A consistent finding among these studies is that competition (i.e., during early development) often results in resource partitioning among adults. For example, North American sunfish (*Lepomis*) often compete for littoral invertebrates as juveniles and negatively impact one another's growth, survivorship and foraging success (Werner and Hall 1977). Subsequently, ontogenetic habitat or diet shifts (or a combination of the two) result in diffuse resource overlap and reduced inter-specific competition among adults (Werner and Hall 1997; Mittelbach 1984; 1988). The

generality of these findings has caused resource partitioning to be considered evidence of past competition (i.e., between functionally or phylogenetically similar species).

Diverse cichlid assemblages in the east African rift valley are model systems for studying resource partitioning and resource divergence (Goldschmidt et al. 1990; Bootsma et al. 1996; Bouton et al. 1997; Genner et al. 1999). Furthermore, resource-based divergence via polymorphism and phenotypic plasticity is considered a possible mechanism responsible for the diverse adaptive radiations of cichlids (Kocher 2004). However, resource-based divergence as a mechanism of speciation is less well accepted than sexual selection (Kocher 2004). Many of the most convincing examples of sympatric speciation (e.g., among vertebrates) involve cichlid fish (Kocher 2004; Barluenga et al. 2006). Conspicuous disruptive selection via ecological divergence is the leading speciation-initiating hypothesis in all of these examples. Thus the cichlid radiation suggests the importance of resource partitioning and specialization in evolutionary contexts (Martin and Pfennig 2009).

METHODS

An organism's niche is partially a function of its trophic interactions within a community (Elton 1927; Hutchinson 1957). Several methodologies provide useful insight into the trophic niche. Stomach content analysis (SCA) is a traditional method (Hyslop 1980) and provides taxonomically detailed results (e.g., Family-level). However, SCA has several limitations, the most important of which is the varied residence times for different materials in the digestive tract. For example, soft tissues (e.g., invertebrates) often have short residence times and carbonate materials such as bones or shells (e.g., vertebrates or mollusks) often have long residence times. Furthermore, temporal fluxes in resources can create short-term or

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long-term shifts in prey availability. For example, seasonal influx of allochthonous resources (Junk et al. 1989) creates a time-dependent subsidy for many consumers in temperate systems. Large sample sizes over a temporal gradient are necessary to overcome the short-term dietary patterns provided by SCA.

Stable isotope analysis (SIA), primarily of C and N, is also a popular method of investigating trophic niche space (West et al. 2006). Stable isotope ratios fractionate (i.e., change) consistently between prey and consumer. For example, $\delta^{13}C$ is highly conserved across trophic transfers (prey to consumer) resulting in little enrichment (0-0.5‰) of consumer relative to prey (DeNiro and Epstein 1978; Post 2002). In contrast, $\delta^{15}N$ enriches in stepwise increments of 2.5-3.5‰ (Post 2002; Vanderklift and Ponsard 2003) and thus is used to delineate trophic levels. For example, in aquatic systems, if basal resources (i.e., algae, plants) have $\delta^{15}N$ ratios of 6.0‰, herbivores and carnivores are expected to have ~9‰ and ~12‰, respectively. However, there is considerable opportunity for 'mixing'; omnivorous species, for example, that consume diverse prey items that may have contrasting isotope ratios. Mixing models estimate the relative contributions of prey items to consumers (e.g., in trophic studies) and can calculate a unique solution for multiple sources. For example with a dual-isotope mixing model ($\delta^{13}C$ & $\delta^{15}N$), a solution can be calculated for 3 prey items. The Environmental Protection Agencies (EPA) IsoSource mixing model (Phillips and Gregg 2003) estimates the contribution of many prey items (>3) which is particularly useful with generalist species than consume a variety of prey items. Stable isotope analysis is particularly useful because it reflects time-integrated trophic characteristics based on assimilation (i.e., incorporated into muscle tissues). For example, with fishes in a natural system, $\delta^{13}C$ turnover takes 4-6 months in adult fish (Weidel et al. 2011). Thus, SIA

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overcomes the limitations of SCA. However, because many taxa may have similar isotope ratios, SIA provides taxonomically coarse results. For example, taxa within the same trophic guild (i.e., that consume similar resources) often cannot be discriminated. In combination, SCA and SIA provide detailed, time-integrated information about organism's trophic interactions.

CHAPTER 2

TROPHIC ANALYSIS OF TWO SUBTROPICAL SOUTH AMERICAN FRESHWATER
CRABS USING STABLE ISOTOPE RATIOS

ABSTRACT

Crayfish and crabs do not co-occur often. However, the crab families Aeglidae (crayfish morphology) and Trichodactylidae (crab morphology) are sympatric in many subtropical South American streams. I investigated the trophic status of *Aegla uruguayana* (Aeglidae) and *Trichodactylus panoplus* (Trichodactylidae) in a South American subtropical piedmont river (Cuareim River, Uruguay) using $\delta^{13}C$ and $\delta^{15}N$ ratios. Stable isotope analysis revealed that the two crabs have different trophic niches. I estimated the relative importance of prey items using a five-source mixing model. Three fractionation rates (-1, 0, +1 ‰) influenced the estimated assimilation (%) of prey items to consumers. However, the relative importance of prey items was unaffected. *Aegla uruguyana* shifts from herbivore-detritivore to omnivore during ontogeny. Shared morphology between crayfishes and aeglids likely facilitates similar trophic roles, however *A. uruguayana* occupies a much lower trophic position than is typical for crayfishes. *Trichodactylus panoplus* is a strict herbivore-detritivore. In contrast to tropical crabs, they do not engage in carnivory or exploit terrestrial subsidies. In subtropical South American streams, aeglids may be the functional equivalent of crayfishes whereas trichodactylids may fill a trophic role atypical for freshwater crabs.

INTRODUCTION

Invertebrate functional roles in stream ecosystems vary considerably across spatial and biogeographic gradients. In Northern Hemisphere streams, crayfish and caddisflies are often the major taxa driving detritivory (Wallace & Webster, 1996; Covich et al., 1999; Benke et al., 2001; Creed & Reed, 2004; Creed et al. 2009). In tropical streams, shrimp are often the major detritivores (Covich & McDowell, 1996; Covich et al., 1999; Boulton et al., 2008, Cross et al., 2008). In tropical systems that lack shrimp or other shredders freshwater crabs are dominant invertebrate detritivores (Dobson et al., 2002; Zimmerman & Covich, 2003, Lancaster et al., 2008). The trophic niche and community impacts of many invertebrate taxa are well understood (reviewed in Wallace & Webster, 1996; Covich et al., 1999). However, invertebrate communities in many tropical and subtropical regions are poorly studied and information on basic ecology is lacking.

Crayfish are largely restricted to temperate zones, with most of the group's diversity occurring in Neartic and Australasian regions (Crandall & Buhay, 2008). The distribution of Southern Hemisphere crayfishes is associated with the Gondwanan breakup (Toon et al., 2010). Crayfishes are often integral components of stream communities and have been shown to affect plant and animal community composition (Creed, 1994; Creed & Reed, 2004), organic matter processing (Parkyn et al., 2001; Creed & Reed 2004), and transport of terrestrial resources into the aquatic food web (France, 1996). Crayfishes are generally considered omnivorous (Creed, 1994; Bondar et al., 2005) but often have ontogenetic shifts to carnivory (Gutiérez-Yurrita et al., 1998) and may function as predators in some systems (Parkyn et al., 2001).

In contrast, the global distribution of freshwater crabs is largely restricted to Neotropical and Oriental regions (Yeo et al., 2008). Crabs also influence organic matter processing (Hill & O'Keefe, 1992; Lancaster et al., 2008) and transportation of terrestrial resources into the aquatic food web (Covich & McDowell, 1996; Lancaster et al., 2008). Crabs are typically omnivorous in tropical streams (March & Pringle, 2003; Lancaster et al., 2008), although ontogenetic shifts from carnivory to herbivory-detritivory may be common (Hill & O'Keefe, 1992; Marijnissen et al., 2009). Many species forage on land (Dobson, 2004) and often exploit unusual terrestrial resources such as ants (Lancaster et al., 2008) and palm fruits (A. P. Covich, personal communication). Both crayfishes and crabs are particularly key components of high gradient stream communities (March & Pringle, 2003; Creed & Reed, 2004; Lancaster et al., 2008). Crayfishes and crabs have a conspicuously allopatric distribution and share many functional roles in freshwater communities.

Freshwater crabs are found throughout South American subtropical rivers, one with typical crab-morphology (Trichodactylidae) and the other with crayfish-morphology (Aeglidae) (Fig. 1). In general, these two morpho-types are not thought to co-occur (Rodríquez, 1986), so this study provides an interesting system for a comparative analysis of their trophic niches in sympatry. In allopatry, crayfishes and crabs often have similar trophic and functional roles. In sympatry, that scenario seems unlikely, particularly considering that these taxa occur in high densities (Collins et al., 2006; 2007), ensuring frequent interactions. The trophic roles of both groups of crabs are poorly understood. Bueno & Bond-Buckup (2004) described two aeglids in southern Brazil as omnivorous and noted increased carnivory in larger individuals. Collins et al. (2007) reported *Aegla uruguayana* and *Trichodactylus borellianus* in an Argentinean stream to be herbivore-algivores. Castro-Souza & Bond-

Buckup (2004) found that there was no seasonal variation in the trophic role of aeglids. This probably due to the historically dry climate associated with subtropical South America (Iriniondo 1999) restricting seasonal resource fluxes. Furthermore, crabs are important prey items for a variety of aquatic and terrestrial animals in South American streams (Collins et al., 2006; Collins et al., 2007; Bond-Buckup et al., 2008) and are thus potentially important links between aquatic and terrestrial ecosystems.

I investigated the trophic niche of an aeglid (*Aegla uruguayana*) and a trichodactylid (*Trychodactylus panoplus*) in the Rio Cuareim, a subtropical South American piedmont stream in northwestern Uruguay using stable isotope ratios. My objective was to compare the trophic niches of both crab morphologies (crayfish and crab) with those in systems where they occur in allopatry, such as temperate streams (crayfishes) and tropical streams (crabs). I hypothesize that aeglids and trichodactylids occupy distinct niches and that the convergent morphology of aeglids and crayfishes would be conducive to them occupying similar trophic niches, thus trichodactylid crabs may thereby occupy a niche somewhat atypical of crabs.

METHODS

The Rio Cuareim is located in subtropical South America (Iriniodo, 1999) and drains northwestern Uruguay and southwestern Brazil and is a major tributary to the middle Rio Uruguay (Fig. 2). I sampled crabs (Fig. 1) and potential food items from the upper Rio Cuareim in Uruguay. I recorded carapace length (CL) for *Aegla* and carapace width (CW) for *Trichodactylus* (according to convention for their respective morphologies). I allowed specimens to clear their guts for 72 h to allow and then froze specimens in the field and later stored them at -80˚C. Whole specimens were lyophilized until dry and ground into a

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homogenous mixture (Anderson & Cabana, 2007). Weighed samples (0.6-1.2 mg) were then transferred into Ultra-Pure tin capsules (Costech Analytical Technologies, Valencia, CA). Stable isotope ratios were analyzed at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Arizona, USA). Isotope values are expressed in delta notation (‰), which represent deviation from universal standards. Delta notation is calculated as $[(R_{sample}/R_{standard})-1] \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ (see DeNiro & Epstein, 1978; Post, 2002). I use a three factor (juvenile and adult *A. uruguayana*, and adult *Trichodactylus panoplus*) one-way analysis of variance (ANOVA) and Tukey's post hoc tests separately for both $\delta^{13}C$ and $\delta^{15}N$ isotopes. We did not encounter juvenile *Trichodactylus panoplus;* their densities may either be extremely low due to predation (Collins et al., 2006; 2007; Bond-Buckup et al., 2008) or they may utilize different habitats (e.g., deep water) that were not sampled.

The most abundant prey items were sampled based on preliminary surveys and with consideration to the prey items described by Bueno & Bond-Buckup (2004). All potential prey items were collected from littoral zones where aeglids and trichodactylids were abundant. Detritus samples are primarily terrestrial leaf-based. The only common macrophyte in the Rio Cuareim is *Hydrilla*, which has a patchy distribution. Because leptophlebiid and hydropsychid samples display different $\delta^{15}N$ signatures (i.e., Phillips et al., 2005), we did not pool them. Collectively, they should represent the spatial range between grazers and carnivores and provide an estimation of larval invertebrate contributions to crab diets.

I used a five-source $\delta^{13}C$ and $\delta^{15}N$ mixing model (Phillips & Gregg, 2003) to estimate percent contribution of potential prey items (Table 1) to *Aegla uruguayana* and

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Trichodactylus panoplus diets. Mass balance tolerance was set to 0.3‰ and source increment

was set to 1% (Hoeinghaus & Davis, 2007). Fractionation is the relative change in stable

isotope ratios across a trophic transfer (from prey to consumer) and is possibly the primary

assumption involved in stable isotope interpretation (reviewed in Gannes et al., 1997; Post,

2002). I corrected for δ^{15}N fractionation using the mean rate of 2.54‰ reported from a meta-

analysis of fractionation rates (Vanderklift & Ponsard, 2003) and accepted for marine crabs

(Hoeinghaus & Davis, 2007). Mixing models are particularly sensitive to the fractionation

value incorporated into the model. Additionally, isotopic fractionation has been shown to be

variable in marine crabs, particularly δ^{13}C fractionation (Frantle et al., 1999). Therefore, I

report mixing model estimations based on three δ^{13}C fractionation corrections (+1, 0, -1‰).

These rates encompass accepted fractionation rates for marine crabs (0‰; Hoeinghaus &

Davis, 2007) and liberal enrichment (+1‰) and depletion (-1‰) alternatives that span the

variation reported in marine crabs (Frantle et al., 1999). Variation in fractionation results

from consuming items with high (i.e., plants) and low (i.e., animals) C/N ratios (Rudnick &

Resh, 2005; Lancaster et al., 2008). For all additional statistical analyses, we used SPSS (ver.

17.0; SPSS, Inc., Chicago, IL). Species were identified using meristics outlined in Martin &

Abele (1988) for *Aegla uruguayana* and Magalhaes (2003) for *Trichodactylus panoplus*.

Voucher specimens are deposited in the Auburn University Natural History Museum and

Learning Center (Auburn, Alabama, USA): *A. uruguayana* (AUM 22709) and

Trichodactylus panoplus (AUM 22710).

RESULTS

Potential food sources displayed distinct isotopic signatures (Table 1) allowing for powerful source discrimination by the mixing model. Juvenile (mean±SD; CL 10.8±1.1 mm) and adult *Aegla uruguayana* (27.27±1.8 mm) displayed significantly different δ^{13}C ratios ($p<0.0001$; Fig. 3), suggesting a shift in the relative importance of C sources. There was no significant difference in δ^{15}N ratios of juveniles and adults ($p>0.05$; Fig. 3), suggesting *A. uruguayana* feeds within the same trophic level in both life stages. Mixing model results suggest that generally two prey items are obligate for each class regardless of the δ^{13}C fractionation rate used in the model (Table 2). Aquatic macrophytes were the only primary food item of juvenile *Aegla* but they secondarily assimilated algae, detritus, and larval insects (Table 2). Adult *Aegla* primarily assimilated detritus and larval insects. *Trichodactylus panoplus* (CW 12.6±2.7 mm) displayed significantly different δ^{15}N ratios than juvenile ($p=0.004$) and adult ($p=0.036$) *A. uruguayana* (Fig. 3). There was a significant difference in δ^{13}C ratios between *Trichodactylus panoplus* and adult *A. uruguayana* ($p=0.005$), but not between *Trichodactylus panoplus* and juvenile *A. uruguayana* ($p>0.05$; Fig. 3). Because there were not distinct size classes for *Trichodactylus* (CW 9.1-15.5 mm), I could not adequately analyze ontogenetic diet shifts. However, using regressions, I found no significant relationship between carapace width and δ^{13}C ($R^2=0.06$, $F_{2,6}=0.33$, $p=0.59$) or δ^{15}N ratios ($R^2=0.18$, $F_{2,6}=1.12$, $p=0.34$), nor did I find a significant relationship between δ^{13}C and δ^{15}N ratios ($R^2=0.08$, $F_{2,6}=0.42$, $p=0.55$). Compared to other invertebrate taxa, both crabs displayed relatively low trophic positions based on δ^{15}N ratios (Fig. 4).

Isotopic fractionation is a primary assumption underlying interpretation of stable isotope analyses. Furthermore, mixing models are particularly sensitive to fractionation

adjustments. In combination, discrepancies and uncertainties about isotopic fractionations provide for complicated interpretation of source contributions to a consumer. My data demonstrate that the proportion (%) of estimated assimilation was influenced by the fractionation rate incorporated into the mixing model. Prey item assimilation often varied 20-30% and up to 50% depending upon assumed fractionation rates (Table 2). However, the relative importance of prey items was unaffected by fractionation assumptions. The most important or obligate (i.e., 1st -99th percentile does not zero) items were conserved across multiple fractionation rates (Table 2). For example, *Trichodactylus panoplus* displayed obligate assimilation of macrophytes and detritus in all models. The relative importance of the two items merely varied depending on the fractionation rate assumed. Therefore, in the case of generalists or omnivores that consume a variety of prey items, the influence of fractionation on diet interpretation is probably less important compared to specialists in which a single prey item is particularly important. In marine crabs, $\delta^{13}C$ fractionation is more variable than $\delta^{15}N$ (Frantle et al., 1999). However, this may be crab- or invertebrate- specific because fractionation of $\delta^{15}N$ is often considered more variable in fishes (Vander Zanden & Rasmussen, 1999; Post, 2002). Freshwater crabs may show particularly high $\delta^{13}C$ variation because they occupy low trophic positions and directly consume basal resources. Collins et al. (2006) reported trichodactylid yearly mean densities of $33.4\pm3.9/m^2$, $14.2\pm9.3/m^2$, and $9.4\pm5.9/m^2$ for *Trichodactylus borellianus* in three rivers in the neighboring Rio Parana drainage (Argentina). I anecdotally observed, but did not quantify similar densities in the Rio Cuareim.

DISCUSSION

Stable isotopes reveal that aeglids and trichodactylids have different trophic niches, but that there is an ontogenetic component to their trophic relationships. *Aegla uruguayana* shifts from herbivore-detritivore juveniles to omnivorous adults. *Aegla uruguayana* share many trophic characteristics with crayfishes and may be functional equivalents in subtropical South American streams. *Trichodactylus panoplus* are superficially similar to shrimps and crabs in tropical systems in that they are herbivore-detritivores. However, in contrast to tropical crabs, trichodactylids do not appear to engage in carnivory. Both subtropical crabs also have lower trophic positions than their temperate or tropical counterparts. Mixing models indicate that fractionation correction does influence the relative assimilation (%) of prey items by freshwater crabs it does not change the relative importance of prey items.

Aegla uruguayana exhibit ontogenetic diet shifts and assimilate a larger proportion of invertebrates in later life stages, a pattern also shared with subtropical *A. platensis* and *A. ligulata* (Bueno & Bond-Buckup, 2004) and temperate crayfish (Gutiérez-Yurrita et al., 1998). *Aegla ligulata* shift from consuming ~8% invertebrates (by volume) to 20% throughout ontogeny and *A. platensis* from 8.5% to 10.5% (Bueno & Bond-Buckup, 2004). This is lower than the estimated 16.5% to 43% for *A. uruguayana* according to our isotopic analyses. However, in crayfish, estimated assimilation of invertebrates can be higher than consumption (Hollows et al., 2002). This is likely due to preferential assimilation of invertebrates compared to lower quality foods (Whiteledge & Rabeni, 1997), such as detritus, which is the primary diet item for *A. uruguayana*. The contribution ranges (1-99th percentile) of invertebrates also suggest that mean contribution data may overestimate assimilation rates. Large contribution ranges provide for ambiguous interpretation of mixing model results

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(Phillips & Gregg, 2003; Benstead et al., 2006). However, with subtropical crabs, obligate prey items explain a large proportion of the diet (60-90%) and have constrained contribution ranges (max-min contributions < 20%), thus allowing for strong inference about dietary patterns.

Enriched δ^{15}N ratios are often associated with increased carnivory (Post, 2003). However, larger *A. uruguayana* display lower δ^{15}N signatures than juveniles despite higher assimilation of invertebrates. This phenomenon has also been shown in crayfishes (Parkyn et al., 2001; Bondar et al., 2005). We assume that incorporation of larger proportions of δ^{15}N-enriched invertebrates is mitigated by larger proportions of δ^{15}N-depleted detritus. In crayfishes, assimilation efficiencies are higher for invertebrates than plant detritus (Whitledge & Rabeni, 1997). Diets consisting of protein-rich invertebrates also result in faster growth rates (Bondar et al., 2005), demonstrating a benefit to consuming animal material rather than plant material. Thus, increased consumption of detritus in adults could be an inadvertent side effect of foraging for invertebrates. This observation is consistent with their affinity to habitats rich in leaf fragments and twigs (Bücker et al., 2008).

Aeglids, like crayfishes, appear to be omnivorous. Ontogenetic shifts to carnivory and reductions in δ^{15}N ratios are also shared characteristics with crayfishes (Gutiérez-Yurrita et al., 1998; Parkyn et al., 2001; Bondar et al., 2005). The convergent morphology between crayfishes and aeglids may be key in these shared functional roles. However, aeglids have distinctly lower trophic positions than crayfishes. Crayfishes typically have higher trophic positions (based on δ^{15}N ratios) than other invertebrates such as mayflies and caddisflies (Parkyn et al., 2001; Bondar et al., 2005), suggesting that they can function as top invertebrate predators in some systems. In contrast, we found that aeglids have lower trophic

positions than mayflies and caddisflies. Indeed, of all invertebrate taxa sampled, only

bivalves and trichodactylids had lower trophic positions. Assimilation of $\delta^{15}N$ and $\delta^{13}C$ may

be decoupled in crabs (Lancaster et al., 2008), thus aeglids may preferentially assimilate

detrital $\delta^{15}N$ relative to crayfishes, particularly considering crayfishes are known to

preferentially assimilate invertebrates relative to plants (Whiteledge & Rabeni, 1997).

Trichodactylus panoplus display trophic characteristics of a herbivore-detritivore.

This is similar to the trophic roles of crabs in tropical systems (Dobson et al., 2002; Dobson,

2004). However, crabs are often opportunistically carnivorous (Dobson, 2004) and

depending on the extent, may be functional omnivores (March & Pringle, 2003) or top-

predators (Marijnissen et al., 2009) in their respective invertebrate communities. Based on

mixing model estimations and their low trophic position relative to other invertebrates, it is

unlikely that *Trichodactylus panoplus* engages in carnivory. Furthermore, trichodactylids do

not appear to exploit terrestrial prey (Collins et al., 2007) and we did not observe

Trichodactylus panoplus on land. Thus, crabs in subtropical South America appear to deviate

from their tropical counterparts. Fidelity to in-situ resources may prevent these crabs from

transporting some terrestrial resources into the aquatic food web. However, predation by

numerous terrestrial predators including birds, mammals, reptiles and amphibians (Collins et

al., 2007 and references therein) provides transport of autochthonous resources to the

terrestrial food web. This difference in food web connectivity produces further contrast to

tropical systems, where crabs frequently forage on land (Dobson, 2004) and predation is

often low (Dobson et al., 2007; Lancaster et al., 2008). This difference is likely because wet

tropical montane environments are more conducive to overland excursions by crabs (Dobson

et al., 2007; Lancaster et al., 2008). The arid prairie environment of Uruguay and southern Brazil (Iriniondo, 1999) likely prohibits this behavior.

Although their feeding behavior is infrequently studied, freshwater crabs are likely important components of many subtropical South American stream ecosystems. *Aegla uruguayana* has trophic characteristics that mirror those observed in Northern Hemisphere temperate crayfishes (Cambaridae, Astacidae; Gutiérez-Yurrita et al., 1998; Bondar et al., 2005; Stenroth et al., 2008) and Southern Hemisphere temperate crayfishes (Parastacidae; Parkyn et al., 2001). These patterns are also consistent with other aeglid taxa (Collins et al., 2007; Santos et al., 2010). Future studies are needed to determine if aeglids are, like crayfish, ecosystem engineers and keystone species (Creed, 1994; Usio, 2000; Creed & Reed, 2004; Dorn & Wojdak, 2004; Feminella & Resh, 1989; Bengston et al., 2008). Furthermore, herbivory in streams can have dramatic effects on competitors as well as on taxa that rely on macrophytes for cover or reproduction (reviewed in Feminella & Hawkins, 1995). Thus, the effects of trichodactylids in South American stream ecosystem processes are likely important ecologically and deserve further study, especially now that their distributions and evolution are increasing being documented (Pérez-Losada et al., 2002; Pérez-Losada et al., 2004; Campos, 2005; Pérez-Losada et al., 2009).

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TABLES AND FIGURES

Table 1. Stable isotope ratios (mean±SD) of potential prey items of *Aegla uruguayana* and *Trichodactylus panoplus* in the Rio Cuareim, Uruguay.

Sources	n	C/N	$\delta^{13}C$	$\delta^{15}N$
Filamentous algae	5	12.1±0.1	-20.6±0.4	7.5±0.6
Terrestrial derived detritus	3	22.9±1.4	-23.5±0.1	3.1±0.3
Aquatic macrophytes	3	23.0±2.2	-16.9±0.5	6.6±0.6
Hydropsychidae	20	5.4±0.1	-21.1±0.4	10.5±0.3
Leptophlebiidae	16	5.7±0.7	-20.8±0.6	9.3±0.2

Table 2. Percent contribution of food items to *Aegla uruguayana* and *Trichodactylus panoplus* diet estimated by a five-source $\delta^{13}C$ and $\delta^{15}N$ mixing model after three $\delta^{13}C$ fractionation corrections: +1: enrichment, 0: no enrichment, -1 ‰: depletion relative to prey. Values are mean contribution (1st-99th percentile in parenthesis). Items with obligate assimilation (contribution ranges do not include zero) are indicated in bold.

Food Source	Juvenile *A. uruguayana*	Adult *A. uruguayana*	*T. panoplus*
-1 $\delta^{13}C$ ‰			
Algae	21.2 (0-61)	0.3 (0-1)*	11.3 (0-35)
Macrophyte	**34.6 (21-46)**	0 (0-0)*	**29.7 (20-39)**
Detritus	**22.5 (11-32)**	**60 (59-61)***	**47.8 (40-55)**
Hydropsychidae	9.3 (0-27)	**39.3 (39-40)***	4.8 (0-15)
Leptophlebiidae	12.3 (0-36)	0.3 (0-1)*	6.5 (0-20)
0 $\delta^{13}C$ ‰			
Algae	15.3 (0-45)	4.9 (0-18)	6.1 (0-21)
Macrophyte	**53.8 (43-63)**	0.9 (0-4)	**48.3 (41-56)**
Detritus	**14.5 (5-22)**	**51.2 (47-55)**	**39.4 (34-46)**
Hydropsychidae	7.6 (0-23)	**29.9 (3-47)**	2.9 (0-43)
Leptophlebiidae	8.9 (0-27)	13.1 (0-43)	3.4 (0-12)
+1 $\delta^{13}C$ ‰			
Algae	9.5 (0-30)	23.6 (0-65)	2.0 (0-8)
Macrophyte	**73.9 (65-82)**	10.5 (0-22)	**65.3 (61-71)**
Detritus	6.9 (0-14)	**38.5 (26-48)**	**31 (28-34)**
Hydropsychidae	4.1 (0-14)	11.8 (0-33)	0.7 (0-3)
Leptophlebiidae	5.6 (0-18)	15.5 (0-43)	1.0 (0-4)

*undefined using -1 $\delta^{13}C$ ‰ correction because the mixture fell out of bounds (i.e., outside the polygon of sources) therefore, listed contributions are after -0.5 $\delta^{13}C$ ‰ correction.

FIGURES

Fig. 1. Preserved specimens of *Aegla uruguayana* in lateral (A/B), dorsal (C) and ventral (D) view and *Trichodactylus panoplus* in dorsal (E) and ventral (F) view. Specimens were collected from the Rio Cuareim, Uruguay. Voucher specimens are deposited in the Auburn University Natural History Museum and Learning Center (Auburn, Alabama, USA): *A. uruguayana* (AUM 22709) and *T. panoplus* (AUM 22710). Scale bar = 1 cm.
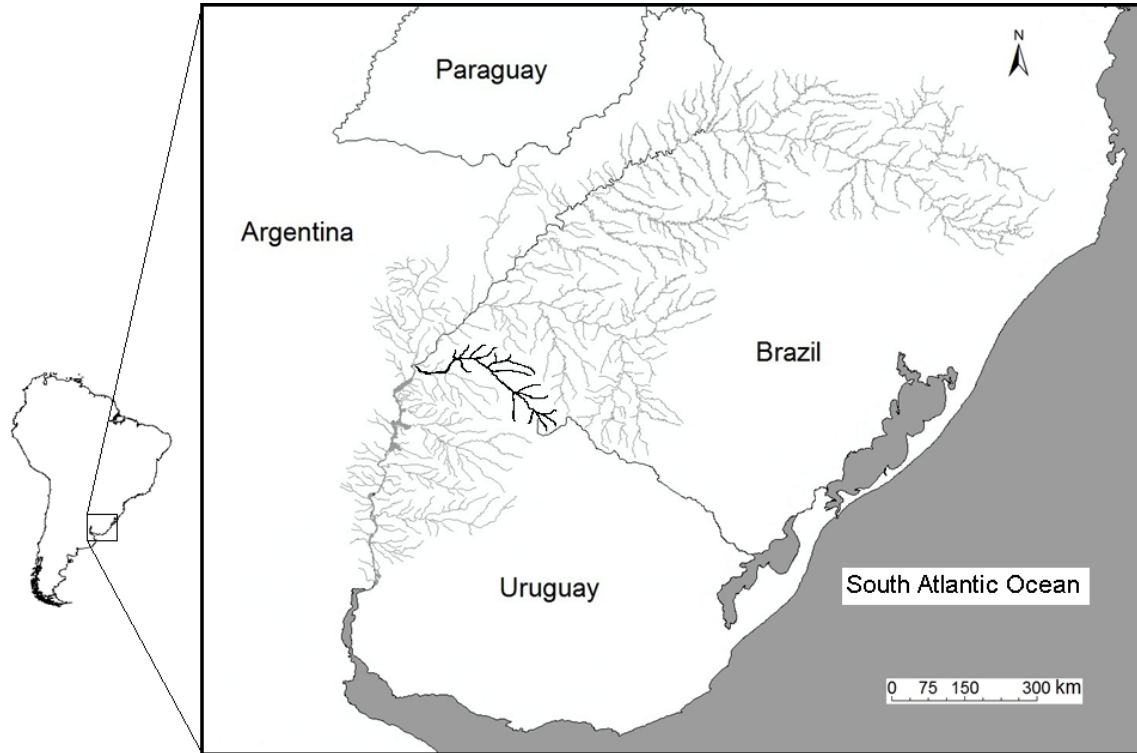
Fig. 2. The Rio Uruguay Drainage and Rio Cuareim basin (bold) located in subtropical South America. *Aegla uruguayana* and *Trichodactylus panoplus* were collected in the headwaters of the Rio Cuareim, along the border of Uruguay and Brazil.
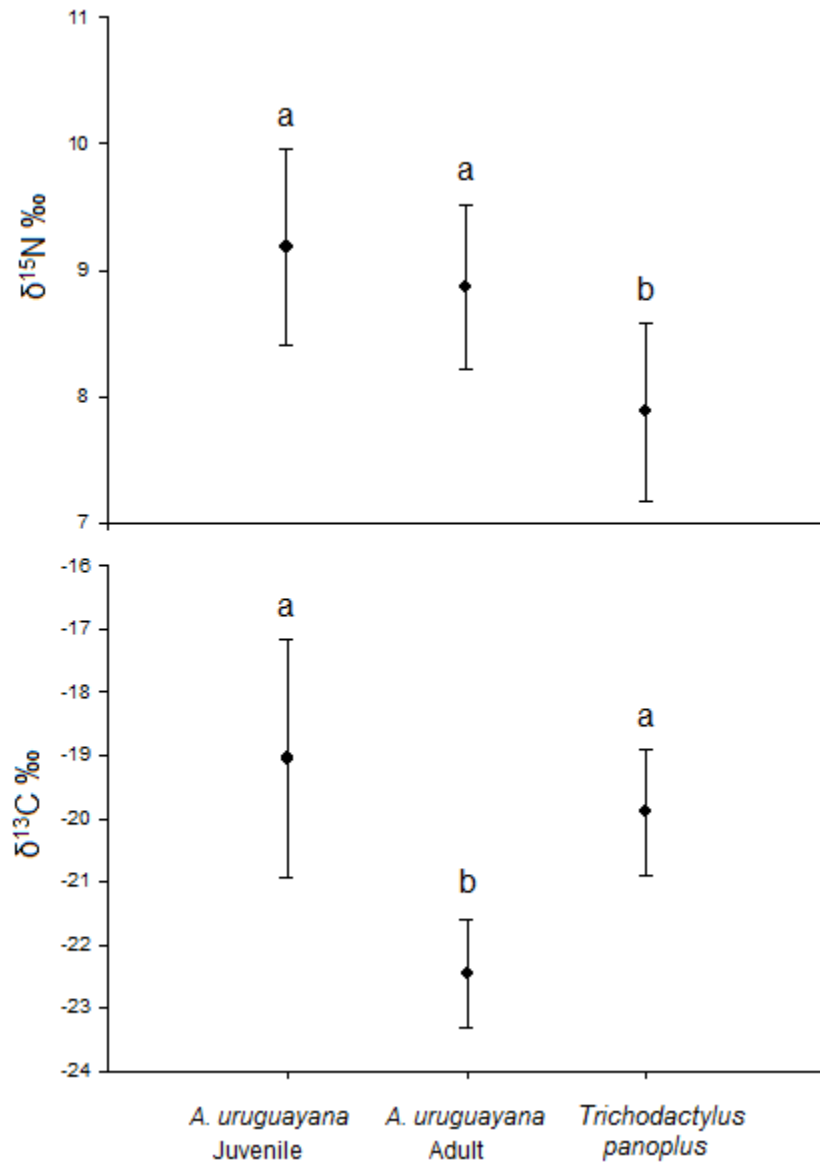
Fig. 3: Results of ANOVA post-hoc (Tukey HSD) comparisons (for $\delta^{15}N$ and $\delta^{13}C$) between juvenile (*n*=9) and adult (*n*=8) *Aegla uruguayana* and (*n*=6) *Trichodactylus panoplus* from the Rio Cuareim Uruguay. Stable isotope ratios are mean±SD. Letters denote significant comparisons.
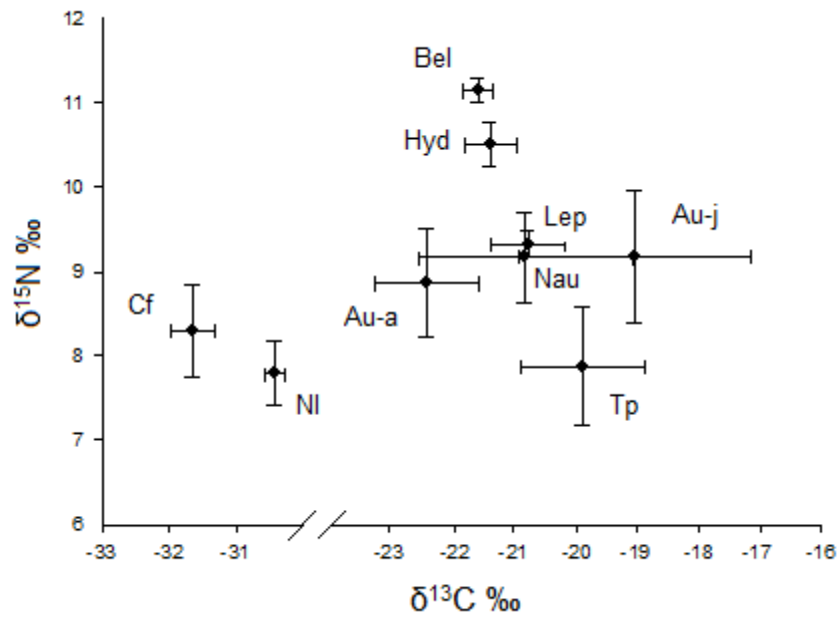
Fig. 4. Mean (±SD) stable isotope ratios of invertebrates in the Rio Cuareim, Uruguay.

Abbreviations are as follows: *Corbicula fluminea* (Cf), *Neocorbicula limosa* (Nl),

Belostomatidae (Bel), Hydropsychidae (Hyd), Leptophlebiidae (Lep), Naucoridae (Nau),

adult *A. uruguayana* (Au-a), juvenile *A. uruguayana* (Au-j) and *Trichodactlys panoplus* (Tp).

CHAPTER 3

TROPHIC GUILD SCTRUCTURE OF A DIVERSE SUBTROPICAL SOUTH
AMERICAN FISH COMMUNITY

ABSTRACT

Characterization of food web structure may provide key insights into ecological

function, community or population dynamics and evolutionary forces in aquatic ecosystems.

The food web structure of temperate and tropical lakes and rivers is well studied. However,

few studies have examined food web structure of subtropical South American river systems.

We measured stable isotope ratios of 23 fish species from the Rio Cuareim a 5th order

tributary of the Rio Uruguay basin. My goals were to 1) determine if subtropical riverine

food webs conform to patterns reported from temperate and tropical rivers, 2) compare

trophic segregation at trophic guild and taxonomic scales and 3) estimate the relative

importance of basal resources supporting fish biomass. Isotopic data demonstrate that the

food web structure of the Rio Cuareim is more similar to tropical rivers than to temperate

rivers. However, this stream displays unique traits that are likely characteristic of subtropical

South American systems. Although the relative frequency of omnivores is similar to tropical

rivers, I found that the Rio Cuareim supports comparatively few detritivorous fishes. Similar

to many temperate rivers, invertebrates are likely the dominant detritovires. Subtropical

rivers uniquely support a disproportionately high frequency of mesopredatory species (e.g.,

Crenicichla). Although community-level isotopic overlap was high, trophic guilds and

taxonomic groups can be clearly differentiated using stable isotope ratios. Understanding

food web structure may be important for future conservation programs in subtropical river systems by identifying top predators, taxa that may occupy unique trophic roles and taxa that directly exploit basal resources.

INTRODUCTION

Regardless of whether researchers are interested in diversity, ecological interactions or economic value, river systems are often defined by the fish species they support. For example, diverse cichlid lineages have defined many tropical drainages, particularly the East African rift valley (Kocher 2004). North American, European and Asian temperate and sub-tropical streams often lack detritivorous fishes and are often characterized by high insectivore and browser diversity (e.g., Cyprinidae, Catostomidae, Percidae; Ibañez et al. 2009). Economically important lineages (e.g., Salmonidae, Centrarchidae) characterize temperate meso-predator guilds (Mittelbach & Persson 1998; Albert et al. 2011) and apex predators are typically large bodied (Mittelbach & Persson 1998).

Tropical rivers support diverse fish communities (Jepsen & Winemiller 2002), and are characterized by a few highly-successful lineages (Characidae, Cichlidae, Loricariidae; Albert et al. 2011). Although tropical systems are species-rich and niche space appears saturated, some species occupy ecological roles that lack redundancy and are thus extremely important to ecosystem function (Taylor et al. 2006). Detritivorous taxa are diverse (Jepsen & Winemiller 2002) and are often important to ecosystem function (Taylor et al. 2006). For example, sucker-mouthed catfishes (Loricariidae) play important functional roles by modifying habitat (Power 1990) and consuming resources that are often poorly exploited by fishes (e.g., wood, macrophytes; Lujan 2009). Mid-trophic level tropical fishes are frequently

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omnivorous (Jepsen & Winemiller 2002) and comparatively few are invertivores (Ibañez et al. 2009) whereas apex piscivores are often large-bodied (Layman et al. 2005).

Basal energy sources ultimately support fish biomass. Communities in mid- or large-sized temperate streams are often supported by autochthonous production and characterized by high algal or macrophyte biomass (Thorp & Delong 1994; Thorp et al. 1998). However, in smaller streams or systems with high climatic fluctuations, allochthonous subsidies may also be important (Junk et al. 1989; Zeug & Winemiller 2008). Large tropical stream fish communities are also often supported by algal biomass (Lewis et al. 2001; Douglas et al. 2005). Autochthonous resources typically support consumer biomass even in systems with large inputs of terrestrial production (Jepsen & Winemiller 2007). Thus, there may be fundamental differences in fish community reliance on basal production sources across biogeographic regions.

Subtropical rivers are poorly studied compared to tropical and temperate systems (Winemiller 1990; Fry 1991; Thorp et al. 1998; Hamilton et al. 1998; Finlay 2001; Lewis et al. 2001; Jepsen & Winemiller 2002; Jepsen & Winemiller 2007; Zeug & Winemiller 2008; Winemiller et al. 2010). Subtropical South American drainages support diverse fish assemblages and display comparatively high degrees of endemism and cryptic diversity (Lucena & Kullander 1992; Rican et al. 2010; Pialek et al. 2011). However, species boundaries, community composition and ecological roles of many subtropical South American fishes are poorly known and our understanding of food web structure in these streams is extremely limited (Zarucki et al. 2010; Serra et al. 2011).

I am not aware of any studies of the food web structure of any subtropical South American streams, although there are analyses of subtropical lakes and estuaries (Garcia et al.

2006; Garcia et al. 2007). The Rio de la Plata Basin, the second largest catchment in South America after the Amazon, displays high levels of endemism (35-65%; Pialek et al. 2011; Albert et al. 2011) and supports diverse ichthyofauna. For example, the three primary tributaries: Rio Paraguay, Rio Uruguay and Rio Paraná support 332, 230, and 330 species, respectively (Reis et al. 2003; Albert et al. 2011). Many Rio de la Plata Basin fishes are believed sister to Amazonian taxa (Reclus 1895; Eigenmann 1906; Eigenmann et al. 1907; Pearson 1937; Carvalho & Albert 2011). This system provides a unique opportunity to investigate food web structure in a poorly-known subtropical stream with physical and possibly functional attributes similar to temperate latitude streams, but with biological communities derived from Amazonian systems.

I investigated the trophic guild structure of the Rio Cuareim, Uruguay, a major tributary to the Rio Uruguay basin, using stable isotope ratios. My objectives were to 1) determine if subtropical rivers conformed to patterns reported from temperate and/or tropical rivers, 2) compare trophic segregation at community and guild-scales (trophic and taxonomic) and 3) estimate the relative importance of basal carbon sources supporting fish biomass.

METHODS

Study Site

The La Plata basin is the second largest river system in South America and the fifth largest in the world with a 3×10^6 km^2 catchment area (Brea & Zucol 2011). It extends from Southern Uruguay to Northern Argentina and includes tropical and subtropical latitudes (Brea & Zucol 2011) and supports a diverse Gondwanan fish assemblage (Maisley 2000).

The Rio Uruguay drainage is the smallest of three main tributaries to the La Plata River, and like most of the La Plata basin, is supported by an extensive groundwater reservoir (Favetto et al. 2005; Brea & Zucol 2011). The Rio Cuareim drains southwestern Brazil and northwestern Uruguay and is a major tributary to the middle Rio Uruguay. The surrounding physiographic region is arid, subtropical prairie (Iriondo 1999; Brea & Zucol 2011). The Rio Cuareim is a clear-water piedmont river characterized by cobble substrates and has diverse and highly endemic fish communities (Lucena & Kullander 1992; Zarucki et al. 2010; Serra et al. 2011, EDB pers. obs.). For more detailed discussion of the study site see Burress et al. (Chapter 1).

Study Design

I sampled fishes in the upper Rio Cuareim basin using a combination of backpack electro-fishing, seines, cast nets and hook and line sampling. Samples were pooled across three sites in the upper basin. Fishes were immediately euthanized and frozen. I lyophilized caudal muscle tissue until dry, ground them into a homogenous powder and packed samples into tin capsules (Costech Analytical Technologies). Stable isotope ratios were analyzed at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Arizona, USA). Isotope values are written in delta notation, which represent deviation from standards: Pee Dee Belemnite (PDB) limestone and atmospheric nitrogen for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Layman et al. (2007) established a methodology for using stable isotopes to provide community-level measures of trophic structure (i.e., niche space). I established niche space using C and N isotope bi-plots to evaluate the occurrence of functional redundancy and, more

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importantly, to identify taxa that may play functionally unique trophic roles. Lack of trophic

redundancy can have dramatic community-wide ecological impacts if species are lost (Taylor

et al. 2006). I used discriminate function analysis (DFA) to determine whether species could

be classified into predefined trophic guilds and taxonomic groups (Order, Family, Species)

based on their stable isotope ratios. I determined trophic guilds for fishes from dietary

references in the literature (Table 1). To estimate the relative importance of basal resources, I

estimated the number of trophic transfers that separate each trophic guild from basal

resources: herbivores and detritivores (1), omnivores (1.5), invertivores (2), and piscivores

(2.5). I then corrected consumer isotope ratios based on fractionation rates of 0.5‰ (δ^{13}C;

DeNiro & Epstein 1978) and 2.54‰ (δ^{15}N; Vanderklift & Ponsard 2003) for each trophic

transfer. Due to assumptions involved with these corrections, I did not attempt to quantify the

importance of each basal resource and instead used the spatial relationships in a CN bi-plot to

estimate their relative importance. I deposited voucher specimens in the Auburn University

Natural History Museum and Learning Center (Auburn, Alabama, USA).

RESULTS

I sampled four basal resources and 23 species of fish (Table 2; Fig. 1). Community-

wide bi-plots of fish C and N isotope ratios display relatively large isotopic overlap between

most fishes (Fig. 1a). In contrast, discriminate function analysis revealed that fishes can be

reliably classified into both trophic guilds (70.3% classification success) and taxonomic

groups (40-58.6% classification success) based on stable isotope ratios (Tables 3 and 4).

Trophic guild membership is significantly associated with δ^{15}N ratios (R^2=0.58, F=164.33,

p<0.0001; Fig. 2) although herbivores display higher than predicted δ^{15}N ratios relative to

other guilds (Fig. 2). Large $\delta^{13}C$ variation within trophic guilds and high overlap between trophic guilds suggest that carbon sources are not useful in identifying fish trophic guilds (Fig. 2).

Approximately 80% of the omnivores were correctly reclassified using stable isotope ratios and only 20% were classified into the invertivore- and herbivore-guilds (10% each). This guild was only second to piscivores and detritivores in reclassification success (86% and 100%, respectively), demonstrating that, despite the isotopically diverse sources consumed by omnivores, they possess a guild-level isotopic identity. Unexpectedly high $\delta^{15}N$ ratios for the herbivore guild resulted in a reduced ability to discriminate herbivores from invertivores (29% of invertivores incorrectly classified as herbivores) and omnivores (25% of herbivores incorrectly classified as omnivores). Trophic guilds also display distinct isotope ratios in tropical South American rivers (Jepsen & Winemiller 2002). Course and fine taxonomic groups can also be classified but DFA was less successful at separating them (Table 3).

Based on my estimated fractionation corrections, algae may support most fish biomass (Fig. 3). However, if the food chain is longer than estimated, or fractionation rates are higher (i.e., Post 2002), the importance of detritus may be equally or more important. Macrophyte carbon only appears important to a few species (Fig. 1a) that may consume them directly (Table 2). I did not collect water samples, so we are unsure of the role of particulate organic matter (POM). However, two filter-feeding bivalves have $\delta^{13}C$ ratios of -31‰ (Chapter 2), suggesting POM is not an important basal resource to fish biomass.

DISCUSSION

My data suggest that subtropical river food webs are more analogous to tropical than temperate rivers. Like tropical systems, the fish assemblage of the Rio Cuareium has a relatively high abundance of omnivorous and mesopredatory fishes but few detritivores. The majority of sampled fish biomass was supported by algal production. Trophic guilds and taxonomic groups display unique isotope signatures in the Rio Cuareim suggesting lack of functional redundancy in some taxa, indicating they may be ecologically important.

Tropical and subtropical South America share about 35% of their fauna (Carvalho & Albert 2011) and our data suggest that those shared fauna may be important to ecosystem function. In Amazonian rivers, *Prochilodus mariae* are abundant and important for carbon flow and ecosystem function because there are no functional analogs (i.e., detritivorous fishes; Taylor et al. 2006) that occur at comparably high densities. In the Rio Cuareim, *Prochilodus lineatus* are isotopically distinct from other species sampled and are the only species to exhibit a strongly detritivorous trophic niche. Furthermore, detritivory is common in tropical systems (Jepsen & Winemiller 2007), but hyper-abundant species may be responsible for most detritus processing (Taylor et al. 2006). Stable isotope analyses indicate that few of the species sampled (i.e., abundant species) are detritivorous in subtropical systems, suggesting that *Prochilodus* may be important to subtropical ecosystem function.

Omnivory is common among fishes in tropical South America and tropical streams often have more omnivores than temperate streams (Jepsen & Winemiller 2007; Ibañez et al. 2009). Nearly half the species sampled in this study were classified as omnivores according to literature and isotope ratios, suggesting that Rio Cuareim omnivory rates are similar to South American tropical streams and higher than Northern hemisphere temperate systems

(Ibañez et al. 2009). Omnivory may be an adaptive response to fluctuations in resource availability or poor resource predictability (Winemiller 1989; Jepsen & Winemiller 2002). This suggests both tropical and subtropical South American streams may have similar variation in resource availability. However, seasonal fluxes of terrestrial subsidies from floodplains in tropical streams (Sensu Winemiller & Jepsen 1998) are unlikely in arid subtropical South America (Iriniondo 1999) and may be replaced by seasonal temperature shifts that influence autochthonous resources (e.g., algae, macrophyte biomass).

Both temperate and tropical systems support numerous piscivorous species (Winemiller 1989; Mittelbach & Persson 1998; Layman et al. 2005). However, the isotopic relationships of predator guilds appear to vary across biogeographic regions. Winemiller (1989) classified *Serrasalmus* (Piranha) as shearers, and *Hoplias* as engulfers based on their feeding modes. In tropical river systems, Jepsen & Winemiller (2002) found that these taxa displayed similar isotopic signatures despite the differences in feeding mode. In contrast, we found that these two feeding modes segregated strongly in δ^{13}C ratios, with the three engulfers (*Hoplias lacerdae*, *Crenicichla celidochilus*, *C. missioneira*) displaying relatively higher proportions of δ^{13}C compared to the two shearers (*Serrasalmus spilopleura*, *Oligsarcus robustus*). Biogeographic variation in the carbon range of basal resources or importance of basal resources likely influences these relationships.

Predator assemblages also reveal differences between temperate, tropical and subtropical systems. Piscivores in temperate and tropical rivers tend to be large-bodied (Mittelbach & Persson 1998; Layman et al. 2005). In contrast, pike cichlids (*Crenicichla*) consume large proportions of fish (Layman et al. 2007; Burress et al. (Chapter 4)) and are modestly sized (99-209 mm SL); indeed they are 2-4x smaller than piscivores in temperate

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(reviewed in Mittelbach & Persson 1998) and tropical systems (Layman et al. 2005). *Crenicichla* also occur in tropical systems, however, species diversity is often low (Layman 2004). I am unaware of a functional equivalent in temperate taxa, especially a taxon that has such high diversity. Temperate sunfishes (*Lepomis*) may be superficially similar in that they are abundant and often co-occur. Subtropical taxa that are more analogous to sunfish might include deep-bodied, laterally compressed forms that are not strictly carnivorous, such as *Gymnogeophagus* or *Australoheros* (Cichlidae). *Micropterus* (black basses) sometimes co-occur and are often strongly piscivorous, but grow 2-4x larger than *Crenicichla* (Mittelbach & Persson 1988) and may be more analogous to subtropical South American *Hoplias* (wolf fish) or tropical South American *Cichla* (peacock bass; Layman et al. 2005).

Our data suggest that the 35% of taxa shared by tropical (Amazonian) and subtropical (Rio de la Plata) drainages are important for ecosystem function. *Prochilodus* may be important for moderation of carbon flow as has been observed in tropical rivers (Taylor et al. 2006). Suites of mesopredatory species, such as *Crenicichla,* that occur in high diversity and abundance in subtropical rivers (Lucena & Kullander 1992; Pialek et al. 2011; Serra et al. 2011) likely control community structure via predation pressure as they do in tropical systems (Reznick et al. 1990; Johansson et al. 2004). Furthermore, armored catfishes (Loricariidae) may be key consumers of resources of limited availability to fish consumers (i.e., wood and macrophytes). Taxa that are unique to subtropical systems (i.e., *Gymnogeophagus, Australoheros*) are largely generalist omnivores, and this may limit their influence on ecosystem-wide processes, in contrast to predators and species that directly exploit basal resources (Reznick et al. 1990; Johansson et al. 2004; Taylor et al. 2006).

Basal resources ultimately support fish biomass. According to our estimate of fractionation corrected isotope ratios for each trophic guild, the Rio Cuareim fish community is largely supported by algae. However, it is also possible that detritus plays an important role in this system. Algal carbon is widely considered the most important carbon source for consumers (Thorp & Delong 1994; Thorp et al. 1998; Lewis et al. 2001; Douglas et al. 2005). Recent evidence suggests terrestrial carbon may be important in some systems (Zeug & Winemiller 2008), particularly when seasonality produces frequent transport of allochthonous resources into the river channel (Funk et al. 1989). Autochthonous material is considered more easily assimilated than allochthonous material (Thorp & Delong 1994) and indeed, few species that we sampled displayed detritivorous isotopic signatures.

Subtropical South American streams share characteristics with northern hemisphere temperate and tropical streams, including algal supported fish biomass. High frequency of omnivory is shared with tropical streams and detrital processing may be primarily performed by invertebrates, like in temperate streams. Charasteristics of apex predators may be unique to subtropical South American streams. Small-bodied piscivores are abundant and diverse in the Rio Cuareim. Specifically, the almost exclusively endemic pike cichlid diversity, with 24 valid species (Pialek et al. 2011 and references therein) and numerous potentially undescribed species (Varella 2011) define subtropical South American ichthyofauna. Furthermore, if predation pressure by pike cichlids in subtropical streams mirrors predation pressure in species-poor tropical systems (Reznick et al. 1990; Layman 2004; Johansson et al. 2004), they are ecologically important and may shape community structure and evolution.

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TABLES AND FIGURES

TABLES

Table 1. Trophic guild assignment for fish taxa and references used.

Trophic guild	Taxa	Reference
Omnivore	*Bryconamericus*	Russo et al. 2004
Omnivore	*Leporinus*	Horeau et al. 1998
Invertivore	*Heptapterus*	Horeau et al. 1998
Omnivore	*Trachelyopterus*	Moresco & Bemvenuti 2005
Omnivore	*Astyanax*	Esteves 1996
Piscivore	*Hoplias*	Winemiller 1989
Piscivore	*Serrasalmus*	Winemiller 1989
Omnivore	*Rhamdia*	Winemiller 1989
Omnivore	*Gymnogeophagus*	Yafe et al. 2002
Omnivore	*Australoheros*	Yafe et al. 2002
Piscivore	*Oligosarcus*	Nunes & Hartz 2006
Detritivore	*Prochilodus*	Taylor et al. 2006
Herbivore	*Hypostomus*	Nonogaki et al. 2007
Herbivore	*Rineloricaria*	Jepsen & Winemiller 2002
Herbivore	*Ancistrus*	Power 1984; 1990
Piscivore/Invertivore	*Crenicichla*	Lucena & Kullander 1992

Table 2. Stable isotope ratios (mean±1 SD) of fishes in the Rio Cuareim, Uruguay. Samples are representative of adult size-classes.

Taxa	n	C/N	$\delta^{13}C$	$\delta^{15}N$
Piscivore				
Crenicichla missioneira	13	3.17	-19.0±0.6	12.2±0.5
Crenicichla celidochilus	10	3.16	-18.9±0.9	12.9±0.1
Hoplias lacerdae	3	3.18	-18.1±1.1	14.1±0.9
Oligosarcus robustus	2	3.25	-20.6±0.7	13.5±0.5
Serrasalmus spilopleura	2	3.21	-20.4±0.6	13.5±0.1
Invertivore				
Crenicichla scottii	11	3.15	-19.9±0.3	11.7±0.2
Crenicichla minuano	18	3.14	-22.1±0.6	11.4±0.5
Crenicichla lepidota	15	3.17	-20.9±1.3	11.3±0.1
Heptapterus mustelinus	2	3.44	-20.6±0.6	12.2±0.1
Crenicichla tendybaguassu	5	3.17	-18.5±0.5	11.7±0.3
Omnivore				
Gymnogeophagus gymnogenys	7	3.84	-17.2±1.3	9.4±0.7
Gymnogeophagus cf. *meridionalis*	4	3.18	-18.9	10.9
Gymnogeophagus meridionalis	1	3.18	-20.6±1.1	10.7±0.1
Australoheros scitulus	5	3.19	-18.3±0.6	10.5±0.3
Astyanax sp.	5	3.27	-21.6±0.6	10.8±0.6
Leporinus amae	3	3.11	-19.5±0.5	10.8±0.6
Bryconamericus iheringi	6	3.44	-19.8±1.0	10.5±0.3
Rhamdia quelen	1	3.15	-21.6	10.9
Trachelyopterus galeatus	1	3.20	-22.1	10.8
Herbivore				
Ancistrus taunayi	5	3.24	-25.6±1.5	11.3±0.5
Rineloricaria cf. *misionera*	3	3.30	-18.0±0.2	11.1±0.4
Hypostomus uruguayensis	1	3.16	-16.7	9.9
Detritivore				
Prochilodus lineatus	2	3.82	-21.9±0.6	8.3±0.5

Table 3. Classification success (%) from discriminate function analysis of trophic guilds

(according to the literature) and taxonomic groups using stable isotope ratios (δ^{13}C and δ^{15}N).

Model	Guild	Order	Family	Species
Reclassification success (%)	70.3	58.6	40	51.7

Table 4. Reclassification success of trophic guild membership of fishes in the Rio Cuareim, Uruguay using discriminate function analysis. Guild membership is based on literature and predicted guild membership is based on stable isotope ratios (δ^{13}C and δ^{15}N).

Guild	Predicted Guild Membership (%)				
	Piscivore	Invertivore	Omnivore	Herbivore	Detritivore
Piscivore	85.7	14.2	0	0	0
Invertivore	14.5	52.7	3.6	29.1	0
Omnivore	0	10.5	78.9	10.5	0
Herbivore	12.5	0	25	62.5	0
Detritivore	0	0	0	0	100
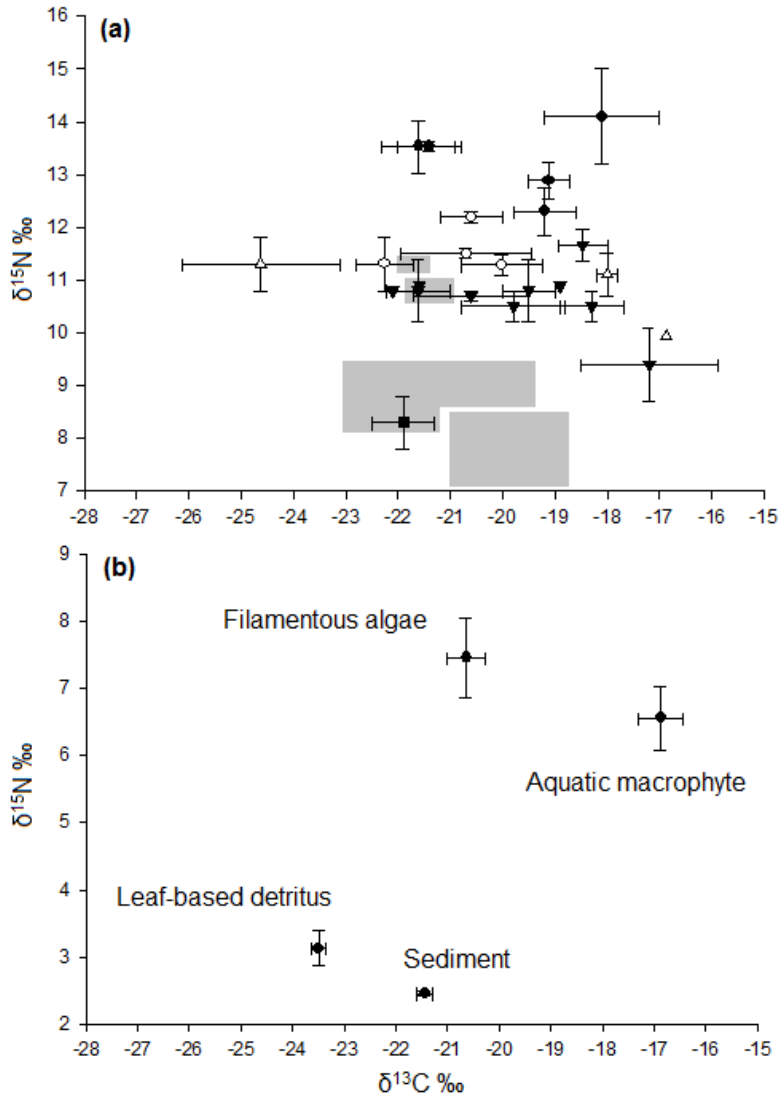
Figure 1. Stable isotope (mean±1 SD) bi-plot showing the trophic relationships of a) fishes: piscivores (◆), invertivores (○), omnivores (▼), herbivores (△), detritivores (■) and b) basal resources in the Rio Cuareim, Uruguay. Shaded areas represent trophic space occupied by invertebrates (see Chapter 2).
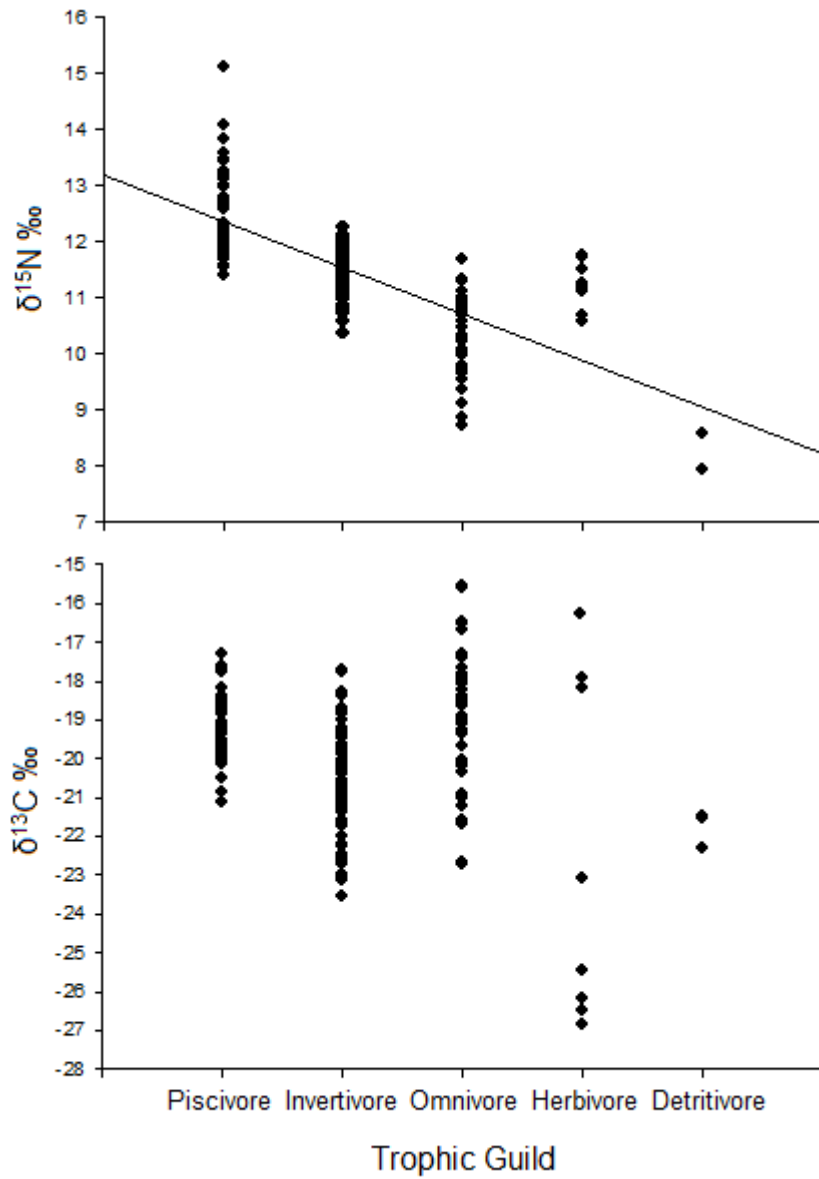
Figure 2. The isotopic relationship between trophic guild and δ^{15}N ratios for fishes in the Rio Cuareim, Uruguay. Each point represents an individual. Guild assignment is based on previous literature and stable isotope ratios (δ^{13}C and δ^{15}N).
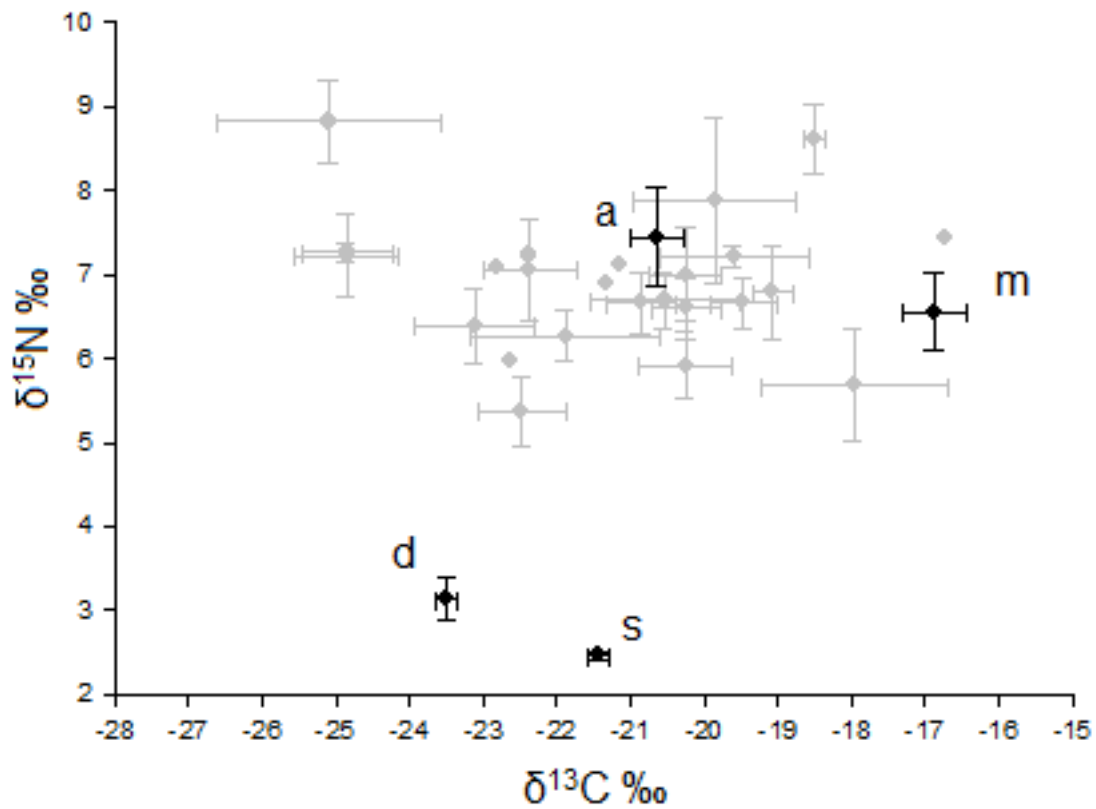
Figure 3. Stable isotope bi-plot (mean±1 SD) of basal resources: algae (a), macrophytes (m),

detritus (d), and sediment (s). Gray-scale points are fishes after correction for fractionation

based on an estimated number of trophic transfers: herbivores and detritivores (1), omnivores

(1.5), invertivores (2), and piscivores (2.5).

CHAPTER 4

TROPHIC RELATIONSHIPS AMONG SIX SYMPATRIC PIKE CICHLIDS
(CICHLIDAE: *CRENICICHLA*) IN A SUBTROPICAL SOUTH AMERICAN STREAM

ABSTRACT

The mechanism by which species cope with high degrees of resource overlap among diverse assemblages of functionally similar congeners is poorly understood. The utility of ontogenetic niche shifts in reducing competition has only been reported in limited contexts (e.g., between two species). I analyzed $\delta^{13}C$ and $\delta^{15}N$ ratios of six sympatric mesopredatory fishes (*Crenicichla*) along an ontogenetic continuum in a subtropical river. Isotopic overlap was high in early life stages (young of year and sub-adults) and diffuse in later life stages. Four species displayed ontogenetic niche shifts, which reduced isotopic overlap with congeners in all cases. Additionally, all species displayed reductions in isotopic variability and $\delta^{13}C$ range in later life stages, suggesting trophic specialization has an ontogenetic component. Stomach content analysis and mixing model estimations of prey contributions confirm high trophic fidelity and low diet overlap among sympatric *Crenicichla*. In combination, dietary niche shifts and trophic specialization reduce dietary overlap in later life stages.

INTRODUCTION

Competition is common among closely related species that often have conflicting resource needs (Werner and Hall 1977, Mittelbach 1984, Osenberg et al. 1992). For example,

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when competition for resources is high in early life stages ontogenetic niche shifts often reduce resource-based competition in later life stages (Mittelbach 1984, Osenberg et al. 1992). Our understanding of trophic interactions in is largely confined to comparisons of two species (Mittelbach 1984, Osenberg et al. 1992) and in response to species introductions (Crowder 1984). However, resource partitioning is often considered evidence of past competition (Pianka 1974), thus resource partitioning has been well studied at community-scales (Goldschmidt et al. 1990, Bootsma et al. 1996, Bouton et al. 1997, Campbell et al. 2003).

Stable isotope ratios are useful in trophic studies because they reflect dietary patterns over long time periods (Weidel et al. 2011). An organism's niche is in part derived from its trophic interactions (Elton 1927) and stable isotopes represent the accumulation of trophic pathways through an organism. Measures of niche width (Bearhop et al. 2004) and niche size (Layman et al. 2007) have been developed using stable isotope ratios. Thus, stable isotope studies provide time-integrated insight into niche characteristics of an organism. Ontogenetic niche shifts by fishes have been shown using stable isotopes (Grey 2001, Post 2003, Genner et al. 2004). However, diet shifts were frequently associated with intraspecific contexts such as increased growth and survivorship after switching to piscivory (Mittelbach and Persson 1998, Post 2003) and not into a broad community-wide context.

I investigated the isotopic relationships of six sympatric pike cichlids (*Crenicichla*) along an ontogenetic continuum in a sub-tropical river. I chose this system because it contains a diverse community of highly similar species, both morphologically (Lucena and Kullander 1992) and phylogenetically (Pialek et al. 2011), among which resource-based competition should be important. My objective was to investigate the trophic relationships of

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these species across an ontogenetic continuum and compare niche size and trophic roles among this diverse assemblage of sympatric congeners.

METHODS

Study site

The Rio Uruguay is a species-rich river that encompasses tropical and subtropical latitudes (Brea and Zucol 2011) and supports notably high endemism (Lucena and Kullander 1992, Kullander et al. 2010). The Rio Cuareim drains southwestern Brazil and northwestern Uruguay and is a major tributary to the middle Rio Uruguay. The surrounding landscape is subtropical and dry (Iriniondo 1999) and is supported by an extensive groundwater reservoir (Brea and Zucol 2011) and consists of open prairie and ranch land. The Rio Cuareim is a clear-water, piedmont stream and is characterized by a cobble substrate and fish biomass is largely supported by autochthonous-derived carbon (Chapter 3). The Rio Cuareim basin supports a diverse suite of fishes, many of which are species of conservation concern, including many Rio Uruguay endemics (Soutullo et al. 2009, Zarucki et al. 2010, Kullander et al. 2010, Serra et al. 2011). The Rio Cuareim shares many species with the upper Rio Uruguay, and should therefore be representative of the entire drainage (Zarucki et al. 2010).

Study species

The Rio Cuareim supports six species of pike cichlid: *C. celidochilus* (CRCE), *C. lepidota* (CRLE), *C. minuano* (CRMIN), *C. missioneira* (CRMIS), *C. scottii* (CRSC) and *C. tendybaguassu* (CRTE) (Serra et al. 2011). *Crenicichla* are morphologically constrained to

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an elongate body shape (Lucena and Kullander 1992), are generally considered rheophilic (Kullander et al. 2010), and display genetic divergence of only 1-2% (Kullander et al. 2010, Pialek et al. 2011). These species (except for *C. lepidota*) are endemic to the Rio Uruguay drainage (Lucena and Kullander 1992) and are distributed throughout the upper, middle and lower Rio Uruguay (Kullander et al. 2010, Pialek et al. 2011, Serra et al. 2011). *Crenicichla* are among the most abundant fishes in subtropical South America, particularly in tributaries (Lucena and Kullander 1992; Kullander et al. 2010). All six species are sympatric even at microhabitat-scales and often form mixed species aggregations (Serra et al. 2011, Pialek et al. 2011) suggesting that habitat segregation does not occur.

The predation impacts of *Crenicichla* are well studied, particularly their effects on guppy life history (Reznick et al. 1990, Johansson et al. 2004). Additionally, these studies have largely focused on a single species in Trinidad. Thus, our insight into their ecology is extremely limited in scope considering the importance and success of the group throughout South America. *Crenicichla* is the most speciose genus of Neotropical cichlid (Cichlidae) with 85 valid species (Pialek et al. 2011). The diet of several Amazonian species has been described (Layman et al. 2005, Gibran et al. 2006; Montana and Winemiller 2009), however, *Crenicichla* occur in comparatively high diversities in subtropical drainages. There they also display high degrees of sympatry and endemism (Lucena and Kullander 1992, Kullander et al. 2010), providing an interesting system to study their ecology in a comparative context.

Sampling

I opportunistically sampled fishes (Fig. 1) in the upper Rio Cuareim basin (S 30º46, W 056º02) using a backpack electro-fisher, seines, casting nets and hook-and-line. I rinsed

and froze tissue samples for stable isotope analysis, and preserved stomachs for dissection. The most abundant potential prey items according to preliminary surveys (both fishes and invertebrates) were manually sampled for stable isotope analysis. Two functionally different prey fishes were sampled: cichlids (benthic) and characids (surface-oriented). Characids were further divided by sampling a carnivorous taxa (*Astyanax*) and herbivorous taxa (*Bryconamericus*). Basal resources were also sampled (e.g., algae, detritus, macrophytes).

Stomach content analysis

Stomachs were injected with and preserved in 10% formalin and were dissected in laboratory. I identified contents to family, quantified items using graduated cylinders (Layman et al. 2005) and calculated percent by volume (%V) and percent occurrence (%O) for each item. Additionally, individuals of *C.minuano* and *C. missioneira* were surveyed for reproductive viability via dissection and were classified as mature or immature. Individuals classified as mature were gravid or otherwise possessed enlarged gonads (Nikolsky 1963; Chapter 5).

Stable isotope analysis

Algae and macrophyte samples are homogenized sub-samples from multiple mats/plants (Table 1). Debris was removed from algae using a microscope, however, I consider my samples inclusive of associated microorganisms (i.e., Zeug and Winemiller 2008). Lyophilized caudal muscle of fishes and whole invertebrates were analyzed for natural abundance δ^{13}C and δ^{15}N isotopes at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Arizona, USA). Isotope values are expressed in delta notation

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(‰), relative to standards: Pee Dee Belemnite (PDB) limestone and atmospheric nitrogen for

$\delta^{13}C$ and $\delta^{15}N$, respectively. Consumer C/N ratios were below 3.5 (3.04-3.26), therefore we

did not correct for lipid content (i.e., Post et al. 2007). I used the IsoSource mixing model

proposed by the Environmental Protection Agency (Phillips and Gregg 2003) to estimate the

relative assimilation of prey items by consumers. The IsoSource mixing model is popular in

ecological studies (Hoeinghaus and Davis 2007, Zeug and Winemiller 2008, Taylor and

Batzer 2010) and is useful because it can estimate the contributions of many sources. This

utility is particularly valuable when estimating the diet of generalist consumers that feed on

numerous prey items. The model also has several limitations based on fractionation

assumptions and source discrimination (Phillips and Gregg 2003, Benstead et al. 2006). Dual

isotope mixing models also assume that both isotopes are assimilated equally (Lancaster et al.

2008). Collectively, stomach content analysis, and dual-isotope mixing models should

provide detailed and time integrated insight into consumer trophic niches. Sources were

chosen a priori using stomach content analysis. If an item represented less than 1% of the

stomach contents by volume, it was not included in the mixing model to avoid erroneously

attributing prey items to consumers (i.e., algae, allochthonous prey). For *Crenicichla*

tendybaguassu, I lacked adequate stomach content data, thus we referenced data from the

literature (Lucena and Kullander 1992). To test the robustness of our models to source

discrimination (the models ability to distinguish sources; Phillips and Gregg 2003, 2005) I

used discriminate function analysis, where classification success indicates isotopic

distinctness of sources.

I corrected for isotopic fractionation using rates from comprehensive experiments or

meta-analyses: 0.5‰ for $\delta13C$ (DeNiro and Epstein 1978, Post 2002) and 2.54‰ for $\delta15N$

(Vanderklift and Ponsard 2003). These fractionation rates adjusted all consumer isotope ratios into the polygon of sources (see Benstead et al. 2006) thus producing solvable models. The popular δ15N fractionation rate of 3.4‰ (Post 2002) did not provide solvable models for any species (δ15N ratios were often near basal resources). To prevent spatial variation from influencing the stable isotope analyses, fishes were sampled from a limited area (5 km reach in the upper Rio Cuareim basin) with uniform surrounding land-use. Community assemblages, physiogeography and land-use are conserved across the Rio Uruguay drainage (Iriniondo 1999, Zarucki et al. 2010, Serra et al. 2011) thus our results should have broad application to these species entire distribution range.

To evaluate ontogenetic diet shifts I classified fishes into three life stage classes: young of year (YOY), sub-adult and adult. Classes were based on maximum body size (standard length- SL) for each species according to Lucena and Kullander (1992): young of year (<30%), sub-adult (30-70%), and adult (>70%) for all species except CRLE. CRLE is the smallest species and classes had to be adjusted: young of year (<50%), sub-adult (50-80%) and adult (>80%). The age classes are approximations based on the size at which they are reproductively viable as noted during the dissection of stomach contents (reproductively viable = adult). The YOY size restrictions are based on extensive field observations by the author. I also tested relationships between body size (SL) and $\delta^{13}C$ and $\delta^{15}N$ ratios for each species using linear regressions. Body size was measured according to Lucena and Kullander (1992). We compared niche width (Bearhop et al. 2004) and niche size (Layman et al. 2007) between species. *Crenicichla tendybaguassu* are particularly rare (Serra et al. 2011) thus I did not sample enough individuals to calculate niche size, niche width or analyze ontogenetic diet shifts. However, I include them in the bi-plots for reference. Both niche width (Bearhop

et al. 2004) and niche size (Layman et al. 2007) measures are sensitive to sample size and disparity between sample sizes. With the exception of *C. tendybaguassu*, which I do not analyze using these methods, our sample sizes are similar for each species (Table 2). All statistical analyses were computed using SPSS (ver. 19.0; SPSS, Inc., Chicago, IL). Voucher specimens of all fish and some invertebrates are deposited in the Auburn University Natural History Learning Center and Museum (Auburn, Alabama, USA).

RESULTS

Body size was not correlated with δ^{13}C ratios in any species (all comparisons $p>0.05$), however, body size was significantly associated with δ^{15}N ratios in 4 of 6 species: CRCE ($n=18$, $R^2=0.905$, $F=152.276$, $p<0.0001$), CRMIS ($n=21$, $R^2=0.734$, $F=52.298$, $p<0.0001$), CRSC ($n=15$, $R^2=0.490$, $F=12.475$, $p=0.004$), and CRLE ($n=21$, $R^2=0.746$, $F=55.9$, $p<0.0001$). Niche widths (δ^{13}C SD), niche size (convex hull area) and δ^{13}C range varied between species and were associated with the diversity of prey consumed (Table 3). Although I did not sample YOY for every species, isotopic overlap generally decreased in later life stages (Fig. 1).

Stomach content analysis revealed that a single prey item often represented a large portion of the diet suggesting trophic specialization in those species (Table 4). Few prey items were obligate contributions to the mixing model (contribution ranges do not include zero), suggesting they may be particularly important to those species (Table 5). Discriminate function analysis successfully classified 87, 82, 100, 91, 91, and 100% of the prey items used in the mixing model for CRCE, CRLE, CRMIN, CRMIS, CRSC, and CRTE, respectively, demonstrating the isotopic distinctness of prey items. This suggests that our mixing model

should be robust to source discrimination (Phillips and Gregg 2003, 2005). A slight discrepancy between stomach content and mixing model estimates was observed because of the method in which I sampled potential prey items (e.g., abundant items). Several species exploited prey items that were scarce (i.e., Gerridae, Odonata), which I was not able to include in the models. However, these items were not primary prey items for any species, thus should have a limited impact on data interpretation.

The best predictor of dietary diversity (number of distinct prey items consumed) was niche size (r=0.661), followed by $\delta^{13}C$ range (r=0.401) and $\delta^{13}C$ SD (r=0.299), although our limited replication (species) prevented any comparisons from being statistically significant (all p>0.05). The number of distinct prey items consumed was significantly correlated with the maximum proportion of an item (N=5, r=0.882, p=0.048). The three measures of trophic niche varied between species and were not always agreement (Table 3).

Isotopic segregation in *Crenicichla* is achieved primarily via $\delta^{13}C$ and secondarily via $\delta^{15}N$ segregation (Table 2, Fig. 1). Turnover of $\delta^{13}C$ in fish muscle tissue in natural circumstances is very slow (Weidel et al. 2011). Coupled with stomach content data that support varying trophic roles, I do not consider isotopic turnover a major limitation to the broad application of our findings over long time-scales.

DISCUSSION

Resource partitioning has been shown in fishes at community-scales (Goldschmidt et al. 1990, Bootsma et al. 1996, Bouton et al. 1997, Campbell et al. 2003). However, most examples of resource partitioning occurring in response to competition have dealt with closely-related species (Mittelbach 1984, Osenberg et al. 1992). This suggests that

interspecific resource interactions are most important among functionally similar species (i.e., congeners). Investigations into such interactions are largely limited to two-species models (Mittelbach 1984, Osenberg et al. 1992). I demonstrate resource partitioning among a diverse group of congeneric species, and place their trophic relationships into an ontogenetic perspective. Ontogenetic diet shifts may play a key role in resource segregation in later life stages.

Convex hull areas (Layman et al. 2007), δ^{13}C SD and δ^{13}C range (Bearhop et al. 2004) provide insight into the niche size of organisms. However, they do not always present a consensus. For example, only two items were consumed by *Crenicichla celidochilus*, but their niche size (i.e., Layman et al. 2007) is larger than species that consume 11 and 13 unique prey items. The almost complete switch to piscivory in *C. celidochilus* is probably responsible for the large ontogenetic variation in δ^{15}N ratios (Post 2002) and also inflates the convex hull area. For species with less dramatic ontogenetic shifts, the different measures present a better consensus. Although I was unable to analyze niche width or niche size for *Crenicichla tendybaguassu* (see Methods) their unique morphology provides some insights into their diet. Hypertrophied lips are typically associated with generalist predators that feed upon rocky surfaces (Arnegard and Snoeks 2001, Elmer et al. 2010). Thus this species potentially has the most specialized feeding mode.

According to the competitive exclusion principle (Hardin 1960) functionally similar species cannot occur in sympatry because competition should result in either the extinction of the less competitive species or a divergence of niches (Pianka 1974). Indeed, our data demonstrate that *Crenicichla* exploit different food resources and that trophic divergence is generally greater in later life stages. Collectively, shifts in mean isotopic signatures

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(primarily δ^{15}N) and reduced isotopic variability (primarily δ^{13}C) reduce isotopic overlap in later life stages. Body size (SL) was positively correlated with δ^{15}N ratios in four species suggesting increased exploitation of fishes with increased age (Post 2002). Isotope variability decreased in later life stages, suggesting increased trophic specialization. Isotopic variability infers either reliance on a broad range of food resources or variation in the relative importance of food items within a cohort, because of potential for isotopic mixing of sources (Phillips and Gregg 2003). In general, greater isotopic variability suggests a greater variety of food items (e.g., generalist). Conversely, low isotopic variability suggests exploitation of a narrow range of food items, or a fixed relative importance of different sources (e.g., specialization). The combination of δ^{15}N enrichment and decreased isotopic variability in later life stages results in reduced isotopic overlap. Ecologically, this suggests that species may be more specialized in later life stages.

These species grow to similar sizes (Lucena and Kullander 1992), co-occur in the same habitats and often form mixed species aggregations (Serra et al. 2011, Pialek et al. 2011). Consequently, differences in size and habitat segregation do not explain the niche shifts or resource segregation. Resource partitioning is often considered an evolutionary response to past competition (Brown and Wilson 1956, Pianka 1974, Mittelbach 1984). Considering the well-established importance of trophic interactions among closely related species (Mittelbach 1984, Osenberg et al. 1992) resource divergence may function to reduce competitive interactions among a diverse group of congeners. Experimental designs and investigations into resource limitation would further elucidate potential trophic interactions of these species.

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TABLES AND FIGURES

TABLES

Table 1. Stable isotope ratios (mean±1 SD) for basal resources in the Rio Cuareim, Uruguay.

Basal Resource	C/N	$\delta^{13}C$	$\delta^{15}N$
Macrophyte	23.0±2.2	-16.9±0.5	6.6±0.6
Detritus	22.9±1.4	-23.5±0.1	3.1±0.3
Filamentous Algae	12.1±0.1	-20.6±0.4	7.5±0.6
Sediment	8.1±0.1	-21.4±0.2	2.5±0.1

Table 2. Stable isotope ratios (mean±1 SD) for sympatric *Crenicichla* in the Rio Cuareim, Uruguay. All size classes are pooled. Standard lengths (SL) are mean (range). Standard deviation of C/N <0.05 in all cases.

Species	*n*	SL (mm)	C/N	δ^{13}C	δ^{15}N
Crenicichla celidochilus	18	105 (43-165)	3.16	-18.9±0.7	12.3±0.9
Crenicichla cf. *lepidota*	21	80 (43-109)	3.17	-20.6±1.3	11.0±0.5
Crenicichla minuano	18	91 (70-139)	3.14	-22.1±0.8	11.4±0.5
Crenicichla missioneira	21	111 (48-163)	3.17	-19.1±0.6	11.9±0.5
Crenicichla scottii	15	122 (79-180)	3.15	-19.8±0.6	11.6±0.3
Crenicichla tendybaguassu	5	86 (82-91)	3.17	-18.5±0.5	11.7±0.3

Table 3. Five different measures of the trophic niche: δ^{13}C SD and δ^{13}C range (Bearhop et al. 2004), convex hull area (Layman et al. 2007) and the number of distinct prey items consumed and the proportion of the primary prey item. Prey items include only those that represent >1% of the stomach contents by volume. All measures include all size classes and thus represent the ontogenetic trophic niche.

Taxa	δ^{13}C SD	δ^{13}C Range	Convex Hull Area	Number of Prey Items Consumed	Maximum Proportion of Prey Item
Crenicichla celidochilus	0.7	2.52	5.25	2	90.6
Crenicichla lepidota	1.3	4.08	4.72	11	32.6
Crenicichla minuano	0.8	2.87	3.26	8	48.1
Crenicichla missioneira	0.6	2.14	3.37	6	37.7
Crenicichla scottii	0.6	2.47	1.45	13	26.9
Crenicichla tendybaguassu	0.5	--	--	--	--

Table 4. Stomach content analyses (% by volume / % occurrence) for *Crenicichla celidochilus* (CRCE), *C. missioneira* (CRMIS), *C. scottii* (CRSC), *C.* cf. *lepidota* (CRLE) and *C. minuano* (CRMIN) sampled from the Rio Cuareim, Uruguay. Copepods, cladocerans, and ostracods are pooled under microcrustacea. Adult chironomids, Trichoptera, and Ephemeroptera are pooled under terrestrial insects. Items representing 0.1-0.99 percent by volume are indicated <1. Items representing < 0.1% by volume are not listed. Primary items are highlighted in gray.

Taxa	CRCE	CRMIS	CRSC	CRLE	CRMIN
	n=20	n=31	n=19	n=26	n=19
Fishes					
Cichlidae	<1 / 45	33.9 / 35.5	1.1 / 15.8	<1 / 19.2	<1 / 10.5
Characidae	90.6 / 35	37.7 / 48.4	14.9 / 31.6	32.6 / 34.6	
Loricariidae	<1 / 5				
Auchenipteridae		<1 / 3.2			
Pimelodidae			3.9 / 5.3		
Unidentified	<1 / 15	4.6 / 9.7	4.9 / 10.5		
Invertebrates					
Decapoda					
Aeglidae		<1 / 6.4	16.6 / 10.5	1.5 / 3.8	
Trichodactylidae		16.9 / 6.5	26.9 / 31.6	2.3 / 15.4	
Microcrustacea					<1 / 21.1
Gastropoda					
Cochliopidae					3.1 / 26.3
Ampularidae					6.7 / 36.8
Ansilidae					6.9 / 42.1
Odonata					
Gomphidae		1.8 / 6.5	9.1 / 15.8	19.8 / 26.9	
Libellulidae			7.2 / 5.3		
Coleoptera					
Elmidae				<1 / 11.5	
Psephenidae			<1 / 5.3		
Heteroptera					
Gerridae				30.7 / 23.1	
Hemiptera					
Naucoridae				2.0 / 3.8	
Chelicerata					
Lycosidae	< 1 / 20			1.9 / 11.5	
Diptera					
Chironomidae	<1 / 20			<1 / 38.5	6.9 / 7.8

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Table 4. Continued.

Taxa	CRCE	CRMIS	CRSC	CRLE	CRMIN
Trichoptera					
Leptoceridae		<1 / 16.1		2.4 / 26.9	7.4 / 68.4
Hydropsychidae			1.3 / 26.3		
Ephemeroptera					
Leptophlebiidae		3.6 / 48.4	5.5 / 63.2	1.2 / 19.2	
Baetidae	6.3 / 40	<1 / 41.9	2.2 / 42.1	3.5 / 38.5	6.9 / 68.4
Caenidae	<1 / 42.1		1.7 / 31.6	<1 / 15.4	11.4 / 42.1
Terrestrial Insects				19.3 / 23.1	
Bivalvia					
Corbiculidae			3.5 / 15.8		48.1 / 26.3

Table 5. Percent isotopic contributions of prey items to *Crenicichla* diets estimated using a dual-isotope mixing model. Values are mean percent contribution (1st – 99th percentile). *C. tendybaguassu* (CRTE), *C. celidochilus* (CRCE), *C. missioneira* (CRMIS), *C. scottii* (CRSC), *C. lepidota* (CRLE) and *C. minuano* (CRMIN). Aeglidae and Trichodactylidae are pooled under Crustacea.

|	CRTE	CRCE	CRMIS	CRSC	CRLE	CRMIN
Fishes						
Cichlidae YOY	32 (17-48)	-	29 (8-48)	7 (0-21)	-	-
Bryconamericus	-	71 (48-96)	19 (0-59)	11 (0-38)	0.3 (0-2)	-
Astyanax	-	11 (0-29)	11 (0-37)	11 (0-37)	0.6 (0-3)	-
Crustacea	-	-	13 (0-36)	31 (3-59)	6 (0-10)	-
Larval Insects						
Leptophlebiid	22 (0-55)	18 (0-46)	14 (0-48)	23 (0-72)	11 (0-41)	37 (0-75)
Hydropsychid	22 (0-54)	-	13 (0-45)	17 (0-54)	2 (0-8)	29 (0-60)
Terr. Insects	-	-	-	-	77 (48-92)	-
Mollusca						
Corbiculidae	-	-	-	-	3 (0-9)	23 (20-25)
Plant Material	11 (0-23)	-	-	-	-	11 (3-18)
Algae	13 (0-32)	-	-	-	-	-
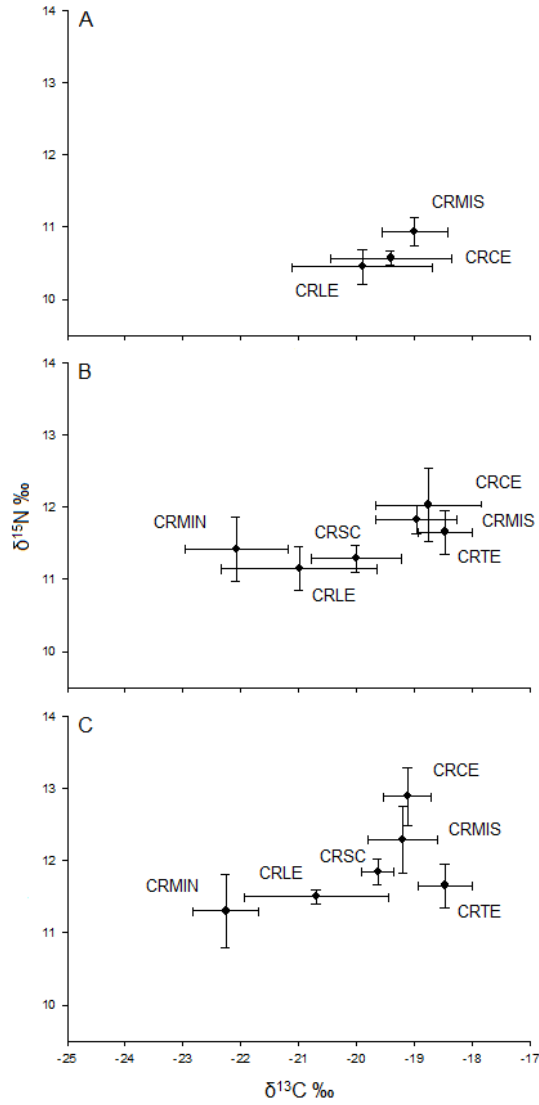
Figure 1. Stable isotope ratios (mean±1 SD) of A) young of year *Crenicichla* (*n*=10), B) sub-adult *Crenicichla* (*n*=55) and C) adult *Crenicichla* (*n*=38): *Crenicichla celidochilus* (CRCE), *C. missioneira* (CRMIS), *C. minuano* (CRMIN), *C. scottii* (CRSC), *C. lepidota*, and *C. tendybaguassu* (CRTE).

CHAPTER 5

ECOLOGICAL SPECIATION OF A RIVERINE SPECIES FLOCK

ABSTRACT

The role of ecological divergence in evolutionary processes in poorly understood.

Resource-based divergence may have important functions in both allopatric and sympatric

speciation events. Here, I demonstrate that a diverse species flock that displays poor genetic

divergence also exhibits discrete skull and lower pharyngeal jaw shapes and discrete trophic

niches. These data demonstrate the presence of morphological and ecological species well

before genetic divergence. Furthermore, the discrete nature of both morphology and trophic

niches suggests that species mate assortatively. Using a combination of biogeographic

evidence and modeling, I demonstrate the improbability of an allopatric circumstance and

propose that the radiation of a diverse riverine species flock occurred via ecological selection

during sympatry.

INTRODUCTION

The role of disruptive ecological selection in speciation events is currently a

contentious topic in ecology (reviewed in Rundle & Nosil 2005). Ecological selection has

been shown to create genetic barriers in experimental systems (Rice & Hostert 1993) and is

generally accepted as playing a role in adaptive radiations (Schluter 2000; Coyne & Orr

2004; Rundle & Nosil 2005). Lacustrine cichlids are evolutionary model systems (Kocher

2004; Seehausen 2006) and encompass the best-known examples of sympatric speciation:

African rift valley (Kocher 2004; Salzburger & Meyer 2004), Cameroon (Schiewen 2005) and Central America (Barluenga et al. 2006; Geiger et al. 2010). Sexual selection is considered the primary mechanism to African rift valley cichlid diversity (Kocher 2004), which may or may not be ecologically based (Rundle & Nosil 2005). Disruptive selection for food resources is a less widely accepted mechanism (Kocher 2004) but African rift valley lakes are coincidently model systems for resource partitioning studies (Goldschmidt *et al.* 1990; Genner et al. 1999; Sturmbauer *et al.* 1993; Bootsma *et al.* 1996). Resource segregation has also been a key factor in other examples of sympatric speciation (Barluenga *et al.* 2006).

Species flocks are monophyletic assemblages of closely related species that occur in sympatry and display high degrees of endemism (Salzburger & Meyer 2004). Examples of species flocks, much like sympatric speciation, have been described in lacustrine systems (Kocher 2004; Salzburger & Meyer 2004; Geiger *et al.* 2010). Recently, riverine species flocks have been found in subtropical South America (Lucena & Kullander 1992; Kullander *et al.* 2010; Pialek *et al.* 2011).

Pialek *et al.* (2011) showed that a diverse assemblage of pike cichlids (Cichlidae: *Crenicichla*) display <2% genetic divergence and are monophyletic. This species flock is endemic to the Rio Uruguay drainage and sympatric, often forming mixed species aggregations (Kullander *et al.* 2010; Pialek *et al.* 2011; Serra *et al.* 2011). This system presents a unique opportunity to evaluate ecological selection because a diverse assemblage of species displays intra-specific genetic variation, indicating they are early in their divergence. This species flock also includes two species that are genetically indistinguishable (Pialek *et al.* 2011), yet these species are readily identifiable in the field based on phenotype

and meristics (see Lucena & Kullander 1992). Indeed, because there are no dispersal barriers and because these fishes are proficient at navigating rheophilic obstacles (Kullander *et al.* 2010), several authors have proposed that this species flock may have resulted from sympatric speciation (Kullander *et al.* 2010; Pialek *et al.* 2011). Here, we use a combination of morphological analyses of the whole body and lower pharyngeal jaws, coupled with stomach content and stable isotope analyses to investigate the possible role of ecological selection in the radiation of a diverse species flock.

METHODS

Sampling protocols and stable isotope and stomach content analyses are as previously outlined in Chapter 4.

Morphometrics

I used a shape principal component analysis (PCA) of the whole body and lower pharyngeal jaw to investigate biologically meaningful shape differences between species. I used 12 landmarks that describe the shape of the body and 10 landmarks that describe the shape of the lower pharyngeal jaw. I superimposed landmarks and aligned them with the generalized Procustes superimposition procedure (Bookstein 1991; Monteiro & Reis 2000) producing consensus configurations for the whole body and lower pharyngeal jaw. I used thin-plate splines to calculate interpolation functions (principal warps) among landmarks. I preformed principal component analyses over the partial warp matrices and generated uniform component scores with the software tpsRelw, Version 1.49 (Rohlf 2010) that represent the most important shape differences among species. To control for size effects, we

performed a MANCOVA using centroid size as a covariate. Centroid size was calculated using the tpsRegr software (Rohlf 2011).

RESULTS

Stomach content analysis revealed that species exploit different food resources (Chapter 4). *Crenicichla missioneira* is a generalist carnivore, consuming two functionally different fishes (cichlids and characids), large littoral crustaceans, and larval invertebrates. In contrast, *C. celidochilus* is a characid specialist (91% of diet) and *C. minuano* is a mollusk specialist (70% of diet). I examined the stomachs of five *C. tendybaguassu* and found insect larvae, snails, plant material and algae. Stomach content analysis of *C. tendybaguassu* by Lucena & Kullander (1992) reported insect larvae, snails and adult insects. The hypertrophied lips of *C. tendybaguassu* provide evidence of foraging via grazing rock surfaces and suggest an omnivorous diet (Arnegard & Snoeks 2001; Elmer *et al.* 2010).

Each species has distinct stable isotope ratios (Fig. 2c) indicating they derive nutrients from different sources. Mixing models confirm stomach content data and demonstrate that these species are assimilating C and N from different prey items (Chapter 4). The omnivorous diet of *C. tendybaguassu* is resolved by the contribution of plant material in the mixing model (see Chapter 4). The model had no solution without the inclusion of plant material as a source, indicating assimilation of plant material was the only explanation for the combined low $\delta^{15}N$ and enriched $\delta^{13}C$ ratios (*C. tendybaguassu* is more $\delta^{13}C$ enriched than all other potential prey items).

Although body shape is generally conserved between species (Fig. 2a), trophic structures, such as the skull and jaws, demonstrate a high degree of inter-specific variation (Figs. 2b and 3) and suggest the importance of functional adaptation. Jaw orientation is also related to trophic function (Figs .1 and 3) and varies greatly between species. While *C. minuano* has isognathous jaws, *C. celidochilus* and *C. missioneira* have prognathous lower jaws and *C. tendybaguassu* has prognathous upper jaws (Fig. 1). Lower pharyngeal jaw shape is dramatically different among species (Figs. 3 and 4) confirming the distinct trophic roles revealed by dietary analyses. Two species that are genetically indistinguishable: *C. minuano* and *C. missioneira* (Pialek *et al.* 2011) also display different body sizes at maturation (Fig. 5).

DISCUSSION

The *Crenicichla* species flock displays traits characteristic of ecological selection: morphological changes restricted to trophic structures, divergence of the pharyngeal jaws, diet partitioning and trophic specialization. Furthermore, discrete morphological and ecological species without transitional forms suggests that assortative mating is strong. I suggest that disruptive selection for trophic divergence played a key role in the radiation of this diverse assemblage.

Although rarely demonstrated, ecological selection likely leads to speciation (Schluter 2000; Coyne & Orr 2004; Rundle & Nosil 2005). Resource segregation is often associated with examples of sympatric speciation (Barluenga *et al.* 2006). Lacustrine species flocks, in particular, are model systems for both evolutionary (Salzburger & Meyer 2004; Kocher 2004) and ecological partitioning (Goldschmidt *et al.* 1990; Genner *et al.* 1999; Sturmbauer

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et al. 1993; Bootsma *et al.* 1996). Our data suggest that ecological selection has likely caused the radiation of a riverine species flock. In this closely related assemblage of pike cichlids, species display strict partitioning of dietary resources and several species display trophic specialization. Both diet partitioning and trophic specialization are expected to contribute to disruptive ecological selection (Martin & Pfennig 2009). Moreover, this type of incongruence between morphological and phylogenetic divergence is consistent with ecological selection (Roy *et al*. 2007) and suggests that the radiation originated via resource partitioning (Schluter 2000; Roy *et al.* 2007; Martin & Pfennig 2009). For example, two of the most genetically-divergent species (*C. celidochilus* and *C. missioneira*) have highly conserved whole-body morphology while the two species with the least genetic divergence (*C. missioneira* and *C. minuano*) display the most dramatic morphological and ecological divergence. The strongly species-specific pharyngeal jaw morphology and associated trophic roles suggest that these trophic roles are fixed across temporal scales.

I show distinct skull and pharyngeal jaw shapes and dramatically different dietary patterns in the two species that are genetically indistinguishable (*Crenicichla missioneira* and *C. minuano*; Pialek *et al.* 2011). Pharyngeal jaws are highly associated with trophic function (Liem 1874; Galis & Drucker 1996). High plasticity of the pharyngeal mill and associated musculature indicate that the structures' shape corresponds with functional use (e.g., dietary patterns) (Mittelbach *et al.* 1999; Hegrenes 2001). Although skull shape is considered conserved, diet-induced skull plasticity is also well documented (Meyer 1987; Wimberger 1991; Wintzer & Motta 2005) demonstrating the ecological basis for divergence of internal and external trophic structures. Most importantly, these two species display distinct trophic niches; *C. minuano* eats bivalves and snails while *C. missioneira* eats fish and invertebrates

(Chapter 4). Furthermore, these two species differ in their size at maturation. The divergence of form, function and reproductive traits suggests that disruptive selection for exploiting varying trophic resources may have resulted in reproductive incompatibility (Kocher 2004) or facilitation of assortative mating (Doorn *et al.* 2009). The lack of intermediate forms, in respect to both form and function, strongly suggests that assortative mating is well established. Otherwise, discrete species-specific traits would rapidly erode (Coyne & Orr 2004).

The genetic similarity among this species flock, their comprehensive sympatric distribution throughout the Rio Uruguay drainage, and their tendency to form mixed species aggregations (Lucena & Kullander 1992; Kullander *et al.* 2010; Pialek *et al.* 2011; Serra *et al.* 2011) presents a possible scenario for sympatric speciation. The timeline for the divergence of these species is coincidently congruent with current estimations for the radiation of African rift valley species flocks (~2 My; Kocher 2004). Pialek *et al.* (2011) report a single vicariant event (Mocona Falls) that separates the upper and lower Rio Uruguay. Kullander *et al.* (2010) provide evidence that, above Mocona falls, there are no additional dispersal barriers and argue the high likelihood of downstream dispersal by these rheophilic species. The four species analyzed here occur above and below Mocona Falls, providing a scenario in which an allopatric separation of the two genetically-indistinguishable species (*C. missioneira* and *C. minuano;* Pialek *et al.* 2011) is highly unlikely. However, the complex biogeographic history of the Rio Uruguay drainage (Albert & Carvalho 2011) may prevent researchers from ever absolutely discounting the role of allopatric speciation in this riverine species flock.

The frequency of resource partitioning among sister taxa (Barluenga *et al.* 2006; Roy *et al.* 2009; Martin & Pfennig 2009), between sympatric congeners (Mittelbach 1984; Mittelbach 1988; Osenberg *et al.* 1992) and among species flocks (Goldschmidt *et al.* 1990; Genner *et al.* 1999; Sturmbauer *et al.* 1993; Bootsma *et al.* 1996) collectively elucidate the important role that ecological selection can play in adaptive radiations. Particularly impressive is the growing evidence for the rapid diversification that can result from disruptive ecological selection (<2 My; Salzburger & Meyer 2004; Kocher 2004; Barluenga *et al.* 2006).

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TABLES AND FIGURES

Figure 1. Live representatives of A) *Crenicichla minuano*, B) *C. tendybaguassu*, C) *C. missioneira* and D) *C. celidochilus.*
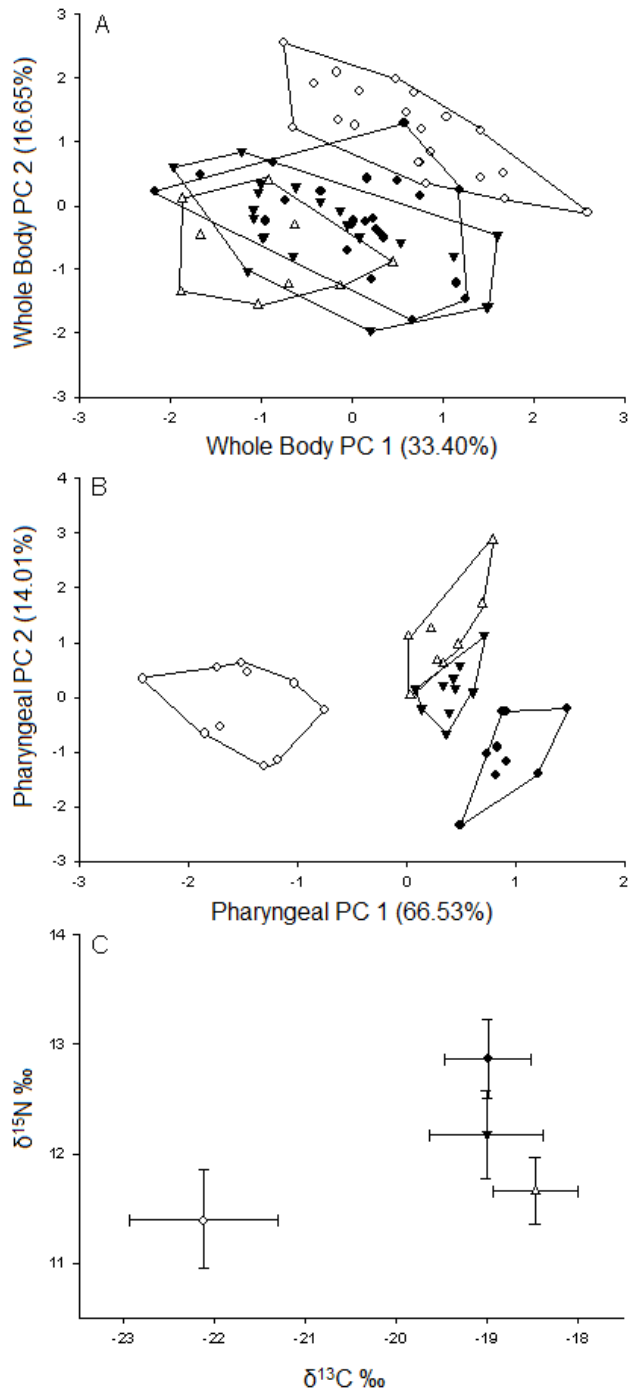
Figure 2. Shape principal component analysis of the whole body (A), lower pharyngeal jaw
(B) and stable isotope (mean±SD) bi-plot (C) of *Crenicichla minuano* (○), *C. celidochilus*
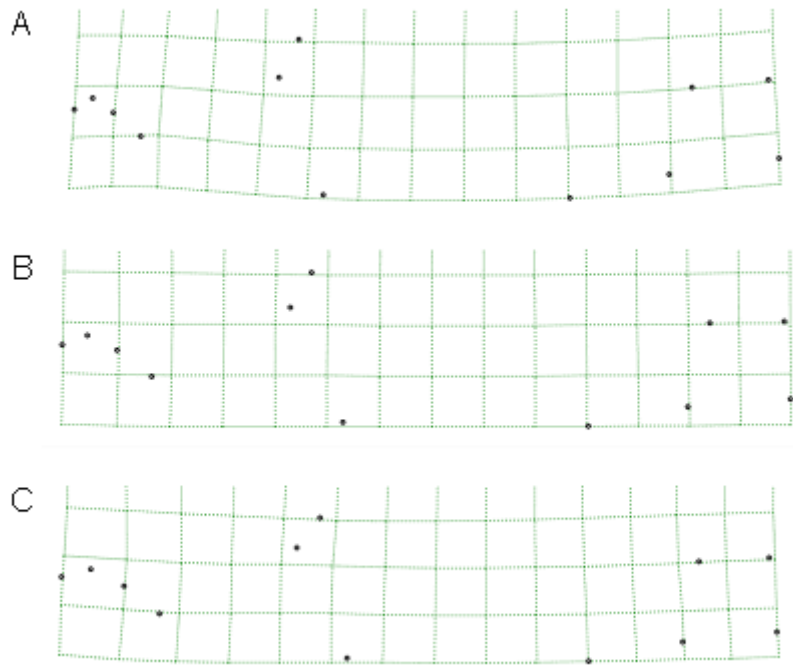(●), *C. missioneira* (▼), and *C. tendybaguassu* (Δ).

Figure 3. Warp transformation grids displaying important shape changes between

Crenicichla species: *Crenicichla minuano* (A), *C. celidochilus* and *C. missioneira* (B) and *C. tendybaguassu* (C). Major shape changes are limited to trophic structures (skull and jaws).
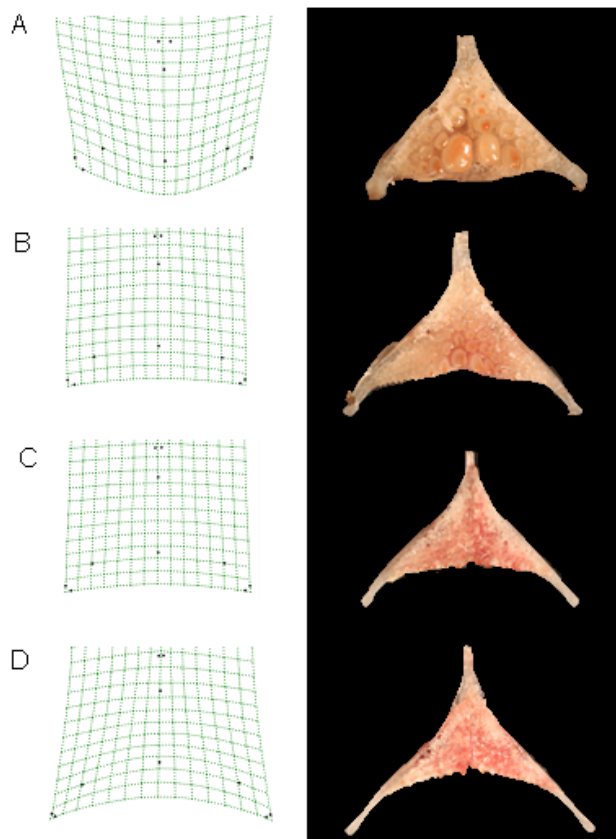
Figure 4. Warp transformation grids for the lower pharyngeal jaws of *Crenicichla minuano* (A), *C. missioneira* (B), *C. tendybaguassu* (C) and *C. celidochilus* (D) representing the continuum between well developed bone with molariform teeth (A) to a reduced plate with small conical teeth (D). Pharyngeal jaws are in occlusal view.

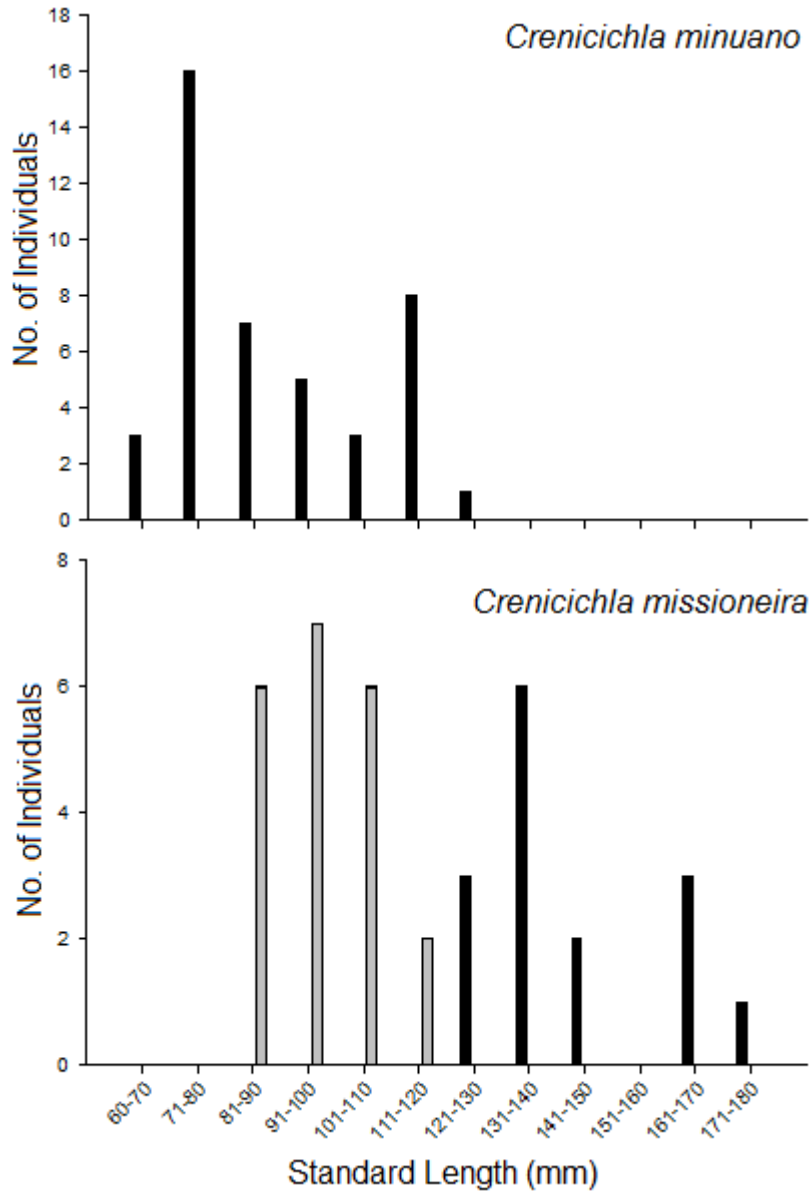Figure 5. Body size distribution of mature individuals of *Crenicichla minuano* and *C. missioneira*. Thirty-seven individuals (61-139 mm) of *C. minuano* and 41 individuals (42-163 mm) of *C. missioneira* were surveyed for reproductive viability via dissection and were classified as mature (black bars) or immature (gray bars). Individuals classified as mature were gravid or otherwise possessed enlarged gonads (Nikolsky 1963).

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