

## Density, body size and diet overlap of four fish species in artificial reservoirs in the Pampas, Uruguay

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**Abstract.** Despite being an important habitat for biodiversity, autoecology studies of fish in artificial water reservoirs in the Pampas grasslands are scarce. The present study explores aspects of population ecology and trophic interactions of four fish species inhabiting these habitats at the hilly area of the Pampas, Uruguay. We performed a seasonal sampling of four permanent lentic water bodies by means of trawls, between May 2012 and February 2013, and studied fish sizes, abundances and diets. The species analyzed were *Astyanax laticeps*, *Cheirodon interruptus*, *Hyphessobrycon anisitsi* and *Australoheros* sp. The highest densities of the assemblage occurred in autumn and winter, with a strong decrease towards the summer. Considering the temporal distribution of sizes and densities, we could infer that there are different reproductive periods in the assemblage. We also found similarity of preys, which suggests the occurrence of competition between most of the species, except for *H. anisitsi*, which integrates terrestrial trophic paths. Our observations reveal the occurrence of an interesting ichthyological community in the studied artificial water reservoirs, which coexistence is based on a differentiation of ecological traits. Maintaining these environments of anthropogenic origin in good condition will contribute to the protection of the native freshwater biota.

**Keywords:** *Astyanax laticeps*, *Australoheros* sp., *Cheirodon interruptus*, *Hyphessobrycon anisitsi*, trophic ecology.

### Introduction

With more than 4500 known species, Neotropical freshwater fish are the most diverse in the world (Reis et al. 2003, Lévêque et al. 2007), yet large information gaps are present in both their diversity and their autoecology. It is estimated, for example, that the actual number of species could exceed 8000 by a wide margin (Bertaco et al. 2016). Although the ichthyofauna of Rio Grande do Sul and surrounding areas are considered among the best studied on the continent, the ecological aspects have mostly been focused on assemblages of streams, rivers, large lentic bodies and wetlands (e.g. González-Bergonzoni et al. 2009, Laufer et al. 2009a), while there is scarce data on anthropic environments with agricultural use (e.g. Dala Corte et al. 2016).

The Pampas is a very interesting region of South America, due to its unique ecosystems, mainly grasslands, and a high biodiversity. Uruguay and southern Brazil are characterized by this landscape, housing a significant number of fish species (Abell et al. 2008). The landscape of the eastern hilly region in Uruguay is home to an important wildlife diversity - a mixture of characteristic species (for some it is their southernmost distribution limit) and species of wider distribution (Brazeiro 2015). Due to its topology and rocky soil, Aceguá remains in a good conservation state, not yet affected by the agricultural advance. A common practice in this region is the damming of small natural water channels for the formation of reservoirs, mainly used for extensive livestock. These reservoirs consist of small lentic water ecosystems, providing environment for a large number of native species. However, despite the role that these water reservoirs play in maintaining local biodiversity, little is known about their biological communities.

In order to understand the native fish assemblages we must deepen the knowledge of the different species' traits (e.g. Laufer et al. 2009a). In this sense, the abundance and body size of the different species in an assemblage are very informative about the structure and function of the whole

aquatic communities. These traits, together with diet can inform us about the energy flow and structure of food webs (Arim et al. 2010, Kati et al. 2015). In the above-mentioned artificial reservoirs, the demographic and functional differences among co-occurring fish species can help us to understand the community mechanisms of coexistence. This coexistence of different small omnivorous fish species could be mediated by niche differentiation, where competing species use the environment differently—i.e. resources, spatial or temporal partitioning—(Brönmark & Hansson 2005).

We studied the fish assemblage of Pampas water reservoirs in north-eastern Uruguay. Here we report the results of a comparative seasonal study of population density, body size and diet of the most abundant fish species in these systems. We hypothesize that fish assemblage of these systems have niches segregations, which should be reflected in temporal differences in their respective abundance and a limited diet overlap.

### Material and Methods

#### Study Area

The present study is based on fish specimens collected in the area of Aceguá (Fig. 1), Cerro Largo Department, located at northeast Uruguay (31°54'S; 54°07'W). Aceguá is an upland area (approximately 220 meters a.s.l.) at the borderline with Brazil, and belongs to the Uruguayan eco-region Gondwanic Sedimentary Basin (Brazeiro 2015). Two large basins drain this area: the Laguna Merín and the Río Negro. The study reservoirs are located in many private farms, where land use is mainly cattle graze and small-scale agriculture. Historically, a common practice in this area was disrupting the natural creeks and water drainages to generate artificial reservoirs (by damming), in order to provide watering for livestock. Although these reservoirs alter the normal water flow, they act as environments for various species, such as invertebrates, fish, amphibians and reptiles, and as foraging sites for mammals and birds. At this area, seven lentic water bodies were sampled, but fish presence was found in only four of them. These four reservoirs are permanent and deeper than 1.5 m, with surface areas ranging 420 to 2700 m<sup>2</sup>.

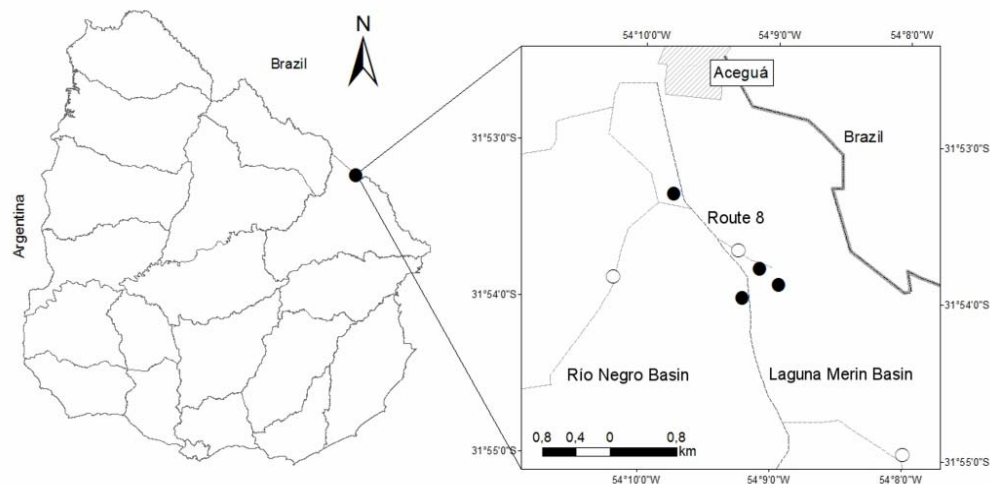


Figure 1. Study site location at the north-eastern Uruguayan borderline. Filled points correspond to sampled pond with fish presence and unfilled point refer to sampled pond without fish presence. Continuous grey lines mark the Uruguay-Brazil border and the dotted lines indicate the main roads. Route 8 matches with the watershed, to the right of the Merín Lagoon basin and left of the Río Negro basin.

### Sampling

Four seasonal samplings were performed on May, July and October 2012 and February 2013, with the help of a trawl net (5 m x 1 m, 5 mm mesh). One of the four ponds was only sampled from July to February. In each pond, two trawls were performed, one at the larger diameter axis and another in the minor diameter axis. The sampled area was recorded in order to standardize the sampling efforts (ranging 70 to 100 m<sup>2</sup>). The fishes were collected and euthanized with an overdoses of lidocaine hydrochloride, then fixed in formalin 4% and deposited at the Ichthyological Collection of Museo Nacional de Historia Natural, Montevideo (MNHN 3379 to 3389 and MNHN 3403 to 3466). At the laboratory, the collected fishes were identified (following Almiron et al. 2004, Azpelicueta & Loureiro 2009, Teixeira de Mello et al. 2011, Salas et al. 2012, Ottoni 2013, Serra et al. 2014), counted and measured with a digital calliper: standard length (SL, from snout tip to peduncle end). For large samples, only a subsample of 80 randomly selected specimens was measured, considering that this amount of individuals is more than enough to stabilize the variation in SL. Density was estimated as the relation of the total number of collected individuals per sampled surface.

### Diet analysis

We analysed the gut content of the fish specimens collected in spring (October 2012), when resource availability is highest. For the samples exceeding 20 individuals, a random sub-sample of 20 specimens of each species was analysed. Fish were dissected and their stomach content was extracted and determined under binocular microscope using local identification keys (Costa et al. 2006, Mugnai et al. 2009, Thorp & Covich 2009). Each prey was counted in order to obtain its abundance at individual stomach.

### Data analysis

Differences in densities were tested by a two-way variance analysis (ANOVA), using species and season as factors, followed by a Tukey post hoc test. Due to the lack of normal distribution of the SL data, we performed a non-parametric Kruskal-Wallis test. Then, SL differences between the four sampled seasons were tested for each species and for each season considering species identity. In both cases, a post hoc test was performed to evaluate differences between pairs of seasons and species (Sokal & Rohlf 2009). Then we explored the differences in SL for the two most frequent species in the spring sample (*A. laticeps* and *C. interruptus*, for which we had enough sample size to perform statistical analyses), considering density as a response vari-

able in the Kruskal-Wallis test. In all cases,  $\alpha = 0.05$  was considered significant (Sokal & Rohlf 2009).

In order to assess the differences in diet composition between different fish species, we performed a Correspondence Analysis (CA). This method analyses graphically the association between the frequencies of each prey item in gut content, grouping similar fish species with this criteria. Association between preys and species was tested by a Chi-square test (Legendre & Legendre 2012). For this analysis, the diet data was grouped into higher taxonomical groups, as follows: Acari, Araneae, Chironomidae, Cladocera, Coleoptera, Formicidae, Heteroptera, Hirudinea, Others (minor frequency preys), Other Crustacea (excluding Cladocera) and Rotifera.

Diet richness was evaluated by a rarefaction procedure using iNEXT package (Hsieh et al. 2014). This analysis is a robust method for the comparison of different sized samples (Chao & Jost 2012). Niche overlap was estimated through Pianka's index, for each pair of fish species. This index evaluates the overlap as the use of common resources (equally available) between two species (Krebs 1999). To verify the presence of non-random patterns in niche overlap, we calculated and statistically tested against null models using EcoSimR package (Gotelli et al. 2015). The program performed 5000 randomizations to create pseudo communities, and then statistically compared the mean niche overlap values for all species pairs, in these randomized communities (null model) with those in the observed data matrix (using algorithm ra3). Interspecific food partitioning might be occurring if the observed mean overlap values are significantly lower than those expected by the null model, whereas similar foraging patterns (corresponding to niche overlap) lead values higher than those expected by chance (Winemiller & Pianka 1990). All statistical analyses were made using R software (R Core Team 2016).

## Results

### Density and body size

In the sampled reservoirs we found four fish species, three belonging to the family Characidae, *Astyanax laticeps* (Cope 1894), *Cheirodon interruptus* (Jenyns 1842), *Hyphessobrycon anisitsi* (Eigenmann 1907) and one to Cichlidae, *Australoheros* sp., possibly *Australoheros* aff. *minuano* (Řičan & Kullander 2008) (Table 1, Fig. 2). Densities statistically differed between

Table 1. Densities of fish species in reservoirs of Aceguá, Cerro Largo, Uruguay. Area and the density (measured as individuals per drag meters) are reported for each species in autumn (A), winter (W), spring (Sp) and summer (Su). NA=data not available.

System	Area	<i>A. laticeps</i>				<i>Australoheros</i> sp.			
		A	W	Sp	Su	A	W	Sp	Su
1	443	0.5	0.2	0.3	0.2	0	0	0	0
2	419	1.7	0.3	0.2	0.1	0	0	0	0
3	2736	5.6	0	0.1	0.2	0	0	0	0
4	2535	NA	97.8	23.6	21.4	NA	76.8	1.2	2.1
System	Area	<i>C. interruptus</i>				<i>H. anisitsi</i>			
		A	W	Sp	Su	A	W	Sp	Su
1	443	40.6	82.8	76.8	7.0	29.4	35.5	6.2	22.1
2	419	90.5	40.9	71.4	19.6	0	0	0	0
3	2736	2.7	1.9	22.9	6.1	0	0	0	0.5
4	2535	NA	0	0	0	NA	0	0	0



Figure 2. Fish species studied in the locality of Aceguá, Cerro Largo Department, Uruguay. A. *Astyanax laticeps*; B. *Cheirodon interruptus*; C. *Hyphessobrycon anisitsi*; D. *Australoheros* sp. A, B and C are pictures of live specimens and D is fixed in formalin (MNHN 3413).

species (Two way ANOVA,  $F=4.8$ ,  $d.f.=3$ ,  $p<0.005$ , Fig. 2), but not between seasonal samples (Two way ANOVA,  $F=2.0$ ,  $d.f.=3$ ,  $p=0.1$ ). The most abundant species throughout the year was *C. interruptus*, reaching its maximum density in autumn and spring (more than 40 individual per sampled meter). The post hoc analysis showed that *H. anisitsi* and *Australoheros* sp. had significantly lower densities than *C. interruptus* and that *A. laticeps* had an intermediate density, not statistically different from the others. Although there is no statistically significant pattern, it was established is notorious that in summer the assemblage presents the lowest density of the year (Fig. 3).

Standard length ranged from 9.7 to 91.9 mm for the whole assemblage. We observed SL differences among species for the four explored seasons, according to Kruskal-Wallis test results (Table 2). Despite the fact that size distribution among species changed in every season, *A. laticeps* and *Australoheros* sp. remained as the largest species and *H. anisitsi* as the smallest (Table 2). There was a significant decrease in mean SL for the three Characidae from autumn to winter. Then, mean SL increased from winter to spring in *A. laticeps* and *H. anisitsi*, and from spring to summer in *C. in-*

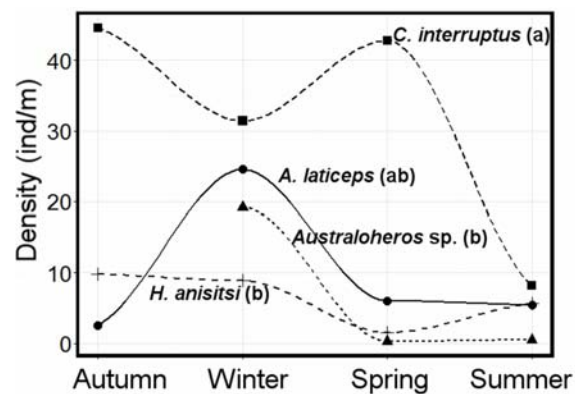


Figure 3. Average density (measured as individuals per drag meters) in each sampling of the different fish species in studied ponds. Letters indicate significant differences between species ( $F_3 = 4.8$ ;  $p<0.005$ ).

*erruptus*. On the contrary, *Australoheros* sp. exhibited a decreased body size in the period going from spring to summer (Fig. 4).

Regarding the density-body size relation of the two most

Table 2. Average SL for fish species in each season, Chi square and  $p$ -value result of Kruskal-Wallis test. Letters denote significant differences in SL (mm) between species.

	<i>A. laticeps</i>	<i>Australoheros</i> sp.	<i>C. interruptus</i>	<i>H. anisitsi</i>	$X^2$	$p$
Autumn	56.29 <sup>a</sup>	-	32.93 <sup>b</sup>	34.67 <sup>b</sup>	282.2	<0.005
Winter	30.33 <sup>a</sup>	37.72 <sup>b</sup>	30.86 <sup>c</sup>	30.01 <sup>a</sup>	60.9	<0.005
Spring	35.23 <sup>a</sup>	46.00 <sup>a</sup>	29.26 <sup>b</sup>	32.30 <sup>b</sup>	53.6	<0.005
Summer	34.20 <sup>ab</sup>	31.66 <sup>ab</sup>	33.64 <sup>a</sup>	30.23 <sup>b</sup>	10.8	<0.05

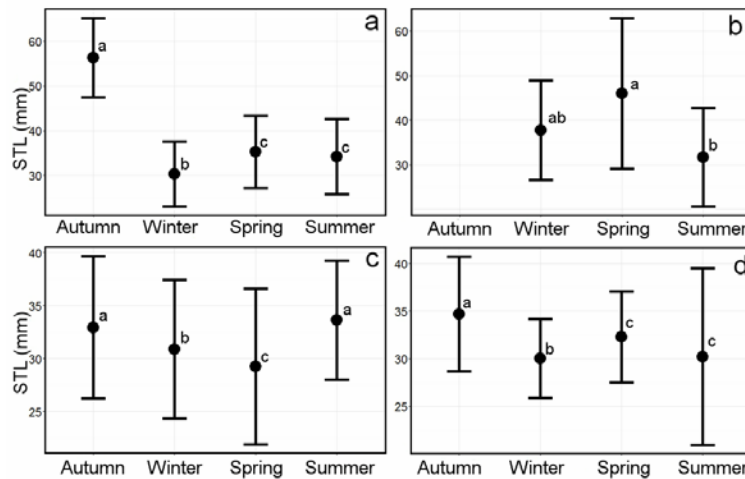


Figure 4. Average SL (mm) with standard deviation associated in each season. a. *A. laticeps* ( $X^2_3=307.6$ ,  $p<0.0001$ ); b. *Australoheros* sp. ( $X^2_2=11.0$ ,  $p<0.005$ ); c. *C. interruptus* ( $X^2_3=72.8$ ,  $p<0.0001$ ); and d. *H. anisitsi* ( $X^2_3=49.84$ ,  $p<0.001$ ). Different letters between seasons indicate significant differences in SL.

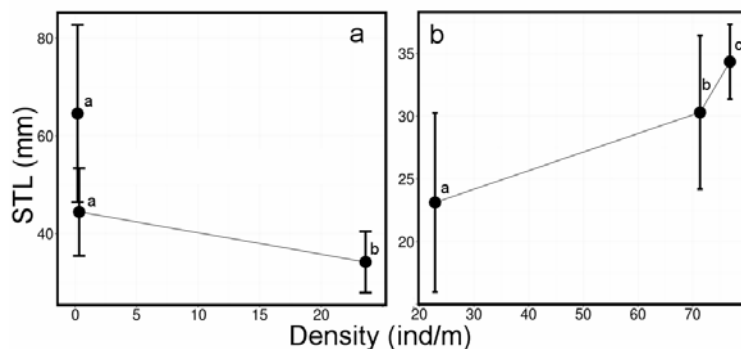


Figure 5. Mean standard length (SL) of: a. *A. laticeps* and b. *C. interruptus* at different densities in Aceguá, Cerro Largo, Uruguay. Different letters indicate significant differences in SL (Kruskal-Wallis test, *A. laticeps*:  $X^2=14.8$ , d.f. =2,  $p<0.001$ ; *C. interruptus*:  $X^2=183.7$ , d.f.=2,  $p<0.001$ ).

frequent species in spring, *A. laticeps* and *C. interruptus*, we obtained opposing results (Fig. 5). On the one hand, *A. laticeps* showed a pattern of negative relation between these two variables (Kruskal-Wallis test,  $X^2=14.8$ , d.f.=2,  $p<0.001$ ), whereas *C. interruptus* showed a positive relation (Kruskal-Wallis test,  $X^2=183.7$ , d.f.=2,  $p<0.001$ ).

#### Diet

We identified 533 prey items (excluding algae that were not quantified) in a total of 122 fish stomachs. The most abundant prey was zooplankton, especially cladocerans and rotifers, followed by aquatic Coleoptera (Table 3). Most of the preys were primary producers and aquatic invertebrates, except for the terrestrial spiders and ants. Algae were found in 39% of the stomachs of the assemblage, with a high frequency in *Australoheros* sp.; 50 % of this species individuals consumed this item. *Astyanax laticeps* is the species that consumed the highest number of prey, followed by *Australoheros* sp. In both cases, Cladocera was the most abundant item. Algae and cladocerans are the only items that were consumed by all four species.

The contingency table analysis showed a statistical significant association between preys and fish species ( $X^2=308.0$ , d.f.=30,  $p<0.0001$ ). In the correspondence analysis, the axis 1 (involving 76.6% of the variance) segregated *H. anisitsi* from the remaining species. In this analysis, *H. anisitsi* was associated with terrestrial preys (spiders and ants), beetles and other aquatic macro-invertebrates (especially dragonfly nymph). While the other three species were related to a zooplanktonic consumption, *H. anisitsi* consumed a greater diversity of invertebrates, including terrestrial and larger aquatic preys. Then the second axis (involving 18.0% of the variance) separated *C. interruptus* from the remaining fish, due to its association with other crustaceans, such as amphipods and calanoid copepods, and leeches (Fig. 6).

Prey richness differed between species. In the cut point of the rarefaction extrapolation, *H. anisitsi* and *A. laticeps* showed higher richness than *Australoheros* sp. and *C. interruptus*. Even though *A. anisitsi* had greater richness in stomach contents, the differences with *A. laticeps* were not significant, but considering the confidence intervals, differences were significant with the other two species (Fig. 7).

Table 3. Frequency of prey consumed by each fish species.

	<i>A. laticeps</i>	<i>Australoheros</i> sp.	<i>C. interruptus</i>	<i>H. anisitsi</i>
<i>N</i>	30	12	60	20
<i>N prey</i>	376	86	19	52
Rotifera	0.80		0.08	0.05
Cladocera	10.80	6.75	0.13	0.40
Copepoda (Cyclopoida)	0.03			
Anfipoda	0.03		0.02	
Belostomatidae			0.02	
Corixidae	0.17			0.20
Notonectidae	0.03			
Coleoptera	0.57			1.25
Coleoptera (larva)	0.03			0.05
Chironomidae (larva)		0.25	0.03	0.20
Odonata (larva)				0.05
Formicidae	0.03	0.08		0.10
Acari			0.02	0.15
Araneae				0.15
Nematoda	0.03			
Hirudinea		0.08	0.02	

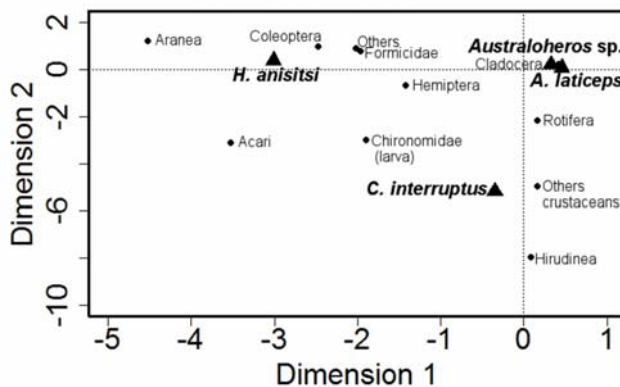


Figure 6. Correspondence analysis of diet items and the fish species (bold) sampled in Aceguá. The Axis 1 involves 71% of the variance, while the second Axis involves 18% of the variance.

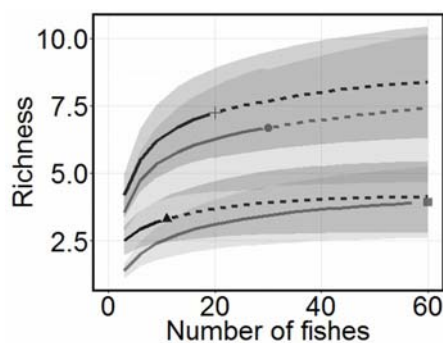


Figure 7. Rarefaction curve of diet by each fish species sampled in Aceguá in October 2012. The solid lines are the result of rarefaction analyses, dashed lines are the extrapolation and shading indicates the confidence intervals at 95%. The cross refers to *H. anisitsi*, the circle to *A. laticeps*, the triangle to *Australoheros* sp. and the square to *C. interruptus*.

The pseudo community analysis indicated that the probability of observing by chance, a mean niche overlap index of 0.20 (simulated mean) or less, is 0.99, indicating lack of niche partitioning. The observed mean overlap index of 0.60 was

statistically significant and greater than the simulated mean ( $p < 0.005$ ). *Hyphessobrycon anisitsi* had the most singular diet. Pianka's index of niche overlap indicated that *H. anisitsi* diet did not overlap with any of the other species (Pianka's index  $\sim 0.3$ ). The other three species showed a high diet overlap (Pianka's index  $> 0.8$ ). This overlap was greatest between *A. laticeps* and *Australoheros* sp. (Pianka's index = 0.99; Table 4).

## Discussion

We report for the first time an annual variation in density, body size, diet composition and overlap of the following four fish species: *A. laticeps*, *C. interruptus*, *H. anisitsi* and *Australoheros* sp., which occur in sympatry at the Pampas reservoirs in the hills of north-eastern Uruguay. The studied environments are located in two large basins modified by anthropic activities, but free from the intensive agriculture pressures. The fact that these four fish species are highly abundant and persistent in the studied water reservoirs provides an idea of their important ecological role. These water reservoirs are also habitat for a rich native biodiversity of

Table 4. Pianka's index of niche overlap for the fish assemblage present in Aceguá. The index compares the overlap in food items for each pair of species.

	<i>Australoheros</i> sp.	<i>C. interruptus</i>	<i>H. anisitsi</i>
<i>A. laticeps</i>	0.995	0.849	0.339
<i>Australoheros</i> sp.		0.820	0.289
<i>C. interruptus</i>			0.302

reptiles, amphibians and macroinvertebrates (e.g. Laufer et al. 2009b, Gobel et al. 2013, Laufer et al. 2014). Other fish species recorded at these systems, but not collected in our samples (due to the used sampling method and their low densities) are *Rhamdia quelen*, *Callichthys callichthys*, *Hoplias malabaricus*, *Astyanax eigenmanniorum* and *Synbranchus marmoratus* (unpublished data). The main threat to biodiversity of these ponds is an on-going bullfrog (*Lithobates catesbeianus*) invasion (Laufer et al. 2008, Ruibal & Laufer 2012, Laufer et al. 2018).

The studied systems showed an important variation in fish densities and body sizes between seasons. *Cheirodon interruptus*, the most abundant species, has a wide geographical distribution and tolerance to a wide range of environmental mesotrophic and eutrophic conditions (Jeppesen et al. 2007, Chalar et al. 2013). *Astyanax laticeps*, characteristic of the region and restricted to hilly areas (Azpelicueta & Loureiro 2009), was another abundant species in the system. *Australoheros* sp. and *A. laticeps* showed the largest body sizes and had the same pattern of annual densities (although *Australoheros* sp. does not appear in the first sampling). Finally, *H. anisitsi* was the least frequent species and remained within a narrow range of densities, relatively lower than the others.

Regarding the diet, we observed that the studied assemblage has a relatively wide trophic niche. *Astyanax laticeps* and *H. anisitsi* showed the highest diet richness, estimated by the rarefaction procedure. However, *H. anisitsi* differed from the others in the composition of consumed items, with a greater proportion of allochthonous prey than the rest of the assemblage. Despite its low density, this species diet could be integrating aquatic and terrestrial trophic pathways (Arim et al. 2010, Rooney & McCann 2012). This different foraging strategy is consistent with a study conducted in the state of Parana (Brazil) in which, within an assemblage of four Characid species, its congener *Hyphessobrycon grieni* was also the major consumer of allochthonous prey (Barreto & Aranha 2006). Even studies in more diverse communities, such as in São Paulo, Brazil, showed that *H. anisitsi* integrates a significant fraction of allochthonous prey in its diet (Castro & Carvalho 2014). The evidence of allochthonous preys in its gut content indicates that the genus *Hyphessobrycon* would have a different trophic role than the other Characidae, which should be considered in future studies.

Interestingly, *A. laticeps* and *Australoheros* sp., the pair of species with similar body sizes and intermediate densities, showed a similarity in their diets and thus a high trophic overlap. This could be explained by their similarity in body size, which is a strong determinant of ecological patterns and may even take precedence to other variables, such as the taxonomic or morphological identity (Laufer et al. 2009a,

Arim et al. 2010). This two species could be strong competitors in the studied systems.

Our observations about body sizes and densities allow us to infer reproductive periods within the studied year. *Cheirodon interruptus* presented a peak density in spring and then a decrease in body size in winter and spring, suggesting that the breeding season could occur during this period. This is in agreement with the evidence for Buenos Aires Province (Argentina), where this species presents an extensive spawning season between July and September (Ferriz et al. 2011). *Astyanax laticeps* had the lowest mean body size and a maximum density peak in winter, which may be related to recruitment events at that season. This evidence does not coincide with the existing literature from southern Brazil, suggesting that this species has a prolonged reproduction during spring and summer (Luz-Agostinho et al. 2010). On the other hand, *Australoheros* sp. reached a minimum value in the average SL in summer, although the maximum density was in winter. These differences could be related to its population dynamics and/or seasonal variations in their reproductive behaviour. It is known that this group of cichlids presents territorial behaviour, couple formation and parental care (Alonso et al. 2011, Nelson et al. 2016). Regional evidence for southern Brazil indicates that *A. aff. facetus* reproduction occurs in spring-summer (Luz-Agostinho et al. 2010). Finally, our observations about *H. anisitsi* densities in autumn and winter, and even body size in winter, suggest that this species' recruitment could occur during this period. The available evidence indicates that this species is a multiple spawner, with a plastic reproduction strategy (Gonçalves et al. 2013).

Density-body size scaling is one of the strongest relationships in ecology, predicting an increase in body size at lower population abundances (White et al. 2007). In this sense, while evaluating this relationship for the two most frequent species in Aceguá we found contrasting results. While *A. laticeps* responded as expected by the theory, *C. interruptus* shows an increase in body size at sites with higher densities. Our study does not allow us to draw conclusions about this observation, but it can be hypothesized that other unconsidered phenomena, such as community interactions (e.g. niche overlap) or sampling bias, are responsible for this pattern.

In summary, coexistence seems to be promoted in the studied systems, by important differences in species traits, especially concerning prey consumption from different origins (terrestrial and aquatic) and temporal segregation in densities and body sizes, probably showing different reproductive cycles. Knowing the natural history of the studied species is a necessary tool for their conservation. Although the three species of Characidae have not yet been evaluated according to IUCN criteria, *A. laticeps* conservation is a priority for the Protected Areas National System in Uruguay (Soutullo et al. 2013). We understand that given the agricultural use of water reservoirs and their important diversity, there must be strategies that ensure the coexistence between appropriate land use and conservation.

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