Diet of four annual killifishes:
an intra and interspecific comparison

Gabriel Laufer[1], Matías Arim[1,2], Marcelo Loureiro[1,3], Juan Manuel Piñeiro-Guerra[1], Sabrina Clavijo-Baquet[2] and César Fagúndez[4]

We examined the diet of 4 annual fishes, *Austrolebias viarius*, *Austrolebias cheradophilus, Austrolebias luteoflammulatus* and *Cynopoecilus melanotaenia* inhabiting temporal ponds of southeastern Uruguay, by analysis of stomach contents. Fishes were captured from fifty ephemeral ponds of Castillos Lagoon basin, in the region of the Humedales del Este. We identified 13099 individual prey items extracted from 669 stomachs of the four captured species. In the studied system, annual killifishes represents the most abundant and conspicuous top predators. Killifishes are generalist key predators at the ephemeral ponds of the studied system, consuming mostly aquatic items. Zooplancton represented the bulk of the diet in the four analyzed species, followed by eggs, algae and diatoms. Insects are the next group in prey number, as follows: Diptera larvae (especially Chironomidae and Cullicidae), Ephemeroptera (especially Betidae), and coleopteran larvae (especially Dytiscidae). Acari are also important prey in number. The four fish species differ in diet composition and in diet richness. A general pattern of differences in diet richness among killifish species and demographic groups could be related to variations in body sizes. As top predators annual killifishes are an important component of the temporal pond ecosystems. Understanding the natural history of this species and their communities is necessary in order to conserve them.

Nós examinamos a dieta de 4 peixes anuais, *Austrolebias viarius*, *Austrolebias cheradophilus, Austrolebias luteoflammulatus* e *Cynopoecilus melanotaenia*, que habitam poças temporárias do sudeste do Uruguai, através da análise de conteúdo estomacal. Os peixes foram capturados em cinco poças temporárias da bacia da lagoa Castillos, na região de Humedales del Este. Nós identificamos 13099 itens de presas estraídos de 669 estômagos das quatro espécies. No sistema estudado, peixes anuais representam os predadores de topo mais abundantes e conspícuos. Os rivulídeos são predadores generalistas nas poças temporaries estudadas, consumindo principalmente itens aquáticos. Zooplâncton representou o item principal da dieta para as quatro espécies, seguido de ovos, algas e diatomáceas. Insetos compoem o próximo grupo em número de presas, como segue: larvas de Diptera (especialmente Chironomidae e Cullicidae), Ephemeroptera (especialmente Betidae), e larvas de Coleoptera (especialmente Dytiscidae). Ácaros foram também presas importantes em número. As quatro espécies de peixes diferem na composição e riqueza das dietas. O padrão geral de diferenciação da dieta entre espécies e grupos demográficos de rivulídeos pode ser relacionado à variação do tamanho corporal. Como predadores de topo, os rivulídeos anuais são um componente importante dos ecossistemas de poças temporárias. A compreensão da história natural destas espécies e de suas comunidades é necessária a sua conservação.

Key words: Annual fishes, Foraging behavior, Predators, Food analysis.

[1]Sección Zoología Vertebrados, [4]Sección Ecología, Facultad de Ciencias, Universidad de la República, Uruguay, Iguá 4225 Piso 9 Sur, Montevideo, Uruguay.
[2]Center for Advanced Studies in Ecology & Biodiversity (CASEB) and Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CP 6513677, Chile.
[3]Departamento de Ictiología, Museo Nacional de Historia Natural y Antropología, Juan Carlos Gómez 582, Montevideo, Uruguay.

Introduction

Diet is a basic determinant of organism biology, being related with most of the ecological attributes (Hughes, 1993). Diet has been associated with morphology (Winemiller, 1991), physiology (McNab, 2002), behaviour (Ward *et al*., 2004), population abundances (Brown *et al*., 2004) and dynamics (Berryman, 1999), community structure (Arim *et al*., 2007) and even ecosystem processes (Thébault & Loreau, 2006). As a consequence, knowing species trophic behavior represents a central step in order to advance in understanding its natural history.

Living in time-constrained habitats, such as ephemeral ponds with periodic drying, imposes several constrains to species behaviour, development, and life history (Ludwig & Rowe, 1990). Biological and environmental factors interact to determine the structure of the temporary pond communities (Wilbur, 1987). Mortality risk can be high in these habitats due to desiccation and predation by odonates, beetles and hemipterans (Wilbur, 1987). Animals found in that habitats—mostly macroinvertebrate and amphibian larvae—show adaptations to deal with that particular hydrological regime, such as rapid colonization and growth rate, high phenotypic plasticity (Wilbur, 1987; Marcus & Weeks, 1997; Relyea & Werner, 2000; Mura *et al*., 2003) or the presence of a resistance phase in their life cycle (*e.g.* diapause). Periodic drying excludes species unable to cope with that physical stress, preventing the colonization of key predators such as fishes and some invertebrate (Skelly, 1995). In fact, many amphibians with no anti-fish predator defenses are restricted to ephemeral pools (Kats *et al*., 1988; Peltzer & Lajmanovich, 2004). However, the annual killifishes (Cyprinodontiformes, Aplocheiloidei) are fish species that evolved in that particular habitat, having a resistance phase in their life cycle (Wourms, 1972).

Annual killifishes originated independently in Africa and South America and have evolved to tolerate the desiccation constrains by completion of their entire cycle within less than one year (Costa, 1995a; Nelson, 2006). This adaptation requires notable high growing and development rates (Errea & Danulat, 2001). When ponds dry out, adults die and their eggs remain in diapause buried in the substrate, hatching on the next year, with the return of favorable conditions (Wourms, 1972; Arezzo *et al.*, 2005). While tadpoles have the possibility of regulating their metamorphosis time (exit of the temporary pond) based on the environmental conditions, for annual fishes the only option is the fast growth and maturation because its whole cycle passes in ephemeral ponds. This fishes require lying eggs before the pond dries (Scheel, 1962), so energetic budgets imply growing and reproduction requirements, probably with a great ingestion necessity.

Despite the uniqueness of this life cycle, there is little information about their life history and diet (Costa, 1995a; Arenzon *et al.*, 2001). The ecological and evolutionary contexts of temporal ponds have profound implications in species biology, well studied in amphibians and invertebrates, but not in fishes. The objective of this study was to describe and analyze the diet of four killifish species that coexist in a temporary pond system, in southeastern Uruguay: *Cynopoecilus melanotaenia* (Regan, 1912), *Austrolebias luteoflammulatus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1964), *A. viarius* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1964) and *A. cheradophilus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1964).

Material and Methods

Study Area. The present study is based on diet analysis of individuals collected in a costal lagoon basin which outflows into the Atlantic ocean, southeastern Uruguay, in the region
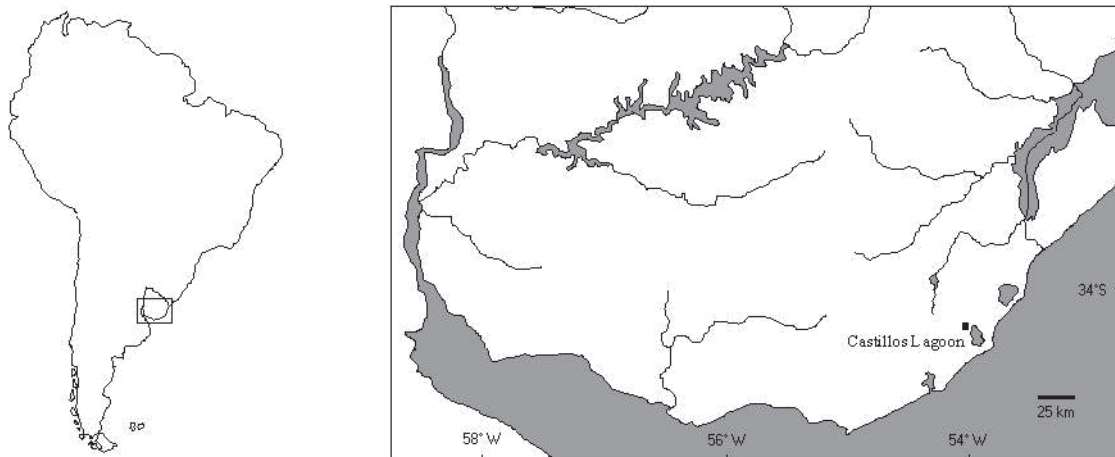

Fig. 1. Map of the study site area. The sampled temporary pools are located in the Castillos Lagoon Basin, about 60 km from Brazilian border.

of the Humedales del Este (Eastern Wetlands), Rocha; declared Biosphere Reserve by UNESCO, program MAB (Fig. 1). A system of 50 temporal ponds was studied, located in grasslands (private farms used for cattle). Ponds consist of ground depressions with a depth that does not surpass 30 cm and a very variable area (from 1 to 25000 m^2). During autumn and winter, these pools are usually water plenty, time at which the pluvial water contributions surpass the losses by evaporation; quickly dried in the beginning of higher spring temperatures. Ponds display an important diversity of organisms, macrophytes and invertebrates as well with different adaptations to this particular hydrological regime. Present vertebrates are fishes and anurans (larvae and adults).

Fish sampling and diet analysis. Fishes were collected in winter of 2006 with hand-net (15 x 20 cm, 1 mm mesh). Fishes were captured and euthanized with a lethal dose of 2-phenoxyethanol and immediately fixed in formaldehyde solution (4%). At laboratory, specimens were identified at the species level, measured with a digital caliper (to the nearest of 0.01 mm) for standard length (STL), and classified in demographic groups according to the presence of visible sexual characters (coloration pattern and fin configuration; Costa, 2006) as follows: juveniles (J), females (F), males (M) or not identified (Ni). The data from those individuals that could not be identified were used for species level analysis, but not for demographic comparisons. All specimens analyzed were placed in the Fish Collection of Facultad de Ciencias, Montevideo (Institutional Code: ZVC-P).

Preserved specimens were dissected and all food contained in their stomach was removed and spread on a glass slide for identification under stereomicroscope. References on aquatic invertebrates and zooplankton were consulted for prey identification (Burgis & Morris, 1987; Consoli & Olivera, 1994; Peña, 2001; Thorp & Covich, 2001; Motta & Uieda, 2004; Costa *et al.*, 2006).

Statistical Analysis. For each species the number of individuals, mean length, number of prey and prey richness were analyzed, discriminating among males, females and juvenile individuals. Differences between frequency variables (fish individuals, prey number and prey richness) were evaluated by G-test at two different levels (Sokal & Rohlf, 1995). First, data were compared between M, F and J of each species. If significant differences were detected, a G-test was realized for each pair of demographic groups (M-F, M-J, F-J). In all G-tests the null hypothesis was an equal distribution among demographic groups (Sokal & Rohlf, 1995). The significance of standard length difference between demographic groups was evaluated by a one-way ANOVA, using Fisher LSD post hoc analysis to evaluate differences between pair of species (Sokal & Rohlf, 1995). STL of *A. viarius* was transformed (STL' = sin^{-1} STL) in order to obtain the necessary test assumptions.

Diversity patterns are strongly affected by the number of observations used in its estimation (Gotelli & Graves, 1996).

Rarefaction is a robust method to describe and contrast diversity patterns when different sample sizes are involved (Arim & Barbosa, 2002). Further, rarefaction is the only diversity measure that is sensitive to rare species and is unbiased by sample size (Magurran, 1988). In the analysis of temporal or interspecific diet patterns, the use of rarefaction represents a robust approach (Arim & Jaksic, 2005) that brings a better insight about diet patterns than diversity indices (Naya *et al.*, 2002). Rarefaction involves the estimation of the expected richness for a series of subsamples from the prey abundance distribution (Gotelli & Graves, 1996). For each species rarefaction diversity curves were estimated for the three demographic groups (M, F, and J). Subsamples where constructed with individual fishes (Gotelli & Colwell, 2001) rather than with individual preys (*e.g.* Naya *et al.*, 2002). For each rarefaction curve, sub-samples from one fish to the effectively observed fish number were taken and species richness retained. This procedure was repeated 10000 times estimating the median richness and confidence interval at 95% with percentiles 0.025, 0.5 and 0.975 (Manly, 1997). This re-sampling method eliminates the assumption of underling distributions in rarefaction estimation (*e.g.* Rosenzweig, 1999). Rarefaction curves where used to contrast diet diversity within species—among demographic groups—and among species—considering species diet as a whole.

Differences in standard length (STL) between species were studied with ANOVA analysis. In order to get normality STL was log10-transformed. A Fisher LSD test was performed to look for differences between species pairs (Sokal & Rohlf, 1995).

For the analysis of differences in diet composition between species, consumed items were categorized in seven classes: Basal items, Primary Consumers (PC: Zooplankton, Molluscs and Other), Predators (P: Adult and Larva), and Non Aquatic items. Differences among species were evaluated by G-test for each item class (Sokal & Rohlf, 1995) and association of killifishes species with diet item categories was analyzed by a Canonical Correspondence Analysis (CCA), using frequencies (Greenacre, 1984). Diet richness composition was contrasted with a rarefaction procedure (Gotelli & Graves, 1996).

Results

We identified 13099 individual prey items extracted from stomachs of the 669 collected individuals of the four fish species. Zooplancton represented the bulk of their diet, followed by eggs, algae and diatoms (Table 1). Insects were the next group in prey number, as follows: Diptera larvae (especially Chironomidae and Culicidae), Ephemeroptera (mostly represented by Baetidae larvae), and coleopteran larvae (especially Dytiscidae). Acari (Hydracarina) were also important prey in number. Other preys such as mollusks and Malacostraca also appear in relative important quantities in the gut content. Non aquatic preys were represented by adult dipterans, Collembolla and Aranea, in relatively low frequencies.

Table 1. Diet of *A. viarius* (*Av*), *A. luteoflamulatus* (*Al*)*, A. cheradophilus* (*Ac*) and *C. melanotaenia* (*Cm*) from temporary pools at Castillos lagoon basin, southeastern Uruguay. Gut content is presented for females (F), males (M), juveniles (J) and not identified individuals (Ni). The first line (N) details the number of analyzed fishes for each species and demographic group.

	Av				*Al*				*Ac*			*Cm*			
	F	J	M	Ni	F	J	M	Ni	F	J	M	F	J	M	Ni
N	234	113	76	63	25	24	59	3	16	4	18	9	9	2	14
Algae	203	109	47	33	7	5	34	0	3	0	0	2	0	8	0
Diatom	248	33	8	1	1	0	7	0	0	0	0	0	0	1	0
Seed	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0
Nematoda	12	10	6	1	3	0	4	0	0	0	0	0	0	1	0
Ostracoda	283	144	128	77	94	48	175	2	2	2	23	12	1	45	2
Cladocera	2643	1167	723	719	82	121	264	20	61	10	30	32	14	56	58
Copepoda	37	26	15	3	4	2	5	4	3	0	0	0	0	0	0
Cyclopoida	195	8	64	4	1	3	36	0	0	0	1	29	0	38	1
Calanoida	990	399	427	376	309	129	277	10	55	6	22	26	2	85	34
Bivalvia	2	0	0	3	0	0	0	0	1	0	5	0	0	0	0
Ancylidae	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	22	0	2	1	0	0	1	0	3	0	6	2	0	0	0
Ampullaridae	5	0	6	0	0	0	3	0	1	0	0	1	0	0	0
Operculum	5	3	3	1	0	0	0	0	0	1	1	0	0	0	2
Oligochaeta	1	0	0	0	3	0	0	0	1	0	1	0	0	0	0
Arthropoda	18	2	5	2	1	0	2	1	0	0	2	0	0	0	0
Amphipoda	9	3	4	3	2	1	10	0	0	1	3	0	0	0	0
Parastacus	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Aranea	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
Hydracarina	66	16	17	10	11	4	12	0	0	1	3	1	0	3	0
Insecta	10	5	5	3	1	0	1	0	0	0	1	0	0	0	0
Dytiscidae (larva)	16	7	9	8	6	4	9	0	0	1	5	0	0	5	0
Haliplidae (larva)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae (larva)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae (larva)	3	4	2	3	0	0	1	0	1	0	1	0	0	1	0
Ephemeroptera (larva)	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae (larva)	34	9	28	18	3	6	8	0	12	0	14	2	0	2	0
Diptera	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tachinidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae (larva)	113	2	13	4	3	1	18	0	1	0	1	5	0	11	0
Tipulidae (larva)	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Culicidae (larva)	17	1	8	3	0	7	32	0	5	1	2	2	0	0	1
Culicidae (pupa)	3	0	0	1	0	0	1	0	0	0	0	0	0	1	0
Culicidae	11	0	0	0	0	0	13	0	0	0	0	0	0	0	0
Ceratopogonidae (larva)	12	11	4	11	2	2	9	0	1	0	3	2	0	2	1
Tabanidae (larva)	25	5	9	1	0	0	9	0	2	0	1	2	0	2	0
Corixidae	0	0	2	1	0	0	1	0	0	0	1	0	0	0	0
Notonectidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Hymenoptera	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Odonata (larva)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Zygoptera (larva)	2	0	1	2	0	0	1	0	0	0	0	0	0	0	0
Anisoptera (larva)	3	0	1	1	0	0	2	0	1	0	0	0	0	0	0
Aphidae	1	0	1	2	0	0	1	0	0	0	0	0	0	0	0
Trichoptera (larva)	4	1	2	1	0	1	0	0	0	0	4	0	0	0	0
Lepydoptera (larva)	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Psocoptera	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collembola	88	0	1	0	0	0	1	1	0	0	0	0	0	0	0
Egg	99	29	83	12	43	24	96	18	71	0	10	1	0	3	0
Egg mass	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cynopoecilus melanotaenia	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Unidentified	17	14	2	1	0	1	3	0	3	0	1	3	1	2	1

Intraspecific analysis. *Austrolebias viarius* has been the dominant species in the system representing 71.21 % of the total number of individuals collected. The proportion of individuals observed in demographic groups differed (G_2=93.10, p<0.05, Fig. 2a), being F most frequent than J followed by M. Males were larger than F, followed by J ($F_{2,420}$= 77.92, p<0.05, Fig. 2b). Total number of prey were higher in F, followed by J an then M (G_2=2441, p<0.05, Fig. 2c) and prey

richness was equal for all stages (G_2=3.575, p=0.17, Fig. 2d). The rarefaction curves shows differences between demographic groups (Fig. 2). Males show the richest diet, followed by F, and then J.

Austrolebias luteoflammulatus were 18.18 % of the total sample. The proportion between F and J did not differ, but M were the most numerous demographic group (G_2=20.60, p<0.05, Fig. 3a). Demographic groups differ at STL

A. viarius
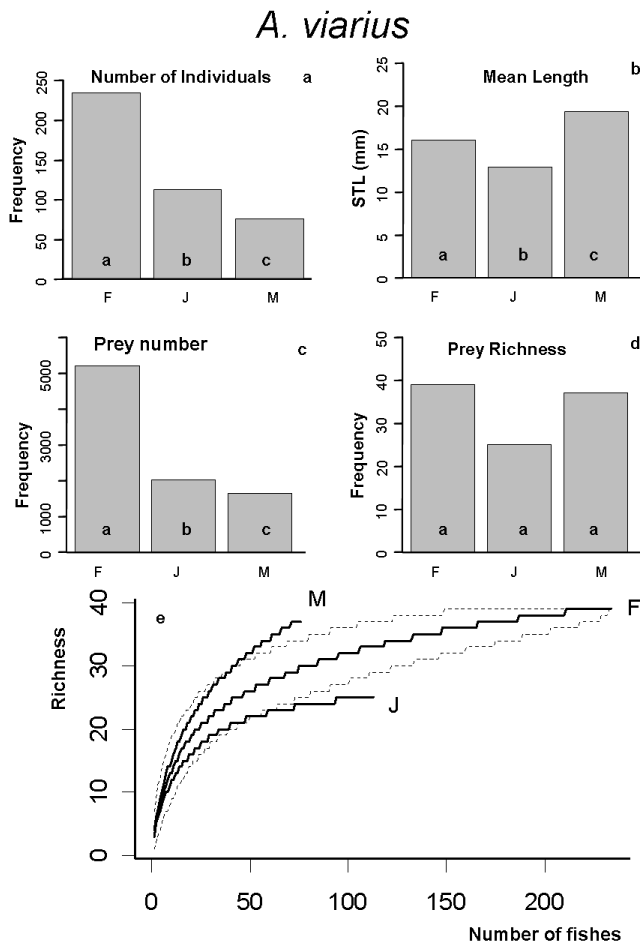

A. luteoflammulatus

Fig. 2. Sampled fishes of *A. viarius* and differences between demographic groups (F, J and M) in: (a) number of collected individuals, (b) body sizes measured as STL, (c) total number of prey recorded at gut analysis, and (d) prey richness at the taxonomic level detailed in Table 1. (e) Rarefaction curves for each class. Dashed lines indicate the confident interval at 95%.
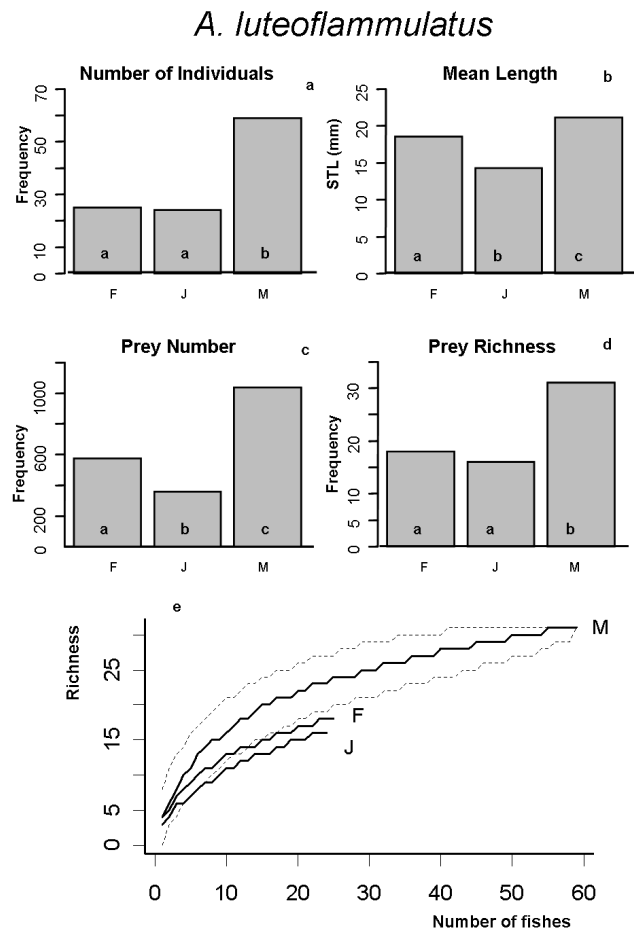
Fig. 3. Sampled fishes of *A. luteoflammulatus* and differences between demographic groups (F, J and M) in: (a) number of collected individuals, (b) body sizes measured as STL, (c) total number of prey recorded at gut analysis, and (d) prey richness at the taxonomic level detailed in Table 1. (e) Rarefaction curves for each class. Dashed lines indicate the confident interval at 95%.

($F_{2,105}$=24.24, p<0.05, Fig. 3b), being M the largest, followed by F and then J. Prey number was significantly higher at adult stages, specially at males (G_2=359.1, p<0.05, Fig. 3c). Differences in prey richness showed the same tendency but it was marginally significant (G_2=5.83, p=0.0541, Fig. 3d). The rarefaction curves show differences between demographic groups, indicating a greater richness for M followed by F and J (Fig. 3).

Austrolebias cheradophilus were 4.21 % of the total sample. M and F were the most frequent demographic groups (G_2=10.20, p<0.05, Fig. 4a). There was no significant difference between M and F number of individuals, and the same pattern is observed in body size ($F_{2,22}$=4.44, p<0.05, Fig. 4b). Prey number was significantly higher at adult stages, especially at F (G_2=198.1, p<0.05, Fig. 4c), but considering prey richness, M and F are equal and J have lower level (G_2=10.60, p<0.05, Fig. 4d). The rarefaction analysis did not show significant

differences for each demographic group, probably due to the small sample size (Fig. 4).

Cynopoecilus melanotaenia were 6.4 % of the total sample. The proportion between demographic groups shows significant differences (G_2=10.90, p<0.05, Fig. 5a) being the adults the predominant classes. In addition, proportions of M and F individuals did not differ. Males presented larger size than females ($F_{2,35}$=10.96, p<0.05, Fig. 5b) and both presented larger sizes than juveniles. A significant large number of prey was observed for M, followed by F and J (G_2=262.9, p<0.05, Fig. 5c). Considering prey richness, M and F did not presented significant differences, while J presented and inferior richness (G_2=10.47, p<0.05, Fig. 5d). The rarefaction analysis did not show significant differences for each demographic group, probably due to the small sample size (Fig. 5).
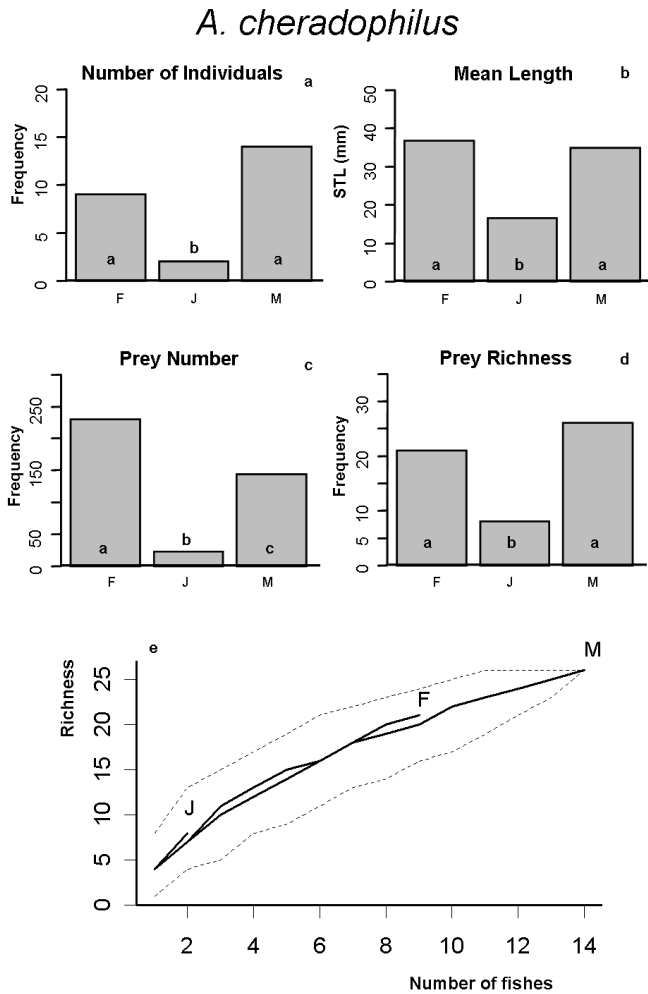
A. cheradophilus

C. melanotaenia

Fig. 4. Sampled fishes of *A. cheradophilus* and differences between demographic groups (F, J and M) in: (a) number of collected individuals, (b) body sizes measured as STL, (c) total number of prey recorded at gut analysis, and (d) prey richness at the taxonomic level detailed in Table 1. (e) Rarefaction curves for each class. Dashed lines indicate the confident interval at 95%.

Fig. 5. Sampled fishes of *C. melanotaenia* and differences between demographic groups (F, J and M) in: (a) number of collected individuals, (b) body sizes measured as STL, (c) total number of prey recorded at gut analysis, and (d) prey richness at the taxonomic level detailed in Table 1. (e) Rarefaction curves for each class. Dashed lines indicate the confident interval at 95%.

Interespecific analysis. The mean standard length STL differs among species ($F_{3,590}$=81.17, p<0.001), being *A. cheradophilus* (mean STL = 34.00 mm) the largest species followed by *A. luteoflammulatus* (mean STL = 18.99 mm). This species is slightly larger than *A. viarius* (mean STL = 15.77 mm) and *C. melanotaenia* (mean STL = 15.74 mm), species that do show significant differences in body sizes at post hoc analysis. The four species rarefaction analysis show no differences between *A. viarius* and *A. luteoflammulatus*; *C. melanotaenia* displays lower, and *A. cheradophilus* higher prey richness (Fig. 6).

Prey group frequencies are different between species. The G-test for the each prey class shows significant differences between killifishes species (Table 2). The graphic resulted from the CCA, reveals differences in prey association with each fish species (Fig. 7). The first axis separates *A. cheradophilus* from the rest of the system species. This
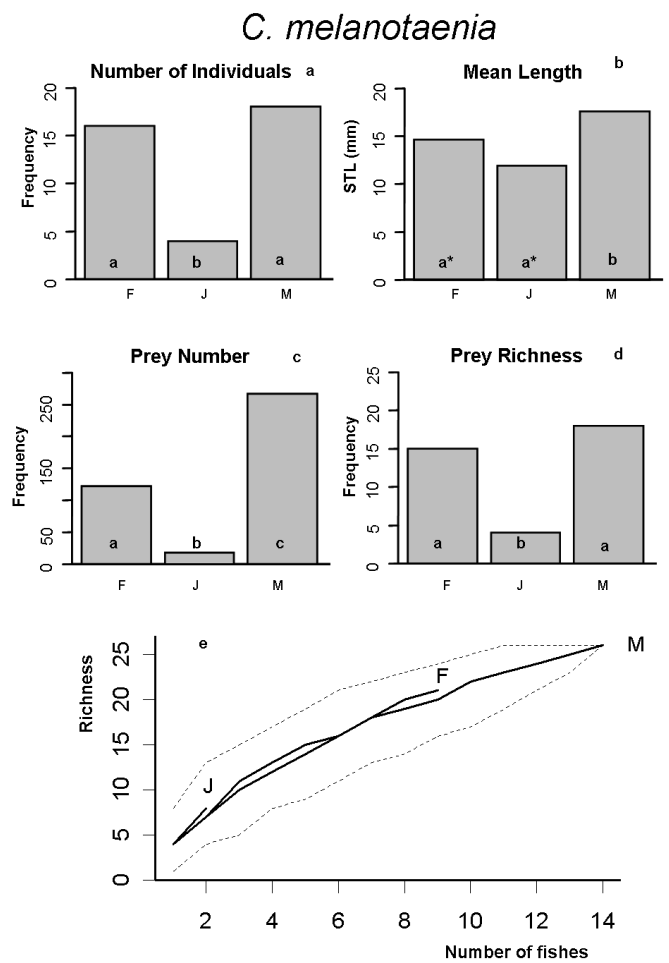
difference is associated with the consumption of mollusk preys. For the rest of the species, despite the differences, there seems to be no important prey association.

Discussion

In our studied system, annual killifishes represents the most abundant and conspicuous top predators, being an important component of the temporal pond ecosystems. In the light of ephemeral habitat, a top predator might have a generalist diet, including the major available quantity of preys. Congruently, our results indicate that the four studied species could be considered as generalist predators, with differences between species and demographic groups, mostly related to size differences. Although there is scarce information about temporal killifish diets, available evidence suggests that this
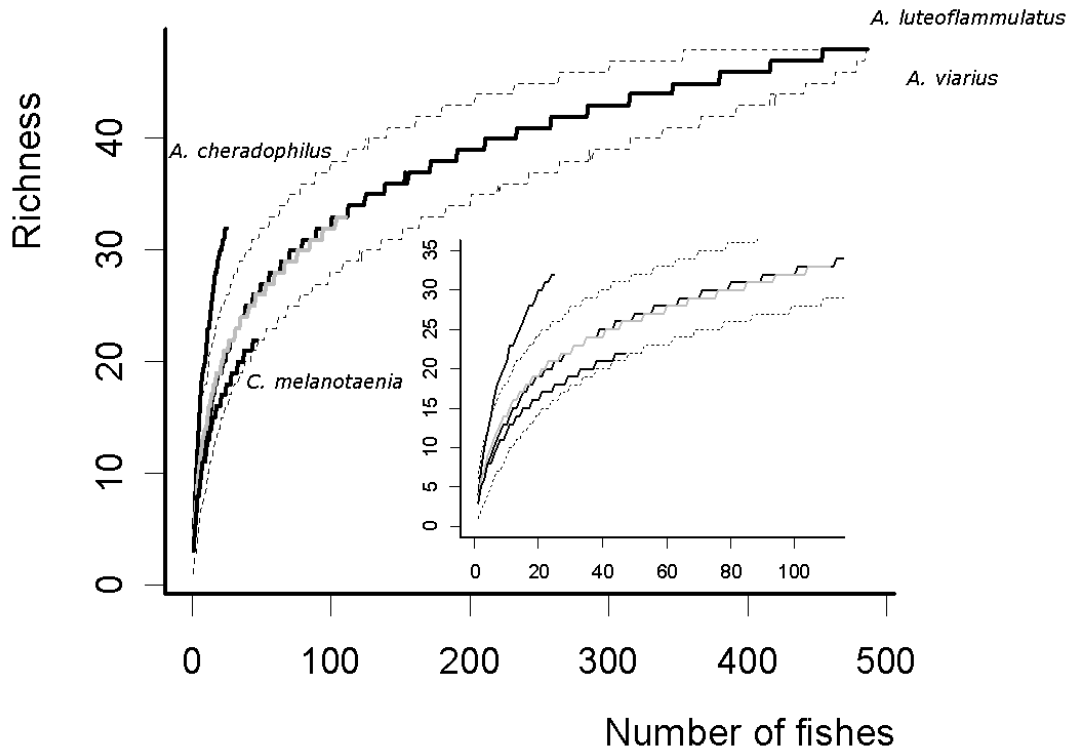
Fig. 6. Rarefaction curves applied for the categorized diet items of *Cynopoecilus melanotaenia*, *Austrolebias luteoflammulatus*, *A. viarius* and *A. cheradophilus*. Dashed lines indicate the confident interval at 95%.
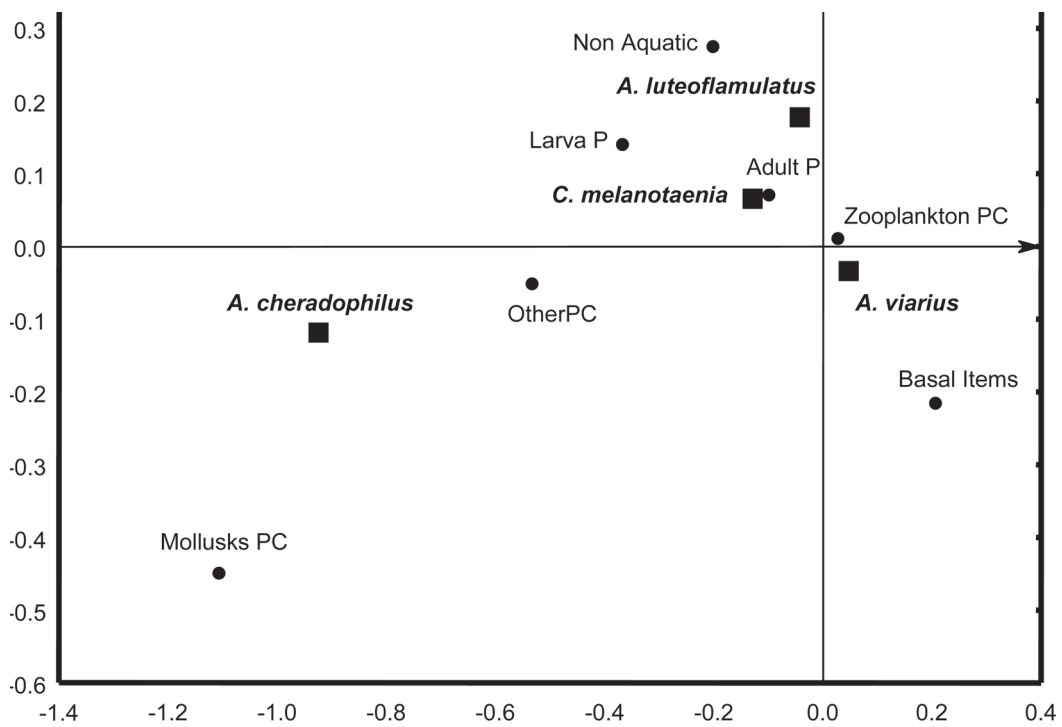

Fig. 7. Correspondence analysis of diet items (%) and the studied killifish species (%): *Cynopoecilus melanotaenia*, *Austrolebias luteoflammulatus*, *A. viarius* and *A. cheradophilus*. The preys were classified in Basal items, Primary Consumers (PC: Zooplankton, Molluscs and Other), Predators (P: Adult and Larva), and Non Aquatic items.

Table 2. Categorized diet items for *A. viarius* (*Av*), *A. luteoflamulatus* (*Al*), *C. melanotaenia* (*Cm*) and *A. cheradophylus* (*Ac*). The preys were classified in Basal items, Primary Consumers (PC: Zooplankton, Molluscs and Other), Predators (P: Adult and Larva), and Non Aquatic items. For each item it is provided the result of G-test (G), the freedom degree (df) and the probability (P), for differences in frequency.

	Av	*Al*	*Cm*	*Ac*	G	df	P
Adult Predator	113	28	4	6	263.8	3	< 0.0001
Larva Predator	144	45	15	16	425.3	3	< 0.0001
Non Aquatic preys	133	54	3	10	428.0	3	< 0.0001
Molluscs Primary Consumers	60	4	5	18	32.7	3	< 0.0001
Zooplankton Primary Consumers	8428	1586	435	215	111033.6	3	< 0.0001
Other Primary Consumers	264	59	22	41	1248.7	3	< 0.0001
Basal Items	689	54	11	3	4363.2	3	< 0.0001

probably represents a common attribute of the group biology (*e.g. Simpsonichthys constanciae* and *Leptolebias cruzi* at Rio das Ostras, Brazil, Costa, 1995).

Observed differences in diet richness among killifish species and demographic groups could be related to variations in body sizes (McLaughlin, 1989; Woodward *et al.*, 2005). Among the studied species, larger killifishes presented more diverse diets (Fig. 6). Within species, males typically presented a more diverse diet than females, and juveniles a poorer diet than adults (Figs. 2e-3e-4e-5e). Evidence suggests that as predator body size increases, larger prey items are progressively added to diet (*e.g.* insect larvae), faster than smaller species (*e.g.* zooplankton) are eliminated (Woodward & Hildrew, 2002). As a result, a larger predator eats virtually all the prey species consumed by smaller predators, adding bigger items available at the system (Woodward & Hildrew, 2002). Observed patterns in all the species herein studied are congruent with an increase in diet richness with body size.

Size dimorphism between sexes has been suggested as a main determinant of diet variation (Shine, 1989; Pearson *et al.*, 2002). Concordant with previous reports, males of *Austrolebias* and *Cynopoecilus* are larger than females (Vaz Ferreira *et al.*, 1964; Costa, 1995; Arenzon *et al.*, 2001). Further, sexual differences in diet are suggested to be favored by natural selection, when morphology or behavior constrains each species to a limited range of resource (Colwell, 2000). The lack of significant differences between the size of the different sexes in *A. cheradophilus* could be related with the reduced statistical power from the low number of individuals collected.

In the studied system, the most common species was *A. viarius*, followed by *A. luteoflammulatus* comprising both species 89.4% of the observed individuals (Table 1). These species were mainly represented by juvenile individuals (Figs. 2a-3a), while less abundant species—*A. cheradophilus* and *C. melanotaenia*—presented greater incidences of adult individuals (Figs. 4a- 5a). These different patterns could originate from asynchrony in hatching times and/or by inter-specific differences in growth rates. Samples were taken at the initial phase of pond life, suggesting that temporal separation in life cycles among species is probably occurring.

This suggest that the largest—*A. cheradophilus*—and the smallest—*C. melanotaenia*—killifish species appeared early in the hydrological period while the middle size species develop later. Inter-specific differences in developing times have not been previously evaluated in annual killifishes. Its consequences could be the strong constraining of developing time, being actually less than the short pond life. This fact reinforces temporal limitations, and so, this cost should surpass costs related to competition or predation (Houston and McNamara, 1999). These strategies could maintain killifishes diversity, despite of the occurrence of four generalist vertebrate predators in a small, time constrained ecosystem (Wilbur, 1987).

Temporal systems are generally identified as a refuge for fish where species sensitive to predation reproduce. The annual killifishes are giving a singular community structure, coexisting with anuran larvae (*Hypsiboas pulchellus*, *Odontophrynus americanus, Scinax squalirostris* and *S. granulatus*, unpublished data). The absences of tadpoles in fish gut content, suggests that predation is not a main determinant of this taxa coexistence, as is the case in other communities (Kats *et al.*, 1988; Alford, 1999). The existence of anti-predation defenses has identified elsewhere as a frequent mechanism of predation avoidance by amphibians (Alford, 1999; Ruxton *et al.*, 2004). However, the large size of the observed tadpoles, always larger than killifishes, indicate that size refuge to predation could be the most important anti predation mechanism in this system (Chase, 1999).

The present article represents the first report of the trophic ecology of four annual killifishes that coexist at the same wetland system. The study attempts to advance in the understanding the natural history of these species and their ecological communities, as a basic tool for conservation and future life history studies. The three *Austrolebias* species are endemic to this region, only having *C. melanotaenia* a wider distribution (D'Anatro & Loureiro, 2005; Costa, 2006). Although being located in a MAB reserve, the system is located in private farms, and future land management practices, can affect the ponds and their dynamics. Further studies concerning species and their communities are necessary in order to conserve this species and their ecosystem.

Acknowledgements

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