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Trophic interactions between a native Catfish (Trichomycteridae) and a non-native species, the Rainbow Trout, in an Andean stream, San Juan, Argentina

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The trophic relationship between Torrent Catfish (Hatcheria macraei) and Rainbow Trout (Oncorhynchus mykiss) was studied in a lotic Andean system of Argentina to examine whether these fish compete for the same food resources. Fishes were sampled seasonally, in autumn, spring, and summer. Hatcheria macraei and O. mykiss presented a carnivorous generalist feeding strategy. Trout diet was dominated by aquatic macroinvertebrates, and terrestrial invertebrates were an important component in summer. Large-sized Rainbow Trout fed on Catfish, which in turn preyed mainly on Chironomidae and Elmidae larvae. During flood periods, the Trout diet, as well as that of the Catfish, was mainly composed of clingers (Elmidae and Chironomidae) and particularly in the case of Catfish, burrowers (Oligochaeta) were found. We found an elevated dietary overlap between Trout and Catfish with seasonal changes. Our results suggest that coexistence of Trout and Catfish depends on input of terrestrial invertebrates, and the feeding of Catfish on small prey.

Keywords: non-native salmonids, terrestrial and aquatic prey, competition, diet

Introduction

Salmonid invaders have led to biotic homogenisation, habitat alteration, and loss of native or endemic species worldwide (Rahel, 2002; Towsend, 2003). Fishes of the Salmonidae family, native to the Northern Hemisphere, have been successfully introduced in the Southern Hemisphere (Crawford and Muir, 2008). In particular, *Onchorynchus mykiss* was introduced in Argentinean Andean streams and rivers in 1904 (Baigún and Quirós, 1985), without previous ecological studies having been conducted to assess the impact of this species on the native fauna.

The Trichomycteridae family is the most diverse group of Catfish in Argentinean Andean ecosystems, with three genera: *Hatcheria, Silvinichthys* and *Trichomycterus* (López et al., 2008). *Hatcheria*, with its single species, *Hatcheria macraei*, exhibits a wide distribution in southern South America in the Andean region of Argentina and Chile, which is relevant for examining phylogenetic information. The range of distribution includes the Atlantic and Pacific drainages (López et al., 2008; Unmack et al., 2009) known to be historically connected because of glacial activity (Unmack et al., 2012). *Hatcheria macraei* is an important environmental indicator of water pollution (Ortiz et al., 2003) because of possible waste dumping of mining activities carried out near rivers of the region.

There are different lines of evidence to explain O. mykiss success in different environments: the phenotypic plasticity of the Trout life cycle (Sloat et al., 2014; Sloat and Reeves, 2014); its generalist carnivorous diet (Di Prinzio and Casaux, 2012; Tagliaferro et al., 2015), including native fishes (Macchi et al., 1999; McIntosh, 2000; Vigliano et al., 2009); the fact that it modifies the use of habitat by native species (Habit et al., 2010; Penaluna et al., 2009); and foraging (Baxter et al., 2004). However, studies that identify mechanisms of coexistence with native species are scarce (Arismendi et al., 2011). Understanding possible trophic overlaps could identify potential risks to native fishes. Morphological differences between O. mykiss and H. macraei may have favored the coexistence. H. macraei is a rheophilic and negatively phototactic Catfish (Menni, 2004) which has a subterminal mouth, and negative allometric growth. This could be related to the small size of benthic prey consumed during all ontogeny (Barriga and Battini, 2009). Onchorynchus mykiss has a robust body with a high caudal peduncle, a large head, a large mouth, and long paired fins (Keeley et al., 2005). Rainbow Trout prey on epibenthic, aquatic drift and terrestrial fauna (Molineri, 2008) the abundance of which is probably influenced by the availability of prey types (Nakano et al., 1999) with different diel periodicity.

There are no existing studies focused on native and non-native fish diet in the Cuyo Andean region to understand the coexistence of these species. The goal of this study was to analyse the trophic relationships between Torrent Catfish (*Hatcheria macraei*) and Rainbow Trout (*Oncorhynchus mykiss*), to assess the competition for food resources, and to generate information that will serve as a tool for conservation and management of the fish populations in the Cuyo Andean region.

Methodology

The Las Burras stream is located in the Cuyo-Desaguadero basin, an ecoregion in the San Juan province, Argentina (31°18'09.7" S, 69°38'58.1" W). It is a freshwater stream at 2,000 metres above sea level, which originates in the Andes and flows into the Castaño river. There are anthropogenic willow forests that generate small artificial oases with varying degrees of waterlogging driven by changes in the water regime. During the study, the average air temperature was 11.8°C in autumn, 14.8°C in spring and 20°C in summer. Mean flow was 0.13 (m³/s) in autumn, 0.07 (m³/s) in spring and 0.093 (m³/s) in summer. During discharge periods (unstable periods), this stream carries a significant sediment load, which causes deep lateral erosion of the streambed. The native H. macraei and the exotic O. mykiss were the only two fish species present in the Las Burras stream.

Fishes were sampled seasonally in December 2012, April 2013, October 2013, May 2014, October 2014, and January 2015, using hand nets (1 mm mesh size) and fishing-rods. Sampling was carried out over a length of 3 kilometers of stream. Samples were obtained every 100 m, for 6 h daily over the course of 2 days, from 10 a.m. to 4 p.m. All possible microhabitats for both species were sampled to capture all fish sizes. It is possible to observe different sizes of Trout present due to water transparency. In the field, fish were fixed in situ with formalin (10%). In the laboratory, stomach and intestine were removed.

Digestive contents were observed under a stereomicroscope in order to identify and measure each prey item. Prey items were determined following the classification of Domínguez and Fernández (2009), and Ross (1982). The volume of each prey item consumed was estimated using the Dunham (1983) formula for the ellipsoid sphere, $V = 4/3\pi (1/2 L) (1/2 W)^2$, where V is volume, L is length of prey and W is width of prey. To analyse the diet, we calculated the frequency of occurrence (%Fi), percentage by number (%N_i), and percentage by volume (%V_i) as follows:

- $%F = (N_i/N) *100$, where N_i is the number of stomachs containing a category and N is the total number of stomachs.
- $%N_i = (N_i/N_t)^*100$ where N_i is the number of prey items i and N_t is total prey items.
- $%V = (V_i/V_t)*100$ where V_i is the volume of prey items i and V_t is the total volume of prey items.

We estimated the index of relative importance (IRI) as I = (F% + N%) * V%. Then IRI was

expressed as a percentage as %IRI = (IRI/ Σ IRI*100) for each prey category. According to this index, prey items were classified into the following categories: Fundamental prey, IRI% > 75%; Secondary prey, 75% > IRI% > 50%; Accessory prey, 50% > IRI%> 25%, and Accidental prey, IRI% < 25%. In order to establish the hierarchy ranking (DJ) of the diet, the highest value of IRI was considered 100%, and other values were calculated as relative percentages. Diet breadth (B) was calculated using the Levins (1968) index: $B = 1/\Sigma p_i^2$ where p_i is the proportion of each prey type i in the diet. Diet diversity was assessed using the Shannon-Weaver diversity index (H). Diet overlap was estimated using the Morisita-Horn index (I_{MH}) . Prey items were assigned to a functional prey group (FPG) based on origin (Terrestrial) or habit: Clingers (with adaptations to adhere to the substrate) and Burrowers (burrowing in soft sediments) following the Hanson et al. (2010) classification. The Kruskal-Wallis test was applied to compare standard fish length, abundance of aquatic and terrestrial prey, and size of Elmidae larvae. The analysis of feeding strategies was characterised by Amundsen's modified graphical method (Amundsen et al., 1996).

The non-metric multidimensional scaling (N-MDS) technique was used to compare the similarity in abundance of prey items in the *H. macraei* and *O*. mykiss diet, in different seasons, using Bray-Curtis distance (Clarke, 1993). Prior to analysis, the prey abundance matrix was $\log (x+1)$ transformed. The hypothesis of similarity between groups was evaluated through a similarity analysis (ANOSIM, 9999 permutations) with a reference value of p < 0.05. A SIMPER analysis was performed to establish potential differences among Trout and Catfish diet composition between seasons providing a ranking that shows which prey items contributed most by percentage to the percentage of dissimilarity (Clarke, 1993). The R Studio Version 1.0.136 was used for the statistical analyses.

Results

Oncorhynchus mykiss and H. macraei were found to be sympatric in the Las Burras stream (Argentina). A total of 50 Trout and 77 Catfish were analysed in autumn, spring, and summer. The standard lengths of Rainbow Trout (H = 0,16; p = 0.9) and Catfish (H = 5; p = 0.06) were similar for all seasons (Table 1).

Table 1. Mean value and standard deviation of standard length (SL), and trophic niche index in Rainbow Trout (*O. mykiss*) and Catfish (*H. macraei*) in the Las Burras stream. Diet assessed as Levin's index (B), Shannon-Weaver diversity index (H) and Richness (S).

	<i>0. n</i>	ıykiss(n = 50)	Н. т	<i>H. macrei</i> (<i>n</i> = 77)		
Season	S	L	n	S	L	n	
Autumn Spring Summer	137 143 149	$\pm 27 \\ \pm 36 \\ \pm 45$	12 17 21	101 = 90 = 106 =	E 22 E 28 E 26	30 30 17	
Diet	В	Η	S	В	Η	S	
Summer Spring Autumn	4.0 1.2 5.7	1.7 1.3 2.1	29 30 23	4.14 2.20 1.67	1.67 0.52 0.93	12 14 19	

O. mykiss consumes prey of terrestrial origin, benthic macroinvertebrates, and vertebrates. *H. macraei* diet consisted of benthic macroinvertebrates, mainly insects, and vertebrates. The total number of prey items (S) observed in *O. mykiss* (46) was twice that observed in *H. macraei* (21).

Rainbow Trout and Catfish showed a generalist carnivore feeding strategy. Prey items with high percentage of volume in stomach contents, consumed by few individuals, were Acriididae ($\overline{X}_{\text{length}} =$ 1.5 cm) in Rainbow Trout, and Austrelmis ($\overline{X}_{\text{length}} =$ 0.3 cm) and Cicadellidae ($\overline{X}_{\text{length}} =$ 0.3 cm) in Catfish. Prey items with low specific abundance, consumed by many individuals, were Formicidae and Elmidae in Rainbow Trout, and Chironomidae and Elmidae larvae in Catfish (Figure 1).

The highest diversity values in the Trout diet were found in autumn (H = 2.1), and in summer (H = 1.67) for the Catfish diet. The Levins index indicated that the highest diet breadth was found to occur in autumn for Trout and in summer for Catfish (Table 1). Formicidae was the best represented family among terrestrial prey. Fundamental prey items in Rainbow Trout were Austrelmis in spring and summer, Elmidae larvae in summer, and Acridiidae and Formicidae in autumn, with Chironomidae larvae as secondary prey items in autumn. The main prey items in H. macraei diet were Chironomidae larvae in spring and autumn, and Elmidae larvae in summer, with Chironomidae larvae as secondary prey items in summer (Table 2). The length of Elmidae larvae, a fundamental prey in Trout $(\bar{x} = 4.82 \pm 1.38)$ and Catfish $(\bar{x} = 3.65 \pm 1.7)$ in



Figure 1. Feeding strategy diagram: Prey specific abundance in stomach content against frequency of occurrence of prey in the diet of (a) *O. mykiss* and (b) *H. macraei*.

summer, showed significant differences (H = 12.17, p = 0.0004). Trout prey on large Chironomidae larvae ($\bar{x} = 3.19 \pm 0.93$), and Catfish, on smaller ones ($\bar{x} = 2.74 \pm 1.09$) with significant differences (H = 8.9; p = 0.0021).

Overlap in the diet of O. mykiss and H. macraei showed a differential response between seasons, estimated with the Morisita-Horn index. Diet overlap was highest in autumn ($I_{MH} = 0.77$) and similar in spring (I_{MH} = 0.63) and summer (I_{MH} = 0.62). ANOSIM showed a significant similarity in the diet between species and between seasons (R statistic = 0.46, P = 0.001). The SIMPER analysis showed that Formicidae (14%) and Chironomidae larvae (39%) were the prey items that contributed the most to diet similarities between Rainbow Trout and Catfish in autumn; Austrelmis adults (33%) and Chironomidae larvae (46%) in spring. While in summer, Austrelmis adults (15%), Formicidae (12%) and Elmidae larvae (26%) were the prey items that significantly contributed to diet similarities (Table 3). The N-MDS representation separated most individuals from trout and catfish. The graphic overlap was observed during the summer (Figure 2).

The abundance of aquatic prey items in the diet of the Rainbow Trout changed significantly with the seasons (H = 8.6; p = 0.01), being more abundant in autumn (59%) and spring (62%). Terrestrial prey did not show significant differences between seasons (H = 2.1; p = 0.33) but increased in summer (42%) when Formicidae were an accessory prey and in autumn (41%) when Formicidae were fundamental prey. *H. macraei* fragments (n = 3) were found in Trout with standard lengths: 187 mm (autumn), 179 mm (spring) and 242 mm (summer).

Clingers were the most frequent functional prey group (FPG) and there were not significant differences between seasons in Trout (H = 5; p = 0.05) and Catfish (H = 5; p = 0.07). Elmidae larvae were the most frequent prey items in the clingers group for Trout (autumn = 66%, spring = 81% and summer = 94%) and Catfish (autumn = 73%, spring = 71% and summer = 88%) in all seasons. Chironomidae larvae were more frequent in the Catfish (autumn = 90%, spring = 93% and summer = 64%) than in the Rainbow Trout diet (autumn = 83%, spring = 48% and summer = 33%). In *H. macraei*, burrowers were more frequent in spring (45%) and summer (30%) (Figure 3), contributing 51% of the diet volume.

Discussion

The main objective of this study was to examine whether H. macraei and O. mykiss compete for the same trophic resources. Native H. macraei and non-native O. mykiss coexist in Las Burras stream in terms of space and time. Where native species are sympatric with introduced salmonids, there is evidence of negative interactions (for example, reduction in growth, abundance, and habitat use) (McIntosh, 2000; Baxter, 2004; Penaluna et al., 2009). Most of the studies carried out in Patagonia find some degree of segregation between native and introduced species, whether trophic, reproductive or in habitat use (Pascual et al., 2002). Furthermore, it has been observed that Trout cause indirect effects on food webs because of the decrease in the biomass of emerging adult aquatic insects (Nakano et al., 1999).

In the environment studied, *H. macraei* coexists with *O. mykiss* individuals with standard lengths of 80 to 240 mm, whereas in a Patagonian river, *H. macraei* coexists with juvenile salmonids with

			0. my	kiss					H. ma	acraei		
	Autu	um	Spri	ng	Sum	mer	Autu	nn	Spr	ing	Sum	mer
Item	%IRI	DJ	%IRI	DJ	%IRI	DJ	%IRI	DJ	%IRI	DJ	%IRI	DJ
Acridiidae	25.8	100	0.02	0.04								
Aphididae	0.05	0.21			0.23	0.66						
Apoidea	0.72	2.79			0.11	0.30						
Austrelmis (A)	6.14	23.74	44.06	100	35.1	99.50			0.06	0.08	0.1	0.18
Ceratopogonidae (P)									1.76	2.47		
Chironomidae (L)	20.8	80.48	20.87	47.4	0.61	1.74	72.78	100	71.4	100	25	63.13
Chironomidae (P)	2.45	9.49	0.10	0.23	0.03	0.08	0.11	0.14	0.41	0.58	0.1	0.15
Cicadeliidae	4.98	19.25			1.98	5.63	0.02	0.02				
Cicadidae					0.07	0.21	0.03	0.04	0.01	0.02		
Curculionidae	1.90	7.33	0.06	0.15								
Elmidae (L)	1.90	7.34	16.21	36.8	35.3	100	10.31	14.2	8.25	11.56	40	100
Embolemidae	0.05	0.19					0.19	0.26				
Ephemeroptera (N)	0.93	3.60	1.41	3.20	1.24	3.53			0.06	0.08	1.2	3.09
Formicidae	20.45	79.06	12.98	29.5	17.5	49.70					0.2	0.39
Gelastocoridae			1.73	3.92								
Ichneumonoidea			0.20	0.45	0.11	0.31						
Oligochaeta	2.76	10.68	0.44	1.01	2.93	8.32	0.12	0.17	16.4	23.00	17	42.67
Phoridae	0.23	0.89			0.02	0.05						
Platygastroidea	0.23	0.89			0.01	0.02						
Sarcophagidae			0.81	1.84	0.12	0.33						
Simuliidae (L)	10.5	40.80	0.67	1.53	0.14	0.40	9.51	13.1	0.64	0.90	4.0	96.6
Simuliidae (P)			0.07	0.17	0.02	0.07	0.16	0.22				
Tenebrionidae			0.03	0.06								
Tephritidae (L)					0.03	0.09	0.00	0.00			0.2	0.44
Tephritidae (P)									0.29	0.41		
Tephritidae (A)			0.08	0.19			0.06	0.08				
Trichoptera (L)	2.16	8.3	0.24	0.54	4.48	12.70	6.61	9.08	0.73	1.03	12	31.19

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348

Garcia et al./Aquatic Ecosystem Health and Management 20 (2017) 344-352

Table 3. SIMPER results between Trout and Catfish diets in different seasons. Average abundance of prey in Catfish and Trout diet, percentage of contribution to diet (Cont. %), and cumulative percentage (Cum. %). Preys that contributed less than 5% were excluded from the table.

			Average abundance	
Autumn	Contrib. %	Cum. %	H. macraei	O. mykiss
Chironomidae (L)	38.9	38.9	62.4	21.4
Formicidae	13.9	52.8	0.0	11.7
Simuliidae (L)	10.1	62.9	4.3	8.7
Cicadellidae	8.0	70.9	0.1	11.7
Trichoptera (L)	7.4	78.3	7.5	2.8
Elmidae (L)	6.0	84.3	5.2	1.8
Austrelmis (A)	5.0	89.3	0.1	5.1
Spring				
Chironomidae (L)	46.3	46.3	116	124
Austrelmis (A)	32.8	79.0	0.1	74.5
Elmidae (L)	8.3	87.4	4.6	28.2
Formicidae	5.2	92.5	0.0	12.4
Summer				
Simuliidae (L)				
Austrelmis (A)	31.5	31.5	0.1	32.0
Elmidae (L)	25.6	57.1	8.2	34.6
Formicidae	12.0	69.1	0.2	10.1
Trichoptera (L)	7.6	76.7	2.8	7.3
Chironomidae (L)	6.5	83.2	5.9	2.4
Cicadellidae	6.3	89.5	0.0	5.1

a standard length of 20 to 100 mm (Barriga et al., 2013). This was the opposite of that observed in three streams in the northwestern region of Chubut province, where large salmonids were the most abundant fish and *H. macraei* was poorly represented (Di Prinzio and Casaux, 2012). Conversely, *O. mykiss* and *Trichomycterus* were always found to be allopatric in streams in the northwestern region of Argentina (Molineri, 2008).

Comparative studies of the use of food resources allow the identification of important interactions that determine community structure. In our study, Chironomidae and Elmidae larvae were fundamental prey in the diet of *H. macraei*. These results could be related to the abundance of larvae in the stream. The greater abundance of Elmidae and Chironomidae larvae may be due to the impact of Trout on macroinvertebrate communities, as observed by Molineri (2008) in subtropical mountain streams in the northwestern region of Argentina. We found that *H. macraei* feeds on small Elmidae and Chironomidae larvae while *O. mykiss* feeds on the larger ones. This can be explained as a consequence of its high morphological specialisation for anchoring to the substrate like other Trichomycteridae (Adriaens et al., 2010), removing the mud and obtaining small prey to avoid predation by Trout. Chironomidae larvae were fundamental prey items in the diet of *H. macraei* in the Caleufú river (Barriga and Battini, 2009), the Chubut river (Ferriz, 2012), and the Manguera stream in summer (Di Prinzio and Casaux, 2012).

Despite the morphological differences between the native and the introduced species, which presupposes different diet compositions, we found dietary overlap between Trout and Catfish in different seasons. In autumn, this was observed in the consumption of Chironomidae larvae. In spring, the overlap was observed in the consumption of adult individuals of *Austrelmis*, and Chironomidae larvae increased their contribution to Trout diet. In summer, overlap was observed in the consumption of Elmidae larvae when the abundance of other aquatic prey items



Figure 2. Non-metric multidimensional scaling graph (2D) using Bray-Curtis distance to show similarity grade of *O. mykiss* and *H. macraei* prey abundance composition grouped by season. Catfish: Autumn (AU_C); spring (SP_C); summer (SU_C); and Trout: Autumn (AU_T); spring (SP_T); summer (SU_T). The proximity of the symbols indicates a higher degree of similarity (Stress = 0.20).

decreased. These results suggest a potential competion for food resources, although additional evidence would be neccessary to confirm this.

Notwithstanding the observed overlap, however, H. macraei and O. mykiss appear to coexist due to the Trout's reliance on terrestrial prey and the consumption of large macroinvertebrates. Trout predation on terrestrial organisms was also observed in other studies carried out in the Patagonian region (Buria et al., 2009; Di Prinzio et al., 2013; Juncos et al. 2014) as well as in Japan (Nakano et al. 1999; Baxter et al., 2004) and New Zealand (Edwards and Huryn, 1995). The input of terrestrial prey from anthropogenic willow forests and riparian vegetation in summer was an important aid for Trout, when the number of aquatic insects declines due to the emergence of aquatic insects and the abrupt drop during discharge periods, as occurs in other Andean streams of Argentina (Molineri, 2008; Scheibler and Debandi, 2008). However, in a canopied stream of the Patagonian Andes, terrestrial prey were a minority in the diet of O. mykiss (Buria et al., 2009), which

selected larger aquatic macroinvertebrates (Buria et al., 2007), and predation on large aquatic macroinvertebrates may reduce the consumption of terrestrial organisms.

On the other hand, fragments of Catfish in the stomachs of the larger Trout. These results were concordant with observations made in different environments of an ontogenic shift in Trout diet from small macroinvertebrates predators towards piscivory as they increase in body size (McIntosh, 2000; Keeley and Grant, 2001; Arismendi et al., 2011). The growth of salmonids is lower in streams than it is in lakes and oceans, and could be affected, while their size is still small, by a predominantly insectivorous diet and with the possibility of feeding on fishes (Keeley and Grant, 2001). It has been pointed out that upon reaching the approximate size of 150 mm, the Trout become piscivorous (McIntosh, 2000). This study determined this size to be larger while very infrequent. The predominantly insectivorous O. mykiss diet and the low forage rate on H. macraei possibly limit its



Figure 3. Seasonal variations in relative frequency of FPG (Burrowers, Clingers and Terrestrial insects) in (a) O. mykiss and (b) H. macraei.

growth in this environment and contributes to coexistence with Catfish.

Information on the use of food resources allows us to generate new hypotheses on trophic interactions. This data helps improve knowledge regarding interactions between native and non-native species, thus enabling the development of conservation and management strategies for the fish populations in the Andean region of San Juan.

Conclusions

We provide new information about the diet of a sympatric population of a native and non-native fish in a stream in the Cuyo-Andean region. We found diet overlap between Rainbow Trout and Catfish. This result suggests potential competition food resources and/or segregation. We for observed that both species consumed Elmidae and Chironomidae larvae. Catfish prey on small Elmidae and Chironomidae larvae and Rainbow Trout, on larger larvae as a consequence of possible competition or as strategy by Catfish to reduce predation risk. Trout consumed terrestrial prey that could represent an alternative food resource when benthic prey decline (summer-autumn) and facilitate the co-occurrence of native and non-native fish.

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