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Species-specific ontogenetic diet shifts among Neotropical *Crenicichla*: using stable isotopes and tissue stoichiometry

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Ontogenetic diet shifts were compared among five sympatric pike cichlids *Crenicichla* in a subtropical South American stream using stable C and N isotopes and tissue stoichiometry (C:N). Within species, stable N isotopes were positively related to body size while C:N showed negative relationships. Stable C isotopes, however, were not related to body size in any species. By modelling the switch to piscivory using gut content-isotope-body size relationships, diet shifts were shown to be species-specific with regard to both rate and degree of piscivory. Compared to other piscivorous lineages, *Crenicichla* appear to be unusually small-bodied (based on maximum body size). Because of their diversity, abundance and dynamic size-structured functional roles, *Crenicichla* may exert broad and complex predation pressures on the aquatic community.

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INTRODUCTION

Fishes often display ontogenetic diet shifts, which can serve to increase fitness (Post, 2003), reduce competition with similar species (Mittelbach, 1984, 1988) or may simply be passive responses to ontogenetic habitat shifts (Genner *et al.*, 2003). Piscivorous fishes display obligate ontogenetic diet shifts because young ones are often physically constrained by size (Werner & Gilliam, 1984; Keast, 1985) and switch to piscivory at later life stages (Mittelbach & Persson, 1998). Although prey size is often positively associated with consumer body size, larger piscivores do not necessarily feed at higher trophic levels (Mittelbach & Persson, 1998; Layman *et al.*, 2005). Within species, however, body size is often correlated with increased piscivory and higher trophic position (Winemiller, 1989; Post, 2003).

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Ontogenetic diet shifts are well studied in fishes, including comparative analyses of many species (Mittelbach & Persson, 1998). Few studies, however, have addressed ontogenetic diet shifts at scales with both ecologically and evolutionarily important contexts.

Neotropical piscivores are diverse, ecologically important and include well-known game and aquarium fishes including peacock bass *Cichla* (Jepsen *et al.*, 1997; Winemiller *et al.*, 1997; Hoeninghaus *et al.*, 2003; Layman & Winemiller, 2004), pike cichlids *Crenicichla* (Reznick *et al.*, 1990; Johansson *et al.*, 2004), piranhas *Serrasalmus* (Winemiller, 1989), characids and catfishes (Winemiller, 1989; Layman *et al.*, 2005). Piscivores generally progress through a series of size-dependent trophic niches and consume larger prey with increasing body size (Mittelbach & Persson, 1998). These transitions are often categorized by shifts from microcrustaceans, to aquatic insects and finally to fishes (Winemiller, 1989), resulting in a dynamic size-dependent trophic niche. Ecological implications of ontogenetic diet shifts by predatory species include increased food web connectivity and broad predation pressure on diverse resources. Additionally, diet shifts effectively increase the niche size of the species by increasing the diversity of resources they exploit.

Piscivores are important components of many tropical stream communities (Jepsen & Winemiller, 2002; Layman *et al.*, 2005; Burress *et al.*, 2013a) and can affect community characteristics such as food chain length (Post & Takimoto, 2007). Fishes can also influence ecosystem processes. For example, they can alter nutrient cycling and influence availability of limiting resources (McIntyre *et al.*, 2007, 2008). When fishes have heterogeneous or patchy distributions, nutrient recycling can create biogeochemical hotspots (McIntyre *et al.*, 2008). In combination, stable isotopes and ecological stoichiometry can reveal energy sources for fishes (Jepsen & Winemiller, 2007), identify top predators (Jepsen & Winemiller, 2002; Burress *et al.*, 2013a) and elucidate how species may affect ecosystem processes *via* nutrient recycling (Vanni *et al.*, 2002). While stable isotope analyses are often employed with top predators (Boecklen *et al.*, 2011), ecological stoichiometry is perhaps more frequently applied to grazing species, where nutrient recycling grazers can alter both biomass and elemental composition of producers (Knoll *et al.*, 2009).

Ontogenetic diet shifts were studied among five sympatric pike cichlids in a subtropical South American stream: *Crenicichla celidochilus* Casciotta 1987, *Crenicichla minuano* Lucena & Kullander 1992, *Crenicichla missioneira* Lucena & Kullander 1992, *Crenicichla scottii* (Eingenmann 1907) and *Crenicichla lepidota* Heckel 1840. This system was chosen because these species are closely related (Kullander *et al.*, 2010; Pialek *et al.*, 2011), morphologically similar (Lucena & Kullander, 1992), sympatric (Serra *et al.*, 2011) and numerically abundant (Burress *et al.*, 2013a). Thus, congeners in this system probably represent species with shared functional limitations (*e.g.* phylogenetic constraints) and in which competition for similar resources may be high (Werner & Hall, 1977; Mittelbach, 1988; Osenberg *et al.*, 1992; Robinson *et al.*, 1993) such that both evolutionary and ecological processes are important. The objectives were to (1) describe and compare ontogenetic diet shifts using stable C and N isotopes and tissue stoichiometry and (2) model the degree of piscivory of each species as a function of body size using gut content-isotope-body size associations.

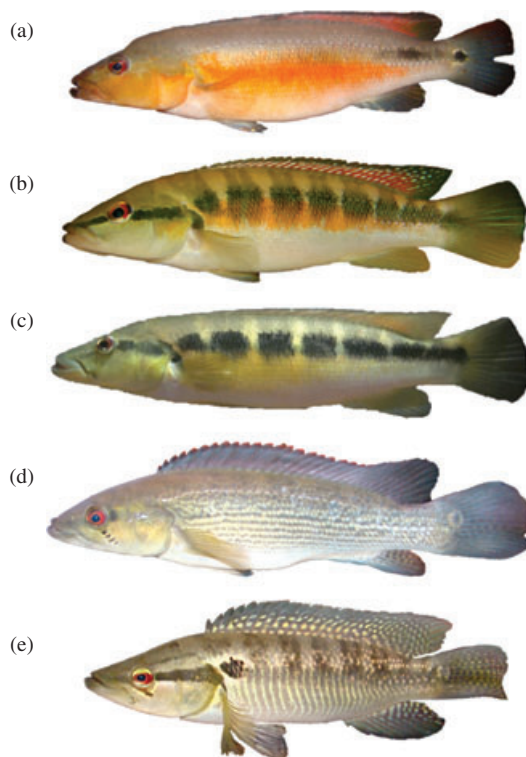


FIG. 1. Representative specimens of (a) *Crenicichla celidochilus*, (b) *Crenicichla minuano*, (c) *Crenicichla missioneira*, (d) *Crenicichla scottii* and (e) *Crenicichla lepidota*.

MATERIALS AND METHODS

The study species all exhibit elongate, tubular shapes and similar maximum body sizes (Lucena & Kullander, 1992). *Crenicichla celidochilus*, *C. missioneira*, *C. scottii* and *C. lepidota* have prognathus lower jaws and *C. minuano* have isognathus jaws (Lucena & Kullander, 1992). They represent three taxonomic groups: *C. celidochilus*, *C. missioneira* and *C. minuano* form a distinct lineage with shallow genetic divergence (<2 million years; Kullander *et al.*, 2010; Pialek *et al.*, 2011) and *C. scottii* and *C. lepidota* represent their own respective lineages (Pialek *et al.*, 2011).

The Cuareim River is a low-gradient piedmont stream that drains southern Brazil and northern Uruguay and is a major tributary of the middle Uruguay River. The fish community is diverse and representative of the Uruguay River basin (Zarucki *et al.*, 2010; Serra *et al.*, 2011). Consumer biomass is primarily supported by algal carbon and *Crenicichla* are mesopredators in this system (Burress *et al.*, 2013a). *Crenicichla* were sampled (Fig. 1) in the upper Cuareim River, Uruguay, in March and November 2010. Species were identified and their standard lengths (L_S) measured according to the method described by Lucena & Kullander (1992). Specimens were euthanized using a lethal dose of 2-phenoxyethanol (Ross & Ross, 2008), preserved on ice in the field and later stored in a -80°C freezer. Additional specimens were preserved in 10% formalin for stomach content analyses (Burress, 2012).

Dissected muscle was lyophilized until asymptotic mass, homogenized and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, U.S.A.). Relationships between L_S and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N were tested to establish the nature of ontogenetic diet shifts. Stable N isotopes reflect consumer trophic position (Post, 2002) and become enriched in stepwise increments [2.5–3.5‰; Post (2002); Vanderklift &

Ponsard (2003)] with each trophic transfer (*e.g.* prey to consumer). Stable C isotopes reflect ultimate carbon sources supporting an organism and typically display low prey–consumer enrichment (*c.* 0‰; Post, 2002). Tissue stoichiometry reflects how an organism recycles nutrients (Pilati & Vanni, 2007). For example, an organism may retain limiting nutrients (*e.g.* N or P) and preferentially excrete others (Vanni *et al.*, 2002). Thus, nutrient recycling by a consumer can be in response to environmental conditions (El-Sabaawi *et al.*, 2012). Additionally, tissue stoichiometry is often similar among consumers (Burruss *et al.*, 2013a) and may be subject to phylogenetic constraints (Vanni *et al.*, 2002). When nutrient limitation is fundamentally different, however, tissue stoichiometry also differs, *e.g.* between plants and animals (Rudnick & Resh, 2005). Thus, in combination, stable isotopes and tissue stoichiometry can track several functional characteristics of an organism.

Species were analysed, pooled and individually. Results were used from stomach content analyses (Burruss, 2012) to model the relationships between piscivory and $\delta^{15}\text{N}$ for each species. Using this relationship, the degree of piscivory was estimated for any given L_S class. All statistics were computed in SPSS (v 20.0; SPSS, Inc.; www-01.ibm.com/software/analytics/spss).

RESULTS

L_S was significantly positively associated with $\delta^{15}\text{N}$ ratios when species were pooled ($r^2 = 0.471$, $F_{1,91} = 81.06$, $P < 0.001$). When species were analysed separately, L_S was significantly positively related to $\delta^{15}\text{N}$ in four of the five species (Table I). Variation in slope (*i.e.* ontogenetic rate; Fig. 2) and total ontogenetic N enrichment was observed (Fig. 2). L_S , however, was not related to $\delta^{13}\text{C}$ in any species (Table I). L_S was significantly negatively associated with C:N when species were pooled ($r^2 = 0.170$, $F_{1,91} = 18.677$, $P < 0.001$). When species were analysed separately, C:N was a significant negative function of L_S in three of the five

TABLE I. Regression statistics for comparisons of body size (standard length, mm) with $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ (‰) and C:N for *Crenicichla* in the Cuareim River, Uruguay

Species	<i>n</i>	Variable	Relationship	r^2	<i>F</i>	<i>P</i>
<i>Crenicichla celidochilus</i>	18	$\delta^{15}\text{N}$	+	0.905	152.71	<0.001
		$\delta^{13}\text{C}$		0.007	0.12	>0.05
		C:N	–	0.242	5.11	<0.05
<i>Crenicichla missioneira</i>	21	$\delta^{15}\text{N}$	+	0.733	52.26	<0.001
		$\delta^{13}\text{C}$		0.002	0.04	>0.05
		C:N	–	0.278	7.31	<0.05
<i>Crenicichla lepidota</i>	21	$\delta^{15}\text{N}$	+	0.746	55.91	<0.001
		$\delta^{13}\text{C}$		0.088	1.83	>0.05
		C:N		0.030	0.54	>0.05
<i>Crenicichla scottii</i>	15	$\delta^{15}\text{N}$	+	0.488	12.39	<0.01
		$\delta^{13}\text{C}$		0.042	0.572	>0.05
		C:N		0.092	1.31	>0.05
<i>Crenicichla minuano</i>	18	$\delta^{15}\text{N}$		0.064	1.09	>0.05
		$\delta^{13}\text{C}$		0.001	0.01	>0.05
		C:N	–	0.575	21.69	<0.001

n, sample size.

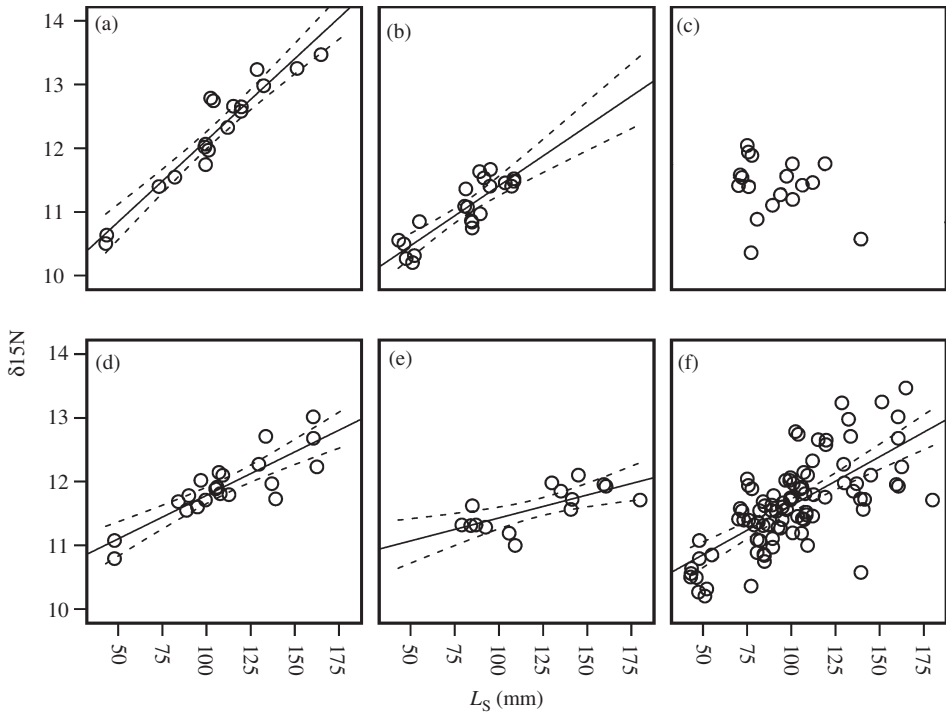


FIG. 2. Plots showing $\delta^{15}\text{N}$ as a function of standard length (L_S) for (a) *Crenicichla celidochilus*, (b) *Crenicichla lepidota*, (c) *Crenicichla minuano*, (d) *Crenicichla missioneira*, (e) *Crenicichla scottii* and (f) all species pooled (see Table I). The curves were fitted by: (a) $y = 0.0256x + 9.5607$, (b) $y = 0.0188x + 9.5304$, (d) $y = 0.0136x + 10.419$, (e) $y = 0.0072x + 10.706$ and (f) $y = 0.0154x + 10.083$ 95% c.i.

species (Table I). The slope of these relationships also varied by species (Fig. 3). Nonetheless, using stable N isotopes and tissue stoichiometry (C:N) in combination, ontogenetic diet shifts were detected in all species.

Of the 208 stomachs analysed, 114 contained prey items and were subsequently used in the model: *C. celidochilus* ($n = 20$), *C. minuano* ($n = 19$), *C. lepidota* ($n = 26$), *C. missioneira* ($n = 31$) and *C. scottii* ($n = 19$). Degree of piscivory (% fish consumed) was significantly positively related to $\delta^{15}\text{N}$ (Fig. 4). Using the proportion of fish consumed, $\delta^{15}\text{N}$ and L_S relationships, the degree of piscivory was modelled as a function of L_S for each species (Fig. 5). This model indicates that *C. celidochilus* diverges in ontogenetic piscivory rate much more quickly than *C. scottii*. In contrast, *C. missioneira* and *C. lepidota* converge at a common degree of piscivory at large body sizes *via* different piscivory rates. This hypothetical convergence is, however, beyond the largest documented L_S for *C. lepidota* (Lucena & Kullander, 1992; Figs 2 and 3). Thus, the model demonstrates that these closely related *Crenicichla* species switch to piscivory at different rates and to different degrees (Fig. 5). For example, *C. celidochilus* is estimated to consume almost exclusively fishes >100 mm while *C. missioneira* consumes fishes of *c.* 160 mm. In contrast, at their known maximum L_S (Lucena & Kullander, 1992; Figs 2 and 3), *C. lepidota* and *C. scottii* are estimated to consume a maximum of 35 and 60% fish, respectively (Fig. 5).

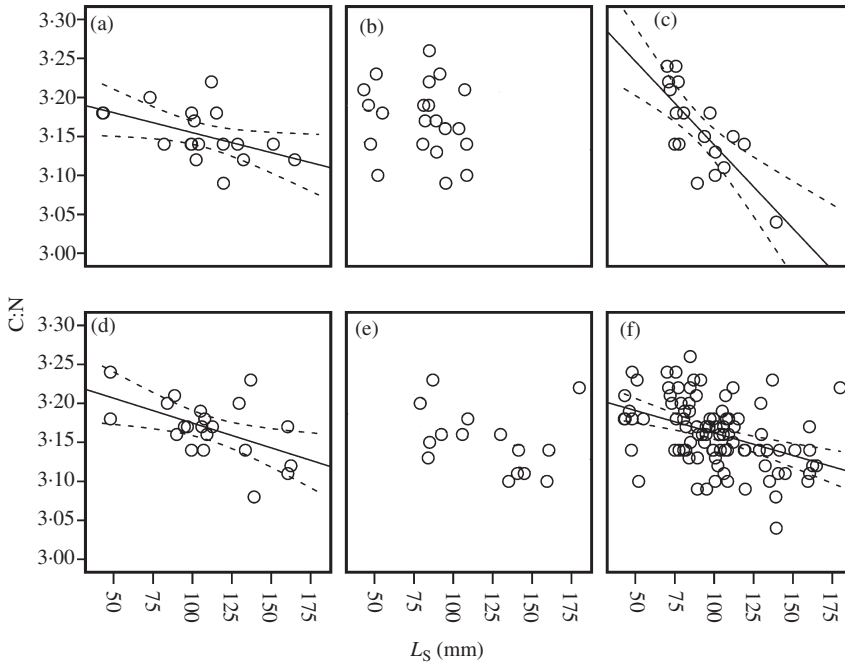


FIG. 3. Plots showing C:N as a function of standard length (L_S) for (a) *Crenicichla celidochilus*, (b) *Crenicichla lepidota*, (c) *Crenicichla minuano*, (d) *Crenicichla missioneira*, (e) *Crenicichla scottii* and (f) all species pooled (see Table I). The curves were fitted by: (a) $y = -0.0005x + 3.2061$, (c) $y = -0.0021x + 3.3538$, (d) $y = -0.0006x + 3.2386$ and (f) $y = -0.0006x + 3.2197$, 95% c.i.

DISCUSSION

Stable C and N isotopes and tissue stoichiometry suggest that five sympatric *Crenicichla* present in this reach of the Cuareim River display species-specific ontogenetic diet shifts. L_S was often related to $\delta^{15}\text{N}$ ratios. Basic stoichiometry (C/N) detected diet shifts in one species (*C. minuano*) that traditional isotope signatures (C and N) failed to detect. Indeed, ultimate carbon sources (based on $\delta^{13}\text{C}$) did not change with L_S in any species. Species-specific diet shifts indicate that each species may exert unique size-dependent predation pressures on the aquatic communities within the Cuareim River.

INTER-GUILD SHIFTS

Switching to piscivory can have profound fitness effects *via* increased growth rates and survivorship (Olson *et al.*, 1995; Olson, 1996; Post, 2003), yet switching rates may be variable across species (Mittelbach & Persson, 1998). This process of ontogenetic diet shifting is perhaps best described as proceeding through a series of ontogenetic niches (Werner & Gilliam, 1984) where numerous prey types are important at different life stages. The present data suggest that the timing and degree of diet shifts among *Crenicichla* are species-specific. *Crenicichla celidochilus* is perhaps the only primary piscivore (Keast, 1985) *via* a fast and complete switch to an entirely piscivorous diet. *Crenicichla missioneira* and *C. scottii* are secondary

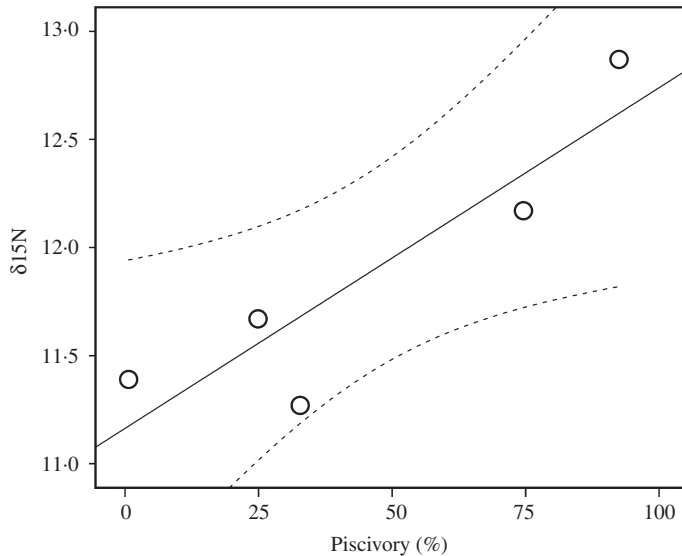


FIG. 4. Relationships between $\delta^{15}\text{N}$ ratios and the proportion of fish consumed (% by volume) according to Burress (2012) in five pike cichlids (O) in the Cuareim River, Uruguay. The curve was fitted by: $y = 0.0163x + 11.184$, 95% c.i.

piscivores (Keast, 1985); these species switch to piscivory more slowly. *Crenicichla lepidota* switches quickly, but not entirely, to piscivory.

Traditionally, stable C and N isotopes have been used to investigate diet shifts in fishes (Post, 2003) and stoichiometry has been employed for tracking ontogenetic patterns in nutrient recycling (Pilati & Vanni, 2007). Here, the utility of C:N in tracking ontogenetic diet shifts in piscivores is demonstrated, where C:N was a negative function of L_S in two piscivores. The ecological implications of diet shifts may be less intuitive than those of stable isotopes, but they may reflect changes in assimilation because C and N may be decoupled (Rudnick & Resh, 2005). Furthermore, the perhaps numerically small (0.3) shifts may be ecologically important, considering that C:N variability among the fish community is low (Burress *et al.*, 2013a).

Because piscivores are constrained by body size early in life and gradually switch to larger prey types with increasing body size, piscivorous fishes often become large-bodied (Mittelbach & Persson, 1998; Layman *et al.*, 2005). For example, largemouth bass *Micropterus salmoides* (Lacépède 1802), chain pickerel *Esox niger* Lesueur 1818 and walleye *Sander vitreus* (Mitchill 1818) often grow to 700–800 mm (Mittelbach & Persson, 1998). These L_S are four times larger than most subtropical *Crenicichla* [120–180 mm; Lucena & Kullander, 1992; this study], despite the fact that *Crenicichla* consume similar proportions of fishes. *Crenicichla*'s switch to piscivory at small L_S may be possible because of an abundance of small-bodied prey in many South American streams (Characidae; Esteves, 1996; Corrêa *et al.*, 2009) that are less demanding of physical limitations (*i.e.* gape). Many *Crenicichla*, including most subtropical lineages, are relatively small-bodied piscivores, with the exception of some large-bodied Amazonian lineages, *e.g.* *Crenicichla lugubris* Heckel 1840 (Layman *et al.*, 2005; Montana & Winemiller 2009). Coupled with their diversity and

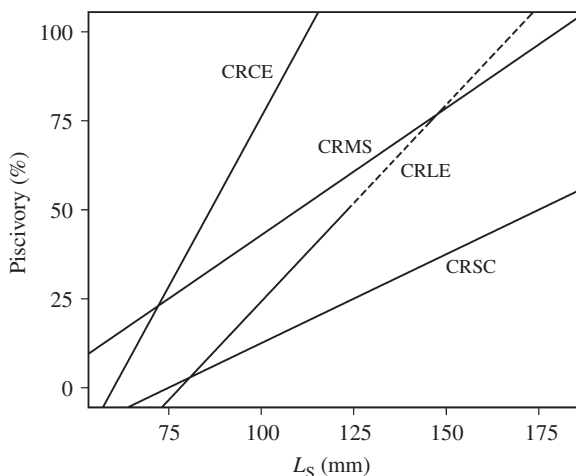


FIG. 5. Model estimating the switch to piscivory based on standard length (L_S), $\delta^{15}\text{N}$ and per cent fish consumed relationships. The relationship between $\delta^{15}\text{N}$ and % piscivory (Fig. 4) was used as a means to convert $\delta^{15}\text{N}$ scores into associated piscivory rates. The estimated piscivory rates were then exchanged in place of $\delta^{15}\text{N}$ (Fig. 2) to create an estimation of the relationship between % piscivory and L_S . The curves were fitted by: *Crenicichla celidochilus* (CRCE) $y = 1.8519x - 110.19$, *Crenicichla missioneira* (CRMS) $y = 0.714x - 28.571$, *Crenicichla scottii* (CRSC) $y = 1.0929x - 84.836$ and *Crenicichla lepidota* (CRLE) $y = 0.5x - 37.5$. Curves become dashed beyond the largest documented size for the species (Lucena & Kullander, 1992; Burress, 2012). *Crenicichla minuano* are not included because they do not consume fishes (Burress, 2012).

abundance in South American streams (Ploeg, 1991; Kullander *et al.*, 2010; López-Fernández *et al.*, 2010; Varella, 2011), *Crenicichla* may fill a relatively dynamic piscivorous niche.

INTRA-GUILD SHIFTS

Of the five species studied, only *C. minuano* displays an intra-guild (or within trophic level) ontogenetic diet shift. Traditional C and N isotope analyses did not detect diet shifts in *C. minuano*. Yet, C:N were negatively related to L_S , perhaps because *C. minuano*'s diet does not include fishes. Instead, *C. minuano* specializes on aquatic insects and molluscs (Lucena & Kullander, 1992; Burress, 2012). Shell crushing entails functional constraints, thus consumption of molluscs is limited by size (Mittelbach, 1984). Bivalves are extremely abundant in the Cuareim River and are $\delta^{13}\text{C}$ depleted compared with other invertebrates (*e.g.* Leptophlebiidae and Hydropsychidae) or fishes (Burress *et al.*, 2013a, b). Thus, it is likely that the diet shift to molluscivory was not detected using $\delta^{13}\text{C}$ because the shift had already taken place prior to the body sizes being sampled (>70 mm). For example, ecologically similar sunfishes *Lepomis gibbosus* (L. 1758) shift to molluscivory at a similar size (*c.* 80 mm; Mittelbach, 1988). Therefore, the ontogenetic shifts detected may be subtle shifts in assimilation, or perhaps even ontogenetic changes in nutrient recycling (Pilati & Vanni, 2007). The latter may particularly be the case considering that a large fraction of their diet is dominated by comparatively poor-quality (*e.g.* molluscs) foods (Burress, 2012).

FUNCTIONAL ROLES

Crenicichla are among the top predators in many South American streams, but may be best characterized as mesopredators (Layman *et al.*, 2005; Montana & Winemiller, 2009; Burress *et al.*, 2013a) and may be important trophic links to larger-bodied predators. *Crenicichla* also feed on a variety of prey items, including fishes (Layman *et al.*, 2005; Montana & Winemiller, 2009; this study), micro- and macrocrustaceans (Gibran *et al.*, 2001; Montana & Winemiller, 2009; Burress, 2012), larval invertebrates (Winemiller *et al.*, 1995), shrimps (Lowe-McConnell, 1969), molluscs and terrestrial subsidies (Burress, 2012). Furthermore, sympatric species often partition dietary resources (Montana & Winemiller, 2009; Burress *et al.*, 2013a). When there is a large size discrepancy between species, they may partition the habitat (Montana & Winemiller, 2009). Subtropical *Crenicichla*, however, often share microhabitats (Pialek *et al.*, 2011; Serra *et al.*, 2011) perhaps owing to their similar body sizes and morphology (Lucena & Kullander, 1992).

Crenicichla often exert significant predation pressure on prey fishes. For example, predation pressure by *Crenicichla* has shaped the evolution of guppy *Poecilia reticulata* Peters 1859 life-history attributes, including schooling behaviour (Seghers, 1974; Magurran & Seghers, 2004a), time budget allocation (Magurran & Seghers, 2004b), body size, colouration (Winemiller *et al.*, 1990) and reproductive investment (Reznick & Endler, 1992). *Crenicichla* is the most speciose genus of cichlids and has a widespread distribution throughout South America (Pialek *et al.*, 2011). Thus, through trophic diversity and ontogenetic diet shifts, *Crenicichla* fill dynamic ecological niches and may have broad and dynamic functional roles in South American streams.

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